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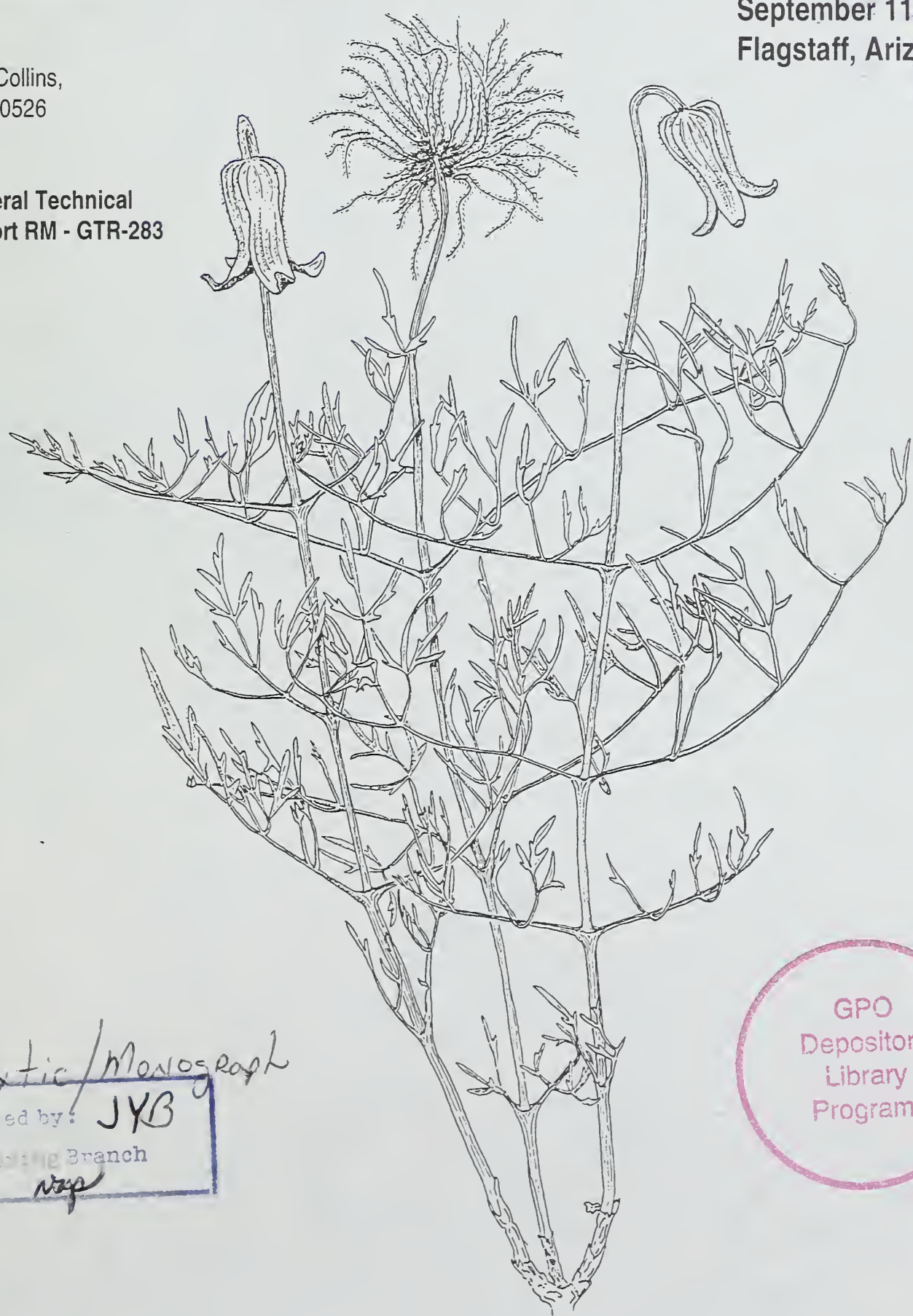
# Southwestern Rare and Endangered Plants: Proceedings of the Second Conference

Rocky Mountain  
Forest and Range  
Experiment Station

September 11-14, 1995  
Flagstaff, Arizona

Fort Collins,  
CO 80526

General Technical  
Report RM - GTR-283



*Analytic/Monograph*  
Received by: *JYB*  
Indexing Branch  
*Wap*

OCT 26 1995

Maschinski, Joyce; Hammond, H. David, and Holter, Louella, tech. eds. 1996. Southwestern rare and endangered plants: Proceedings of the Second Conference; 1995 September 11-14; Flagstaff, AZ. Gen. Tech. Rep. RM-GTR-283. Fort Collins, Co: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 328 pp.

#### Abstract

These contributed papers review the current status of plant conservation in the southwestern U.S. in the current legislative arena, citing specific cases from surveys, and genetic, demographic, and ecological studies. In addition, broad issues affecting the paradigms of conservation of rare plant species in an ecosystem management context are reviewed.

**Key Words:** plant conservation, genetics, demography, reproductive biology, monitoring, endangered species

In order to deliver symposium proceedings as quickly as possible, most manuscripts did not receive conventional statistical, editorial, or peer review. Views expressed in each paper are those of the author and not necessarily those of the sponsoring organization or the USDA Forest Service.

Cover illustration of *Clematis hirsutissima* var. *arizonica* by Anne E. Grodor.

## Southwestern Rare and Endangered Plants: Proceedings of the Second Conference

September 11-14, 1995  
Flagstaff, Arizona

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## Foreword

This conference was a continuation of the brainchild of Robert Sivinski and Karen Lightfoot, who organized the first conference and edited the first proceedings. Because the first conference and proceedings successfully brought people working in the field of plant conservation together, enabled the exchange of ideas, and gave practical assistance to land managers, it was deemed important to have a second conference. Sixty-three oral and poster presentations were made at the conference held September 11–14, 1995, at du Bois Conference Center on the campus of Northern Arizona University.

Contributions to this proceedings were made by researchers working in academia, federal and state agencies, The Nature Conservancy, private consulting firms, arboreta and botanical gardens. The editors made an effort to standardize the format of the papers. We wish to thank all authors.

This Conference and Proceedings were made possible through the generosity of the following sponsors: The Arboretum at Flagstaff, Frances B. McAllister, Navajo Natural Heritage Program, SWCA, Inc., Drs. Henry Hooper, William Gaud, and David Prior of Northern Arizona University, U.S. Fish and Wildlife Service, U.S. Forest Service, Coconino National Forest, Southwest Region, Intermountain Region, Rocky Mountain Forest and Range Experimental Station, Bureau of Land Management—Arizona, Bureau of Land Management—Utah, and Bureau of Land Management—National Office. Special thanks to those who helped to make this conference possible: The staff of The Arboretum at Flagstaff—Laura Wellborn, Steve Yoder, Beverly Stanton, Scott Strohmeyer, Jan Busco, Meg Heim, Kenric Kesler, Tara Bymoen, Jeff Hines; volunteers Judy Kiriazis, Emma Jean Bader, Pat Pedersen, and Matt Ryan; moderators Elizabeth Taylor, Edward Smith, Tad Thiemer, Robert Bellsey, Catherine Gehring, Kenric Kesler, Juanita A.R. Ladyman, Charlie McDonald, Thomas E. Kolb, John Spence, Jon Souder, and Ana Goodman; projectionists and assistants from the School of Forestry. From the Bilby Research Center at Northern Arizona University, Louella Holter deserves our heartfelt thanks for helping to edit and format the proceedings, and Ron Redsteer and Dan Boone for orchestrating the camera-ready copy of the proceedings. Lane Eskew of Rocky Mountain Forest and Range Experimental Station provided technical assistance and encouragement for the publication.

This proceedings is dedicated to Frances B. McAllister, pioneer, community advocate, and heroine, who has dedicated her life to education, conservation, and the display of native plants of the Colorado Plateau.

## About the Editors

Joyce Maschinski is Curator of Plants at The Arboretum of Flagstaff and adjunct professor in the Department of Biological Sciences, Northern Arizona University. She is actively involved in research on rare plant species of the Colorado Plateau and has presented her work in the first *Proceedings of the Southwestern Rare and Endangered Plant Conference*, *The American Naturalist*, *Biological Conservation* and *Conservation Biology*. She organized the Second Southwestern Rare and Endangered Plant Conference.

H. David Hammond taught at Howard University and at The State University College at Brockport, New York, offering a wide variety of botany, plant biochemistry, and evolution courses. He published several papers with his masters degree students on the effect of growth regulators on plant morphogenesis and established herbaria at both institutions. He also spent eight years at the New York Botanical Garden as an editor in the Scientific Publications Department, editing such series as *Flora Neotropica*, *Memoirs of the New York Botanical Garden*, *Advances in Economic Botany*, and others, and was co-editor with David Cambell of *Floristic Inventory of Tropical Countries*. Since retiring, he has volunteered at the Deaver Herbarium (ASC) of Northern Arizona University in Flagstaff.

Louella Holter has many years of experience as an editor at the Bilby Research Center, a multidisciplinary facility at Northern Arizona University.



# Encouraging Conservation of Endangered Plants on Private Lands: A Case Study of Johnston's Frankenia (*Frankenia johnstonii*), an Endangered South Texas Subshrub

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**Abstract:** A study was initiated in 1993 to investigate the status and biology of *Frankenia johnstonii*, which had been reported by local South Texas ranchers and Natural Resources Conservation Service officials to be much more abundant than the original five populations known at the time of listing. In a state that is more than 90 percent privately owned, cooperation with private landowners and access to private property is essential for gathering status information and biological data on rare plants. Developing landowner confidence and working within local rural communities to gain public support were two of the primary goals of this project. This is not an easy task; it takes an abundance of time, listening, compassion, patience, and honesty. Through this initiative, over 25 new populations were located on private lands and landowners became interested in the conservation of this species. Local landowners came together and agreed to preserve this rare species on their private property and discussed options such as local conservation agreements to assure the preservation of *F. johnstonii* in South Texas.

## Introduction

When I, Gena Janssen, first came to work for the Texas Parks and Wildlife Department in 1992, there were tales of an endangered plant, Johnston's frankenia (*Frankenia johnstonii*), down in South Texas that was "everywhere." From local soil conservationists to the ranchers themselves, many residents were claiming that our current data for this species was completely inaccurate. Maybe, community members stated, the species did not need to be listed as endangered at all.

The situation, at first, seemed an easy one to tackle. Just share with us these localities, let us assess the vigor of these populations, and we can then follow through with a reevaluation of the status of the species. However, the difficulty arose with the actual verification of these localities. They were all on private land and no one was willing to divulge any information. The landowners were scared (to say the least). They were fearful of the "government" finding out that they had endangered species on their property, with all the implications they perceived, such as condemnation of property, the government taking over the land, loss of money, no more cattle grazing, and many other scenarios. Herein lies the hurdle. How do you gain the trust of landowners, or an entire community for that matter, to allow for endangered species surveys and studies on their private land? In a state that is more than 90 percent privately owned, cooperation with private landowners and

access to private property is essential for gathering status information and biological data on rare plants. Cooperatively, the Texas Parks and Wildlife Department and Southwest Texas State University submitted a proposal for funding under Section 6 of the Endangered Species Act to attempt to accomplish this very thing and it was funded in 1993.

Johnston's frankenia (*Frankenia johnstonii*) Frankeniaceae is a low-growing perennial subshrub known from Starr and Zapata counties of South Texas and from Nuevo Leon, Mexico. Johnston's frankenia was listed as endangered by the U.S. Fish and Wildlife Service (USFWS) on August 7, 1984 (USFWS 1984), and was listed as endangered by the state of Texas in January, 1987 (Poole and Riskind 1987). At the time of state and federal listing, there were only five verified populations: four in Texas (two in Zapata County, two in Starr County) and one from Nuevo Leon, Mexico. All five populations occurred on private property. No other members of the family Frankeniaceae are listed as threatened or endangered, nor are any members of the family considered federal candidates (USFWS 1993).

Johnston's frankenia was first collected by D.S. Correll in 1966 in Zapata County, Texas. Correll named and described the species in honor of M.C. Johnston later that same year (Correll 1966). In 1973, B.L. Turner described *Frankenia leverichii* as a new species from Mexico (Turner 1973); however,

M.A. Whalen (1987) reduced *F. leverichii* to synonymy under *Frankenia johnstonii*.

This small woody subshrub grows in open or sparsely vegetated rocky, gypseous hillsides or saline flats (USFWS 1988). The soils are usually saline clays or sands. The populations occur within the South Texas Brush Country natural region of Texas (LBJ School of Public Affairs 1978). In Mexico, the one known population of Johnston's frankenia is situated on the transition zone between the Tamaulipan Scrub and the Chihuahuan Desert (Whalen 1980), or the *matorral xerofilo* (scrub of xerophytes) (Rzedowski 1978).

According to the recovery plan for Johnston's frankenia (USFWS 1988), possible threats to the species include specialized habitat requirements, habitat modification and destruction, heavy grazing, and blading and/or bulldozing followed by seeding to buffelgrass (*Cenchrus ciliaris*). Additionally, the Recovery plan for Johnston's frankenia (USFWS 1988) stated that low numbers of the species may be detrimental since there are only about 1,500 plants with no more than a few hundred plants in any of the populations. Low reproductive potential was also cited as a potential threat. According to Turner (1980), the natural habitat seed set is approximately 50 percent and seedlings are rarely observed. Moreover, an added threat to the species is the fact that there are no protected population sites (viz., any sites occurring on federally owned property).

At the time of publication of the Johnston's frankenia recovery plan, lack of data made it impossible to quantify habitat and plant abundance with the precision needed to establish quantified downlisting and delisting criteria. According to the plan, information is needed on specific habitat requirements, population biology, and the population ecology of the species, and continued searches of potential habitat are needed to establish precise limits of the species distribution. Only through landowner outreach could we even begin to try to answer these questions.

The following is not a scientific report—it's a story about *working with people*.

### Initial Landowner and Community Contact

Where do you start? We decided that we would get right to it and contact the landowners of the four known sites in Zapata and Starr counties in Texas. We contacted all by telephone and met with them at the sites. Believe it or not, the very first landowner contacted said, "You want to come

onto the ranch and see my endangered species? Great! When can you come?" (If only they were all so easy.) The remaining three landowners were reluctant, but gracious and accommodating. We spent a lot of time explaining to the landowners that we simply needed access to learn more about the species and that we were also interested in surveying for additional populations to possibly downlist or delist the species in the future.

Since so little was known about the life history and phenology of Johnston's frankenia, we decided to establish monitoring plots on two of these original four known population sites. Using a belt method for perennial plant species by Lesica (1987), we recorded various measures of vigor and fecundity monthly at each site. Although we had no idea at the time, setting up monthly monitoring plots was the best thing we could have ever done. Why? First, we learned about important aspects of phenology and life history. For example, *The Manual of the Vascular Plants of Texas* by Correll and Johnston (1979) states that Johnston's frankenia blooms from November to February; however, we found that it blooms every month *except* November to February. But second, and more important, monthly monitoring put us in the community every month. We became familiar faces—folks you could count on being there at least one week of the month. We were no longer government strangers; we were regulars.

When the monitoring was completed during our weekly visits, we started surveying for new populations on private ranches. This proved to be a difficult and time-consuming task since Texas Parks and Wildlife biologists do not enter onto private land without permission of the landowner and eventually one week a month became two weeks a month. Much time was spent doing the actual legwork to confirm who owned which piece of property and how to get in touch with that person. This meant spending hours at the local tax assessor's office. Most landowners were local and lived either in town or on the ranch. Some were absentees, however, and still other lands were held in trust overseen by a group of lawyers, which made getting permission for access more difficult, but still doable. (If you think asking private landowners for permission for access to look for endangered species is difficult, try asking a group of lawyers. I'm sure the day I called became a Maalox day for them.)

Once we started gaining access to ranches, we found that another technique of determining land ownership was talking with the landowners

whose ranches we were on and asking who their neighbors were. This proved to be one of the most effective ways of verifying landowners and gaining access. Once you get access to one ranch, then you attempt to gain access to the one adjacent, and then the next one, and the next one—sort of a stepping-stone approach. Even this can be complicated at times, however. For example, when asking a landowner who owns the ranch to the west of him, he replied something like this: "Well, it used to belong to Jose Antonio Lopez, but he died and his sons inherited the place. The older son, Jose Jr. did not want anything to do with the ranch, so his brother, Juan, took it over. Jose Jr. moved to Houston and they say he has a very profitable trucking business shipping fresh seafood all over Texas from the Gulf. Juan married Maria De La Garza of San Ygnacio and they had five children, three boys and two girls. Two of the boys are in prison today—drugs or something. The other son went to Texas A & M and now he lives in Brenham, Texas. He's a jailer. One daughter, Carlotta, married Charlie Villareal, the son of the county clerk, and they live in town and have two kids (although they say one is not Charlie's, but he swears it's his). The other daughter, Ana, went to Europe and is into fashion designing now in New York or some place like that. Jose Jr. died back in 1985—heart attack. Juan died just last year. I don't know how. Old age I guess." "So," I said, "does Maria, Juan's wife, own the property?" "Maria?" he exclaimed, "No! Maria left Juan a long time ago for a hunter, who used to lease the place for deer hunting. She married the hunter and I don't know where they live. It broke his heart you know, that hunter was one of his best friends." "So, who owns the property now?" I asked. "Now? Oh, I don't know. I think the bank. I think they foreclosed on it last year after Juan died, because the family didn't pay some loan off." At this point, I did not dare ask which bank.

In rural Texas, one of the best contacts and allies that you can make is the local soil conservationist of the Natural Resources Conservation Service (NRCS). In Texas, there is usually one NRCS representative in each county and they are close to the people and the issues that concern them. However, this relationship, like any other that you are trying to establish when working with endangered species issues, did not come easily. I never gave up, though. I would visit the NRCS office every month and share what I had learned and whose ranch I had been on and whether or not I had found endangered plants. I wanted him

to know that he could trust me and that I would share all my information with him. It took about a year before I felt as though we could actually call ourselves "friends." Today, we work closely concerning ideas about the conservation of endangered plants and the best approaches to use with landowners.

### Getting Over the Rough Spots

It isn't always pretty. Talking with private landowners can be downright scary sometimes. They are scared of and outraged by many of these issues and by gosh they are going to tell you all about it! As I began to meet and get to know more and more landowners, I began to notice that most of them did basically the same thing when they met me: They yelled at me. And then one day it finally hit me as to why they did that. Finally, they had a person, a warm body, in front of them that represented all these endangered species issues that had been scaring them for so long. They just needed to vent, so I let them. They had no one there for them. There was no one there to say, "No, that's not true," or "Yes, that was a very difficult situation for everyone," or "Well, only part of that is true," etc., until now. As time went by, I found that if I let folks speak their peace and listened closely to their concerns, soon we would begin talking like two rational people (although this moment in time took longer for some than for others).

One of the first ranches I gained access to was broken into two pieces with another private ranch in between. The landowner of the two-piece ranch gave me permission to access the other parcel of the property, which was further to the east. In doing so, I had to drive through the ranch in the middle. The owner of the ranch in the middle, however, saw me in my state vehicle driving on "his road" and vowed that if he ever saw me again he would shoot me. Word got back to me in no time. Threats from this man were not to be taken lightly. He had a history of escorting people off his property at gunpoint. The road through his place is actually an easement road that many ranchers use to get to their ranches, which are land-locked, but let's not split hairs. For all practical purposes it was *his* road and he did not want *me* on it. Well, the day soon came when I wanted to use that road again. I was scared, I didn't know what to do. I gathered all my courage, took a deep breath, and I drove down that road and straight to that man's house. I knocked on the door and his wife answered. I introduced myself and asked if I could

she speak with her and her husband for a few minutes. She looked at me, then she looked at my truck. She looked at me again and looked at my truck again. Then, with a look in her eyes like 'Girl, you're crazy', she said, "Yes, come on in. My husband is taking a nap. Let me go wake him up." "Oh, no!" I thought, "First this guy said he would shoot me and now I'm waking him up from his nap! I'm going to be killed!" She came from waking him from his nap and said, "It takes him a while to wake up. I'll make some coffee." It was a July afternoon in South Texas in a house with no air conditioning and I was about to have coffee. Finally, he slowly shuffled into the kitchen, a very old and frail man wheezing of emphysema. The rest of the day is kind of a blur to me now. We talked about the grandchildren and the EPA and the IRS and how his mother's house is underneath Falcon Reservoir because of the government and the drought and the heat and the USFWS and my project and the plant I was looking for and if the cows don't eat your plant, then what's it good for? About five cups of coffee later, not only had he not shot me, but he gave me permission to use his road *and* access to his ranch, as well. Today, it is actually his son who runs the ranch and, yes, they have Johnston's frankenia. And, believe it or not, it is this man's son who has become my biggest supporter in the county. He told me once, "Any girl who is brave enough to go and talk to my daddy and get permission to come on the ranch is okay by me." (Just as an aside, I have since witnessed my first "gunpoint escort victim" last summer. I walked into the NRCS office and there stood a man from the Texas Water Development Board, who was beet red, sweating bullets, wiping the sweat with a handkerchief, standing there surrounded by other people consoling him. When I asked what was wrong, I found that he had just been escorted at gunpoint off of this very ranch I speak of. I started to laugh. This poor man must have thought that I was the cruelest woman in the world. I simply smiled and said, "You should have gone and had coffee with him.")

### Community Outreach and Involvement

In addition to working with local landowners, we also developed community outreach projects and programs for the local community. To reach the general public, we did a series of newspaper articles in the local paper about the project. Additionally, we set up a booth at the county fair. The booth included an extensive pictorial display of all

the rare plants and animals that occur in that region of South Texas in addition to information brochures and live rare plants in pots from the San Antonio Botanical Gardens. We also set up a television that ran constantly with South Texas segments of the Texas Parks and Wildlife television show spliced together. The display was a great success and it gave us the opportunity to talk with people about all the beautiful rarities that occurred right there where they lived.

I love working with children, but I rarely get to do that. So, when a local Americorps volunteer asked me to help her put together a conservation summer program for kids, I jumped at the chance. Together, we developed a four-day summer camp. We each taught hour-long programs and we alternated throughout the day. She taught topics such as water conservation and recycling, while I taught fisheries and wildlife conservation, habitat conservation, the importance of plants and food chains, and endangered species. Each lesson had an activity. For example, after learning about fisheries and wildlife conservation, the children got to make fish prints using real (but dead) fish and tempura paints. They loved it. But not every activity after a lesson was hands on. For example, after learning about the importance of plants, we all sat on the floor while reading *The Lorax* by Dr. Seuss (yes, we are allowed to read this book in Texas). The most rewarding part of the day was always teaching the children about endangered species in Texas. They knew all about pandas, rhinos, and elephants, but they had no idea of the rare and endangered species that occurred right in their own backyard. While talking about another endangered plant in Texas, ashy dogweed (*Thymophylla tephroleuca*), I told the kids that the only place in the world that this plant grows is right here in the area where they live. Upon hearing that, one boy, who was about 10 years old, stood up, and very seriously said, "Then we've GOT to SAVE it!" We should all see things the way 10 year olds do.

### The Landowner Meeting

Two years and approximately 30 newly located populations later, I had reached a pinnacle point. I realized that no matter how many populations I located on private land, trying to downlist or delist this species was still going to be difficult, because the USFWS tends to consider endangered plant populations on private land in Texas as "unprotected." Since the Endangered Species Act

protects listed plants on private land only during activities that use federal funds or permits and since our state law covers less than that, these populations theoretically could meet their demise at any time. We needed something tangible that showed that the threats (land clearing, root plowing, herbicide broadcasting) to these populations would be alleviated. What about a conservation agreement?

I started asking the landowners what they thought about a voluntary conservation agreement. Most landowners indicated that it sounded okay to them, so I decided to set up a meeting with as many of the landowners with the species on their property as I could get. I invited people by mail and announced it in the local newspapers. I was nervous. At this time landowners all over Texas (and the nation) were lashing out against these issues. At one point I got so scared that I did a guest editorial in the local paper stating that this meeting was not a forum for anti-government and anti-endangered species debates, but an opportunity for landowners to make a difference.

We had our meeting in May of 1995, and approximately 50 people attended. I am happy to report that the meeting was civil and productive. We reviewed the legalities of the Endangered Species Act, the differences between the plant and animal laws, and the issues that faced the community. We discussed the conservation agreement and how it would be completely voluntary, their choice, to conserve these plants on their ranches even when they were not using federal money or using a federal permit. After what seemed hours of discussion, I finally asked, "Well, do you all want to do it?" There was dead silence. Then finally one landowner, actually the son of the landowner who wanted to shoot me, said, "I'll do it." Then all the other landowners in the room started shifting in their chairs saying things like, "Well, okay maybe, but we need to see this thing in writing." At that point I promised to write it up with the help of some landowners and I would then share it with everyone to review. This is where I am today.

After the landowner meeting we had a free barbecue dinner outdoors. During the dinner, something happened that touched me so deeply that I will never, ever forget it. I looked up and noticed one of the landowners approaching me. He had his son, who appeared to be early college age, with him. Throughout this time when I was thinking of developing this conservation agreement, it was this very landowner who was now

approaching me, that I thought would probably not do this conservation agreement. He owned only about 10 acres and the Johnston's frankenia was on practically every acre of his property. He and his family are poor (or at least poorer than all the other landowners I had been working with) and that 10 acres is all they have. In the back of my mind I told myself that they would probably never do this and I could understand why. But I was wrong. He and his son came and stood next to me and said "Gena, we want to ask you something. Would it be okay if, you know this conservation agreement thing, if we just did that on half of our property so that maybe we could still build a house or something on the other half?" I got a lump in my throat. I was so moved with emotion, that all I could say was, "Yes, of course you can." He said, "Okay then, next time you're in the neighborhood stop by and we'll mark off which half." I said, "Sure," and they turned around, got into their truck and left.

I still get choked up every time I think about it. It makes me just want to scream to the world, "See! We *can* work these things out!"

## A Learning Experience

I am certainly not the expert, but there are a few things that I have learned through this project. I believe there are five important elements that you need to consider when dedicating yourself to working with private landowners: time, patience, the ability to listen, compassion, and honesty.

It takes time to build a relationship with people. You cannot simply knock on someone's door and ask, "Would you be interested in doing a conservation agreement?" There is a process of building trust and mutual respect that cannot be done in one visit. With time comes familiarity and with familiarity fear and distrust start to fade. I have found, also, that people actually *need* your time. There is no one out there with whom landowners can discuss endangered species issues and concerns and you in turn become their one and only source of accurate, honest information. I find myself explaining over and over to each and every landowner how the Endangered Species Act differs with respect to plants and animals, what is legal and what is not legal, and what is true and what is untrue about what they have heard in the media or from their neighbors. Another important factor about time and building relationships is that they must be maintained. I found that people start to get fearful again when you have not been around for a while—they start wondering what

you're up to. For example, I went on vacation in June, so I was unable to visit the community for the entire month of June. When I came in July, everyone said, "Where have you been? We thought you were never coming back!" The same is true when I neglect to stay in touch often with some landowners. When I do finally call to say hello and update them on the project or whatever, they often make a comment such as, "Yeah, we were wondering what happened to you."

Patience is something that I do not have much of. To practice patience was a learning experience for me. I believe many of us who work for various private and governmental agencies become focused on our performance plans, career goals, field schedules, and calendars, and usually work in over-drive thinking, "I've got to get this done now!" But, nothing goes too fast in rural Texas, and I had to calm down. There is a fine line between being persistent and being a big pain in the neck and unfortunately there were a few times that I learned this the hard way (like the time this woman told me that I was like a wart that needed to be lanced off). Relax. If people are going to do something, they are going to do it in their own time.

If there is one thing that I have done more than any other one thing, it would be listening. And I mean *really* listen. Listening is not simply waiting for someone to stop talking just so you can make your righteous conservation point—you can do that later. Listen with compassion to their opinions, fears, and concerns. You do not have to agree, but at least you can try to understand their point of view. This is their life, their livelihood, their family, and most importantly, *their land*. Be compassionate. You do not need to have all the answers, only understanding. If you want people to listen to what you have to say, you need to be equally as respectful to what they have to say. As time passes you realize that you are really not so different from one another. Your values about conservation are almost identical, you just have different jobs.

Honesty goes a long way. Sometimes honesty is painful when you know the person you are talking to is not going to like what is about to come out of your mouth—but be strong. Do not compromise the truth for the sake of avoiding conflict. I was honest with every person I met about my project, how I needed to do it, the accuracy of the data I needed, etc. When I asked for permission to access private ranches, I did not say I was looking for plants; I said I was looking for endangered

plants. In trying to build trust, I went out of my way to explain what I was doing in detail. I never tried to strike shady deals or triangulate against the federal government. Things like that will only come back later to haunt you. Play by the rules. I never wanted to give anyone the opportunity to find a reason not to trust me. I never crossed a fence line if I was not exactly sure of where I was and I never mapped a population of endangered plants without the landowner knowing exactly what I was doing. In a rural community, if someone feels you have done them wrong, then for all practical purposes, you have done every landowner in that community wrong. News travels fast, bad news even faster, and bad endangered species biologist news travels like lightning! Avoid as many pitfalls down the road as possible—always be honest.

Because of all of the landowner and community outreach, which has taken so much time, heart, and soul, we now have a tremendous opportunity for intensive biological studies that without outreach, would never have been possible. Today we have implemented a grazing enclosure experiment, pollination syndrome studies, and soil seed bank analyses, and in the future we will also initiate a genetic analysis of each population. It seems like such a long, hard way to get there, but in Texas, it may be the only way.

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# Managing and Monitoring Rare and Endangered Plants on Highway Rights-of-Way in Texas

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**Abstract:** Texas is 90 percent private land. One of the major public landholders in Texas is the Texas Department of Transportation, with over 750,000 acres of highway right-of-way. A project was undertaken in 1990 to identify listed and category plants occurring on highway rights-of-way, develop management agreements to protect these species, and establish monitoring plans to assess the effectiveness of the management. To date 26 management/monitoring areas have been established. Preliminary results shows that overall species populations have increased under the agreed-upon management regimes.

## Introduction

Texas encompasses a large area with numerous habitats resulting from widely varying climate, geography, geology, soils, and topography. Because of this, the state supports over 6000 plant taxa with an endemism rate of about 10 percent and has 28 listed species and over 150 category 1 and 2 species. These rare species occur throughout the state, with the exception of the Texas Panhandle. Due to the relatively mild Texas climate and booming economic growth, the human population is increasing rapidly and is displacing or destroying many species and their habitats through urbanization, industrialization, agriculture, or disruption of natural ecological processes. The two main obstacles to protecting plant diversity in the state are the inadequacies of state and federal endangered plant laws and the paucity of easily accessible (i.e., public) land. State law goes no further than to prohibit the unpermitted collection of listed plants and federal law protects plants only on federal lands or federally permitted or funded projects (and these are usually on public lands). Although the percentage of land in private ownership (90%) is quite high, various federal (National Park Service, U.S. Fish and Wildlife Service [USFWS], Department of Defense, etc.), state (University of Texas, General Land Office, Texas Parks and Wildlife Department [TPWD], etc.), and local agencies control the 16,600,000 acres within the public domain (General Land Office 1993). While these agencies are not always required by law to protect listed or category species, they often do so for good public relations, avoidance of listing, or an agency mandate to conserve biodiversity. One often overlooked entity, which owns and manages over 750,000 acres across the entire state, is the Texas Department of

Transportation (TxDOT 1993). While required to protect listed species under the federal Endangered Species Act, this agency usually protects candidates and category species as part of their mission to preserve the natural surroundings. While Texas highways are well known for their impressive wildflower displays, the general public may not realize that these seasonal splashes of color are due not just to the highway beautification program endorsed by Lady Bird Johnson, but to TxDOT's continuing management program of seasonal mowing and judicious herbicide use. Although mowing may have extirpated most woody species from highway rights-of-way, many shorter stature plants remain and prosper under conditions of reduced competition and lessened herbivory. A survey of the Texas Natural Heritage Program (TxNHP) database revealed 150 occurrences of listed or category plants on or within the immediate vicinity of highway rights-of-way. To assure protection for these species a project was initiated in 1990 to identify listed and category plant sites, arrange management agreements with TxDOT, and establish monitoring plots to evaluate demographic trends and their implications for management. Funding was obtained from a USFWS Section 6 grant. To date 26 management/monitoring sites have been established that are found in all areas of the state, with the exception of the Panhandle, and that represent a diversity of habitat types (i.e., swamps, deserts, grasslands, woodlands, etc.). Preliminary results indicate that, overall, species populations have increased under the agreed-upon management regimes.

## Methods

Several thousand plant locality records within the TxNHP database were scanned for proximity



to highway rights-of-way. The physical locations were then searched as time and phenology allowed. When listed or category species were relocated, a meeting was then set up between TPWD botanists and TxDOT personnel. Usually there was a year's lag time between site identification and the TPWD/TxDOT meeting due to schedule conflicts, etc. Meetings were held at the site and TxDOT management as well as rare species requirements were discussed. After reaching agreement on management strategies for both the species and the highway right-of-way, management agreements were drafted, reviewed by all parties concerned, and revised. Management agreements included the location and description of the site (including maps), management techniques currently used, species of concern at the site, recommended management procedures, red flag conditions (conditions that indicate a significant decline in vigor or numbers of individuals within the population at the site), authorship, reviewers, revisors, and dates. Usually monitoring plots were established immediately after the site meeting, using recognized methods (Bowles et al. 1986, Travis and Sutter 1986, Palmer 1987, Pavlik 1987, Spellerberg 1991, Cropper 1993, Given 1994). Monitoring plans were written, including methods, results, general notes, and red flag conditions. Monitoring was conducted on an annual basis, with data being analyzed and compared to previous data to pinpoint trends in vigor and/or demography. If red flag conditions were encountered, an attempt was made to ascertain the cause. If the conditions were the result of TxDOT management practices, the management agreement was revised. Both management agreements and monitoring plans were sent to USFWS as part of the annual Section 6 report.

Each of the 26 sites and species required a different monitoring methodology. Table 1 presents the various taxa, and the demographic and vigor methodology used for each.

## Results

Of the 150 potential sites identified in the Texas Natural Heritage Program database as possibly occurring on highway rights-of-way, 57 were relocated, 15 were either not found or not found to be on a highway right-of-way, and 88 still require verification as of January 1995. Management agreements were initiated for 26 sites and a total of 26 species. Four species have multiple sites and four sites contain two or more species. A total of

33 populations representing 26 species are being monitored. The 26 species include 11 listed species, 3 category 1 species, and 12 category 2 species. Of the 33 populations, 14 have been monitored for 4 years, 11 for 2 years, 5 for 3 years, 2 for 6 years, and 1 for 1 year. As of 1994, 20 populations increased (i.e., exhibited a 10% or greater increase in numbers of individuals between the initial and last census), four decreased (i.e., a 10% or greater decrease in numbers), and eight remained stable (i.e., a less than 10% increase or decrease in numbers). Of the 21 populations monitored more than 2 years, 13 have fluctuated over the years (both increased and decreased in size), including 14 of the 16 populations studied for the longest period (4–6 years). Thus it appears that there is a high likelihood of fluctuating population trends after 3 years. A longer period of study might be required to reveal actual long-term trends in these populations (Given 1994). Vigor data is even more inconclusive, with no trends apparent at all. This is probably due to several factors, such as small sample size, climatic fluctuations, and the brief time period of data collection. Table 2 gives an overview of the demographic trends as of 1994.

## Discussion

Overall, the management agreement–monitoring program has been successful. Although trends continue to oscillate, most populations have increased. Decreases were usually attributable to drought, but occasional abnormal habitat disturbances such as fiber optic cable placement contributed as well. Part of the reason for population increase might be the change in management activities as well as enhanced awareness on the part of the local TxDOT maintenance staff. For example, the initial increase of the *Callirhoe scaberriuscula* population was probably due to a shift in the mowing regime to later in the year after the plants had set fruit. However, most other sites benefited from increased awareness on the part of TxDOT personnel as well as the local community. Many sites were saved from damage by concerned local citizens who noted that road material piles were coming dangerously close to rare plants, or fiber optic cables being placed in highway rights-of-way were trenching through endangered species habitat. Most past damage resulted from a lack of knowledge. If no one knows the significance, the identity, and the location of rare plants, how can they be protected? Once this knowledge

Table 1. Monitored taxa with demographic and vigor methodology.

Taxon (Legal Status*)	Demography	Vigor
<i>Ambrosia cheiranthifolia</i> (LE)	delineated plot, number of stems	number of stems
<i>Amsonia tharpii</i> (C2)	delineated plots, number of individuals, individuals identified	number of stems, number of flowers/fruits per plant
<i>Ancistrocactus tobuschii</i> (LE)	delineated plot, number of individuals	stem diameter, number of flowers/fruits per plant
<i>Asclepias prostrata</i> (C2)	generalized plots, number of individuals, individuals identified	number and length of stems per plant
<i>Aster puniceus</i> ssp. <i>elliottii</i> var. <i>scabri caulis</i> (C1)	delineated plots, number of individuals, large individuals tagged	height, number of primary stems and secondary stems, no. flowers/plant
<i>Callirhoe scabriuscula</i> (LE)	delineated plots, no. of individuals	number of buds, flowers, & fruits/plant
<i>Coryphantha albicolumnaria</i> (C2)	tagged individuals	height
<i>Coryphantha hesteri</i> (C2)	delineated plot, number of individuals, individuals identified	stem diameter
<i>Coryphantha minima</i> (LE)	delineated plot, number of individuals, individuals identified	stem diameter
<i>Echinocereus lloydii</i> (LE)	delineated plots, number of individuals	stem height and diameter, no. of stems and flowers per plant
<i>Echinocereus viridiflorus</i> var. <i>davisii</i> (LE)	delineated plot, number of individuals, individuals identified	number of buds/flowers
<i>Frankenia johnstonii</i> (LE)	generalized plot, number of individuals	diameter, height, and number of flowers/fruits per plant
<i>Genistidium dumosum</i> (C2)	tagged individuals	height, width
<i>Helianthus paradoxus</i> (C1)	delineated plots, number of individuals	number of individuals
<i>Hibiscus dasycalyx</i> (C2)	tagged individuals	height, number of stems, no. flowers/fruits per individual
<i>Hoffmannseggia tenella</i> (LE)	delineated plot, number of individuals, density	number of individuals
<i>Lesquerella thamnophila</i> (C1)	generalized plot, number of individuals	number and length of stems per plant
<i>Nolina arenicola</i> (C2)	tagged individuals	height, diameter
<i>Paronychia wilkinsonii</i> (C2)	delineated plot, number of individuals, individuals identified	areal cover (height X width)
<i>Pediomelum humile</i> (C2)	generalized plot, number of individuals	no. of leaves & flowers/fruits per plant
<i>Salvia penstemonoides</i> (C2)	generally sited plots, number of individuals	number of primary and secondary stems, no. flowers/fruits per individual
<i>Spiranthes parksii</i> (LE)	delineated plot, number of individuals	height, number of flowers per plant
<i>Thelocactus bicolor</i> var. <i>flavidispinus</i> (C2)	delineated plot, number of individuals, individuals identified	plant diameter

Table 1 (continued)

Taxon (Legal Status*)	Demography	Vigor
<i>Thymophylla tephroleuca</i> (LE)	delineated plot, number of individuals, individuals identified	diameter, height, and number of flowers per plant
<i>Tillandsia baileyi</i> (C2)	delineated plots, number of individuals	number of individuals per size class, number of flowering individuals
<i>Zizania texana</i> (LE)	delineated plot, number of individuals, individuals identified	areal cover

\*LE = listed as federally and state endangered; C1 = federal candidate category 1 taxon with enough information available to propose for listing; C2 = federal candidate category 2 taxon under current review for possible listing as either endangered or threatened, but U.S. Fish and Wildlife Service is in need of more information.

Table 2. Demographic trends for species on highway rights-of-way.

Taxon	Site Name	Population Trends*	Study Length (in years)
<i>Ambrosia cheiranthifolia</i>	Carreta Creek	Increasing	2
<i>Amsonia tharpii</i>	McCamey Road	Stable	3
<i>Ancistrocactus tobuschii</i>	Kickapoo	Increasing	3
<i>Asclepias prostrata</i>	Roma	Stable (fluctuating)	4
<i>Asclepias prostrata</i>	Dolores	Increasing (fluctuating)	4
<i>Asclepias prostrata</i>	Tigre Chiquito	Increasing (fluctuating)	4
<i>Aster puniceus</i> ssp. <i>elliottii</i> var. <i>scabriacaulis</i>	Palestine	Increasing (fluctuating)	4
<i>Aster puniceus</i> ssp. <i>elliottii</i> var. <i>scabriacaulis</i>	Ben Wheeler	Increasing	2
<i>Aster puniceus</i> ssp. <i>elliottii</i> var. <i>scabriacaulis</i>	Lake Lydia	Increasing	2
<i>Callirhoe scabriuscula</i>	Ballinger	Increasing (fluctuating)	6
<i>Callirhoe scabriuscula</i>	Maverick	Decreasing	4
<i>Coryphantha albicolumnaria</i>	38 Hill	Stable	3
<i>Coryphantha hesteri</i>	Marathon	Increasing (fluctuating)	4
<i>Coryphantha minima</i>	Marathon	Stable (fluctuating)	4
<i>Echinocereus lloydii</i>	McKenzie Exit	Increasing	3
<i>Echinocereus viridiflorus</i> var. <i>davisii</i>	Marathon	Increasing (fluctuating)	4
<i>Frankenia johnstonii</i>	Zapata	Not applicable	1
<i>Genistidium dumosum</i>	38 Hill	Increasing	3
<i>Helianthus paradoxus</i>	Diamond Y	Increasing (fluctuating)	4
<i>Hibiscus dasycalyx</i>	Cherokee	Stable	2
<i>Hibiscus dasycalyx</i>	Lovelady	Stable	2
<i>Hibiscus dasycalyx</i>	Boggy Slough	Increasing	2
<i>Hoffmannseggia tenella</i>	Bishop	Increasing	2
<i>Lesquerella thamnophila</i>	Tigre Chiquito	Decreasing (fluctuating)	4
<i>Nolina arenicola</i>	Plateau	Stable	2
<i>Paronychia wilkinsonii</i>	Marathon	Increasing	4
<i>Pediomelum humile</i>	Del Rio	Increasing	2
<i>Salvia penstemonoides</i>	Fredrick Creek	Stable (fluctuating)	4
<i>Spiranthes parksii</i>	FM 974	Decreasing	2
<i>Thymophylla tephroleuca</i>	Dolores	Decreasing (fluctuating)	4
<i>Tillandsia baileyi</i>	Sarita	Increasing	2
<i>Thelocactus bicolor</i> var. <i>flavidispinus</i>	Marathon	Increasing	4
<i>Zizania texana</i>	San Marcos	Increasing (fluctuating)	6

\*Increasing = a 10% or greater increase in numbers of individuals or stems from the initial to the final census; Decreasing = a 10% or greater decrease in numbers of individuals or stems from the initial to the final census; Stable = a less than 10% change in numbers of individuals or stems from the initial to the final census; Fluctuating = a population exhibiting both increasing and decreasing trends over the monitoring period.

is gained, proactive management and planning can occur. Occasional mistakes will still happen due to personnel turnover, miscommunication, and rarely, intentional vandalism. However, the overall benefit gained for the conservation of these rare species is much greater as the result of the information sharing among knowledgeable and concerned people. Future work needs to be done, such as contacting the utility companies that have easements along the right-of-way as well as the adjacent private landowners. When all stakeholders in the habitat are made aware of these rare plants, the plants' prospects for long-term survival are more assured.

### Acknowledgments

This study was funded in part by the Texas Parks and Wildlife Department. However, the majority of the funding came from a Section 6 grant from Region 6 of the U.S. Fish and Wildlife Service. Over the years of this project, more people than can be named in this paper worked under less than idyllic conditions on the monitoring plots. We are greatly in their debt for helping collect data. We would also like to thank Rob Sutter and various other staff of The Nature Conservancy for teaching us various techniques and skills as well as reviewing the initial plans. We also owe a great deal of thanks to the numerous personnel of the Texas Department of Transportation, who met us at sites, explained their management practices, and became willing stewards of rare plants of the rights-of-way. In particular we would like to express our greatest appreciation to two TxDOT staff members, David Dunlap and Dennis Markwardt. David's encouragement advanced the task from a single protected right-of-way to a full-scale project.

Dennis has been, and continues to be, a most dependable ally and liaison. His skill at negotiation has spared us many a disagreeable discussion and his ability to get a group of maintenance foremen, bureaucrats, and conservation biologists to reach consensus is probably more responsible for the protection of these plants than all the rest of the work combined.

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# Demography of *Ipomopsis aggregata* ssp. *weberi*, a Rare Plant of Northern Colorado

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**Abstract:** *Ipomopsis aggregata* ssp. *weberi* is known from fewer than 15 sites in the southern Park Range of northern Colorado. Subspecies *weberi* grows on open sites within the subalpine forest. Demographic studies were conducted at four sites to estimate seedling recruitment, survivorship, longevity, and seed production. Approximately 50 percent of seedlings observed in sites free from seed immigration were recruited in the first 2 years. Highest mortalities occurred among seedlings during the first year, as a result of early summer drought. Plants surviving to reproduce lived from 2 to 5+ years, with an average life span of 3 years. An average of 75 flowers was produced per plant, with mean seed sets between 1.4 and 3.3 per flower. Stable populations occur in sites with less than 50 percent cover, suggesting that reduced competition and availability of open sites promote successful reproduction, establishment, and long-term population stability.

## Introduction

Reproductive biology and demography are critical factors in understanding dynamics of rare plant populations (Menges 1991, Simberloff 1988). The purpose of this study was to characterize the demography of *Ipomopsis aggregata* (Pursh) V. Grant subsp. *weberi* Grant & Wilken (Polemoniaceae), which is narrowly restricted to the southern Park Range of north-central Colorado (Grant and Wilken 1986). Subspecies *weberi* is considered environmentally "sensitive" and "potentially imperiled" by the Colorado Natural Areas Program (1995, personal communication). *Ipomopsis aggregata* is composed of six subspecies, which differ strikingly in geographic and ecological distribution, morphological variation, and mode of pollination (Grant and Wilken 1986). Subspecies *weberi* occurs on thin soils of open sites in association with other herbaceous perennials. Such sites are scattered primarily throughout subalpine forest at elevations between 2070 and 3140 m. At least 13 known occurrences have been documented. Subspecies *weberi* is separated from conspecifics by white to pink, salverform corollas, corolla tubes 10–15 mm long and less than 1 mm in diameter, short, broadly flaring throats, and slightly exserted anthers (Grant and Wilken 1986). Plants have basal leaf rosettes prior to flowering and from one to six erect, paniculate flowering shoots, each with 9–18 short, lateral inflorescences.

## Methods

Four study sites, Buffalo, Dumont W, Dumont S, and Walton, were chosen in 1984 to represent a range in altitude and geographical distribution.

Two sites near Dumont Lake, separated by ca. 0.5 km, represent two different aspects, an east-facing slope with a fine-grained substrate and moderate herbaceous cover (Dumont W) and a flat with a rocky to coarse-grained substrate and little vegetative cover (Dumont S). Eight nonadjacent 1 m<sup>2</sup> plots were placed at each of the sites in late August or early September, 1984. Plots were chosen to include at least one fruiting individual. The number of seedlings was estimated in each plot between late May and late June in 1985 and 1986, depending on time of exposure after snow melt. Seedling counts were based on observations made about every 3 weeks. The largest number of seedlings in each plot was considered an estimate of initial cohort size. Although new seedlings were found later in some plots at the end of the first season, these accounted for less than 1 percent of the total number in any plot. Two cohorts recruited as seedlings (1985 and 1986) were followed through reproduction at each site. Sites were visited in May or June and again in August or September (1985–1989) to estimate the number of surviving vegetative rosettes and number of reproductive individuals. All vegetative rosettes in each plot were mapped, using a 10 x 10 cm grid, and observed until death. A total of 417 individuals, beginning with 1-year-old rosettes that survived the first winter, were mapped and followed until death. The probability of survival during each observation period (May–June, August–September) was calculated as a proportion of plants observed during the previous sampling period.

Estimates of shoot height, number of lateral inflorescences, and number of flowers were obtained, using 20 plants outside established plots in each population in 1985. Fruit and seed set was sampled from each of 20 plants in each of 2 different years. Fruit and seed set were recorded from three flowers per plant, each sample taken from a separate lateral inflorescence (lower, middle, upper) on each flowering shoot.

Recruitment of seedlings from the seed bank was estimated from one additional non-adjacent 1 m<sup>2</sup> plot at each site. All reproductive plants within a radius of 2 m from the center of each plot were removed each year from 1985 to 1989. This corresponds to an area three times greater than the mean seed dispersal distance (0.38 m<sup>2</sup>) reported for *I. aggregata* ssp. *aggregata* (Waser and Price 1983). Seeds of *I. aggregata* do not have any specialized means of dispersal. Thus, I assumed that (1) any observed seedlings appearing in subsequent years were recruited from seeds in place in 1985 and that (2) immigration by surface dispersal from outside each plot (by wind or surface runoff during rain storms and snow melt) was minimized. This is an imprecise method in assessing seedling recruitment, but no other in situ method could be devised. For comparative purposes, seedling recruitment was monitored in one of the eight plots at each site used for studying survivorship.

Field observations suggested that the distribution of plants was related to areas free of vegetative cover and rocks. Sites with open ground characterized by fine to medium-grained soils apparently had higher densities of rosettes and reproductive individuals than sites with relatively high levels of vegetative cover or coarse substrates. Consequently, four 100 m x 1 m belt transects were placed along lines that crossed the apparent boundaries of *Ipomopsis* populations. Transects were oriented perpendicular to the perimeter of each population at four points and centered over a reproductive plant at each point. Cover and number of reproductive plants were sampled in 1 m<sup>2</sup> quadrats placed at 6 m intervals along each transect. Cover was estimated by determining the basal area of herbaceous plants, crown cover of shrubs, and rocks visible at the substrate surface, using standard methods (Mueller-Dombois and Ellenberg 1974). Each transect was composed of eight quadrats on either side of the pivotal quadrat, which was not included in the analysis. Cover also was estimated in each of the eight permanent plots established for survivorship

studies. All statistical analyses were performed using log or arcsin (for proportions) transformations, but all reported data are untransformed.

## Results

No differences were found in survivorship between cohorts during the first 2 years at each of the four sites (two-way ANOVA df = 1,56; Buffalo ms = 0.623, F = 3.483, p = 0.067; Dumont S ms = 0.246, F = 0.817, p = 0.489; Dumont W ms = .0048, F = 0.110, p = 0.741; Walton ms = 0.293, F = 2.167, p = 0.147). Consequently, cohort data were combined for each site. Differences between survivorships among cohorts at the end of four successive seasons (two summers, two winters) were tested by a two-way analysis of variance (Sokal and Rohlf 1981), with plots as replicates. The density of seedlings per 1 m<sup>2</sup> ranged from 76.9 ± 35.4 to 219.2 ± 113.2 (Table 1). Probabilities of surviving the first summer ranged from 0.078 to 0.154. Plants surviving the first summer at all sites had high probabilities of surviving successive seasons through the first 2 years of growth. Seedling survivorship during the first summer was significantly higher at Buffalo than at the other three sites (ANOVA df = 3,60; ms = 0.006, F = 3.702, p = 0.016). Although

Table 1. Mean proportions of survivors after four consecutive seasons during first 2 years. Means of proportions with same letter are not significantly different at p = 0.05, df = 56 (unplanned comparisons, Sokal and Rohlf 1981). Mean number of seedlings in first year were 182.1 ± 103.2 (Buffalo), 76.9 ± 35.4 (Dumont S), 161.0 ± 87.8 (Dumont W), and 219.2 ± 113.2 (Walton).

Site	Season	Mean	SD
Buffalo	Summer 1	0.154 <sup>A</sup>	.085
	Winter 1	0.485 <sup>B</sup>	.273
	Summer 2	0.641 <sup>B</sup>	.272
	Winter 2	0.704 <sup>B</sup>	.704
Dumont S	Summer 1	0.103 <sup>A</sup>	.076
	Winter 1	0.720 <sup>B</sup>	.146
	Summer 2	0.905 <sup>C</sup>	.289
	Winter 2	0.921 <sup>C</sup>	.126
Dumont W	Summer 1	0.105 <sup>A</sup>	.047
	Winter 1	0.532 <sup>B</sup>	.302
	Summer 2	0.730 <sup>B</sup>	.348
	Winter 2	0.716 <sup>B</sup>	.373
Walton	Summer 1	0.078 <sup>A</sup>	.048
	Winter 1	0.586 <sup>B</sup>	.316
	Summer 2	0.809 <sup>C</sup>	.213
	Winter 2	0.796 <sup>C</sup>	.222

probabilities of survival increased at each of the four sites with successive seasons, the trend was not significant except at Dumont W and Walton.

Of the 417 plants that survived the first year, at least 229 survived (55%) to flowering. Age at reproduction ranged from 2 to at least 5 years (Table 2). All reproductive plants died following fruiting. The largest age class of flowering plants (106 plants) was 3 years, representing 45 percent of all reproductive plants surviving the first year. Four year olds (52 plants) represented the next highest age class, followed by 2 year olds (37 plants) and 5 year olds (34 plants). At least five vegetative rosettes survived the fifth year without flowering. The density of reproductive plants ranged from 0.4 to 2.7 per plot.

No significant differences were detected among the four sites for shoot height (ANOVA  $df = 3,76$ ,  $ms = 0.001$ ,  $F = 0.106$ ,  $p = 0.956$ ), number of lateral inflorescences per shoot (ANOVA  $df = 3,76$ ,  $ms = 0.006$ ,  $F = 0.667$ ,  $p = 0.575$ ), and number of flowers per plant (ANOVA  $df = 3,76$ ,  $ms = 0.002$ ,  $F = 0.154$ ,  $p = 0.927$ ). Over all sites, shoot height ranged from 21 to 51 cm (means  $\pm$  sd: Buffalo,  $36.1 \pm 9.1$ ; Dumont S,  $34.6 \pm 7.2$ ; Dumont W,  $34.3 \pm 7.9$ ; Walton,  $35.1 \pm 8.3$ ). The number of inflorescences per shoot ranged from 8 to 21 (means  $\pm$  sd: Buffalo,  $13.6 \pm 2.6$ ; Dumont S,  $13.1 \pm 3.0$ ; Dumont W,  $13.7 \pm 3.0$ ; Walton,  $12.9 \pm 3.3$ ), and numbers of flowers per plant ranged from 44 to 122 (means  $\pm$  sd: Buffalo,  $74.1 \pm 17.7$ ; Dumont S,  $76.1 \pm 17.6$ ; Dumont W,  $75.5 \pm 17.5$ ; Walton,  $73.6 \pm 20.2$ ).

No differences in fruit set were found between years at each site (ANOVA  $df = 1,39$ : Buffalo  $ms = 0.009$ ,  $F = 0.039$ ,  $p = 0.844$ ; Dumont S  $ms = 0.002$ ,  $F = 0.193$ ,  $p = 0.667$ ; Dumont W  $ms = 0.001$ ,  $F = 0.067$ ,  $p = 0.797$ ; Walton  $ms = 0.043$ ,  $F = 1.926$ ,  $p = 0.173$ ). Fruit set over all years and sites ranged from 63 to 92 percent (Table 3). Fruit set was significantly lower in both sampling years at Buffalo than at the other three sites. Significant differences in seed set were found between years at each site, but none could be attributed to position on the inflorescence (two-way ANOVA  $df = 2,114$ : Buffalo  $ms = 2.974$ ,  $F = 1.218$ ,  $p = 0.272$ ; Dumont S  $ms = 0.003$ ,  $F = 0.002$ ,  $p = 0.967$ ; Dumont W  $ms = 5.591$ ,  $F = 3.606$ ,  $p = 0.060$ ; Walton  $ms = 1.944$ ,  $F = 2.348$ ,  $p = 0.128$ ). Data (seed set among 3 flowers) were combined for each plant and analyzed by year and site. Mean number of seeds per fruit ranged from 1.4 to 3.3 (Table 3). Differences were found only between the extreme means ( $1.4 \pm 1.7$  at Buffalo in 1986 versus  $3.3 \pm 1.8$  at Walton in 1987).

The number of seedlings observed annually in plots with and without removal of seed sources was calculated as a proportion of total number observed (Table 4). The number of seedlings in the first year ranged from 21 to 150. Proportions of seedlings in removal plots declined steadily over 6 years relative to non-removal plots. Proportions in removal plots ranged from 0.356 to 0.655 during the first year and from 0 to 0.025 during the last year. Proportions in non-removal plots ranged from 0.117 to 0.166 and from 0.136 to 0.289 during comparable years, respectively.

Patterns of seedling recruitment were compared to two expectations. First, a gradual decline in seedling recruitment was assumed, with the largest number predicted during the first year and the smallest in the sixth and last year of the study. Proportions of seedlings in each plot were ranked relative to expectations using Spearman's coefficient (Sokal and Rohlf 1981). Second, uniform recruitment (0.167) was assumed in each of 6 years and compared observed proportions using a Goodness of Fit test (Sokal and Rohlf 1981). Among the four removal plots, three showed a significant correlation with expectations of an annual decline. Among the four non-removal plots, none was significantly correlated. Among all eight plots, only one of the non-removal plots (Walton) was consistent with expectations of uniform recruitment.

Mean cover ranged from 66.6 to 87.2 percent per square meter along belt transects outside of established populations (Table 5). In contrast, mean cover within established populations ranged from 36.6 to 49.7 percent. Reproductive plant density ranged from 0.9 to 1.1 per square meter, including quadrats without reproductive plants. However, with empty quadrats excluded, mean density ranged from 1.2 to 2.3 per square meter. Cover explained 25.1–49.7 percent of the variance in reproductive plant density. Mean cover in permanent plots ( $n = 32$ ) was  $34.1 \pm 21.3$  percent, density of seedlings observed during the first year was  $159.8 \pm 100.7$ , and mean reproductive plant density was  $1.1 \pm 0.6$ . Cover in permanent plots accounted for 16.9 percent of the variance in seedling density and 52.8 percent of the variance in reproductive plant density.

## Discussion

Surviving to reproduce in *I. aggregata* ssp. *weberi* depends primarily on an ability to survive the first growing season following seedling recruitment, rather than on factors imposed during

Table 2. Number of reproductive plants in each age class in four populations of *Ipomopsis aggregata* ssp. *weberi*.

Age Class	Buffalo	Dumont S	Dumont W	Walton	Total
2 years	18	4	7	8	37
3 years	24	13	14	18	106
4 years	14	11	12	15	52
5 years	6	8	6	14	34
>6 years	2	1	1	1	5

Table 3. Mean fruit and seed set in 20 plants from each of four different populations. Means with same letter are not significantly different at  $p = 0.05$ ,  $df = 19$ , respectively (unplanned comparisons, Sokal and Rohlf 1981).

Site	Year	Mean Fruit Set $\pm$ SD	Mean Seed Set $\pm$ SD
Buffalo	1986	0.63 <sup>A</sup> $\pm$ 0.27	1.4 <sup>A</sup> $\pm$ 1.7
	1989	0.69 <sup>A</sup> $\pm$ 0.28	1.9 <sup>AB</sup> $\pm$ 1.7
Dumont W	1985	0.79 <sup>B</sup> $\pm$ 0.19	2.6 <sup>AB</sup> $\pm$ 1.9
	1987	0.91 <sup>B</sup> $\pm$ 0.15	3.3 <sup>B</sup> $\pm$ 1.8
Dumont S	1986	0.83 <sup>B</sup> $\pm$ 0.17	2.1 <sup>AB</sup> $\pm$ 1.4
	1989	0.81 <sup>B</sup> $\pm$ 0.20	2.5 <sup>AB</sup> $\pm$ 1.7
Walton	1985	0.79 <sup>B</sup> $\pm$ 0.25	2.5 <sup>AB</sup> $\pm$ 1.7
	1987	0.91 <sup>B</sup> $\pm$ 0.19	2.9 <sup>AB</sup> $\pm$ 1.6

Table 4. Recruitment of seedlings from the seedbank, calculated as a proportion of total number observed over 6 years in (A) each of four plots from which reproductive plants were removed and (B) each of four undisturbed plots.

Year	Buffalo		Dumont S		Dumont W		Walton	
	A*	B	A*	B	A	B	A*	B**
1985	0.434	0.155	0.477	0.166	0.356	0.117	0.655	0.154
1986	0.270	0.044	0.300	0.196	0.136	0.227	0.209	0.189
1987	0.113	0.108	0.123	0.226	0.271	0.252	0.044	0.098
1988	0.126	0.279	0.041	0.052	0.085	0.039	0.069	0.229
1989	0.050	0.124	0.033	0.072	0.152	0.099	0.022	0.193
1990	0.006	0.289	0.025	0.287	0.000	0.265	0.000	0.136
Total Seedlings:	159	315	243	362	59	453	229	440

\* Spearman's Rank Coefficient of Correlation (proportion by year) in removed plots significant at  $p = 0.05$  ( $df = 5$ ).

\*\* Goodness of Fit test with expectation of uniform recruitment per year significant at  $p = 0.05$  ( $df = 5$ ).



Table 5. Relationship between cover and mean number of reproductive plants at four sites. Mean cover estimated from eight 1 m<sup>2</sup> quadrats outside and within populations along a 100 m belt transect at intervals of 6 m; r<sup>2</sup> = variance in number of reproductive plants per quadrat explained by cover.

Site	Mean Cover Outside Population	Mean Cover Within Population	Number of Reproductive Plants per m <sup>2</sup>	r <sup>2</sup>
Buffalo	87.2 ± 16.6	36.6 ± 20.6	1.0 ± 1.1	0.251
Dumont S	79.6 ± 22.9	49.7 ± 28.4	0.8 ± 1.1	0.381
Dumont W	66.6 ± 23.3	41.5 ± 29.1	1.1 ± 1.2	0.467
Walton	77.0 ± 24.0	48.4 ± 29.8	0.9 ± 1.0	0.497

successive seasons through the first 2 years. From 58 to 92 percent of all seedlings died by the end of the first growing season. In contrast, probabilities of survivorship among vegetative rosettes increased significantly in subsequent years. Rosettes surviving the first summer experienced slightly less survivorship during the first winter (48.5–72%) than those remaining after the second summer (60–90.5%). No clear patterns of survivorship were found among sites. For example, probabilities of surviving the second summer and second winter were significantly greater at Dumont W (an east-facing slope) than at Dumont W (flat terrain), but were not different from that at Walton, a population also located in flat terrain and at a similar elevation. Most seedlings were recruited during the late spring, shortly after snow melt, and seedling recruitment was not observed during the summer months. The geographic distribution of *ssp. weberi* is characterized by heavy winter snow accumulations, early summer droughts, and variability in summer rainfall derived from sporadic, localized storms (Siemer 1977, Dix 1974). Thus, it seems likely that seedling recruitment at all sites generally results from availability of moisture following snow melt rather than summer rainfall, and is followed by high mortality resulting from early summer drought.

Subspecies *weberi* appears to be an obligate monocarp, living 2–5+ years before flowering and fruiting (Figure 1). Monocarpy (semelparity) has been reported for *I. aggregata ssp. aggregata* with age at reproduction ranging from 2 to 7 years (Hainsworth et al. 1984). In contrast, iteroparity was reported in some populations of *I. aggregata ssp. formosissima* (Paige and Whitham 1987) as a

response to reduced levels of pollinators and pollination (< 30–40% fruit set). If a similar mechanism operates in *ssp. weberi*, it would not have been detected, because fruit set did not, on average, fall below 63 percent in this study. Plants of all subspecies of *I. aggregata* grown in an experimental garden, subject to regular watering, a longer growing season, and one winter's dormancy, generally flowered and died within 18 months following germination (Wilken, unpublished data). These studies collectively suggest that both

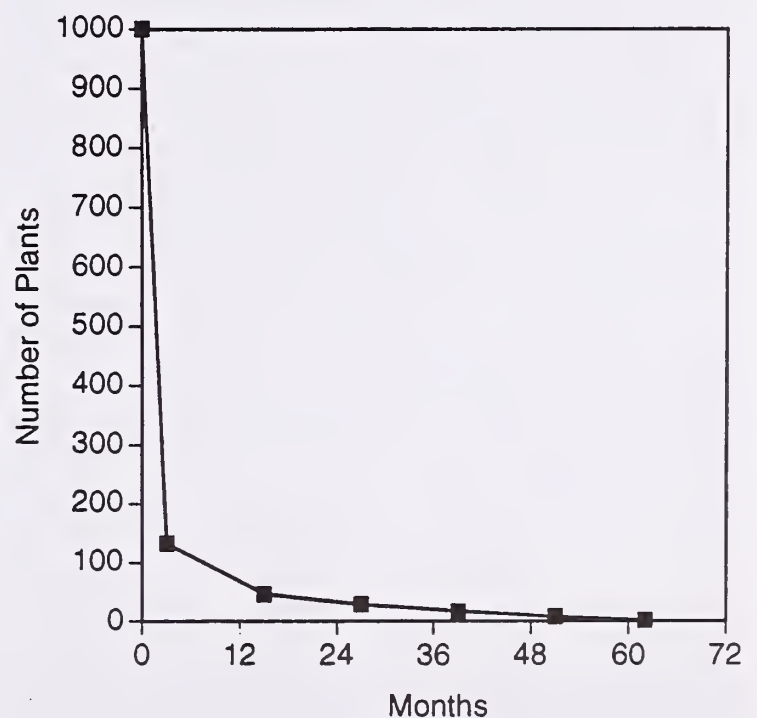


Figure 1. Average survivorship in *Ipomopsis aggregata ssp. weberi*. Data from 1985 and 1986 cohorts combined to show survivorship of a hypothetical cohort of 1000 seedlings.

age at time of reproduction and the balance between semelparity and iteroparity may be related ultimately to accumulated energy reserves, as has been suggested for other monocarps (Werner 1975, Gross 1981, Hirose and Kachi 1982, Klemow and Raynal 1985, Reinartz 1984).

Cover explained less variance in number of seedlings (16.9%) than variance in number of reproductive plants (25.1–52.8%). The unexplained variance in the former may depend on a number of factors, including density of reproductive plants in preceding years, emigration or immigration of seeds, and density of seeds in the soil. In contrast, density of reproductive plants may be more closely related to available space, competitive effects of neighboring plants, and other factors influencing growth of rosettes. In general, populations of spp. *weberi* appear adapted to sites with less than 50 percent cover and are excluded from adjacent sites with greater than 65 percent cover.

Although no attempts were made to estimate densities and longevity of viable seeds in soil samples of natural populations, observations of seedling recruitment in this study suggest that seed banks may become depleted after 5 years. Furthermore, more than 50 percent of observed seedlings appeared in the first 2 years. Overall, the average reproductive plant was about 35 cm tall with 12 lateral inflorescences per shoot and about 75 flowers. The product of estimates for mean reproductive plant density (1.2–2.3 per year) x 75 flowers per plant x overall mean fruit set (0.795) x mean fruit set (0.63–0.91) x mean seed set (1.4–3.3) suggests that, on average, 79 to 518 seeds may fall within an area of 1 m<sup>2</sup>. The predicted numbers exceed the total number of seedlings recruited from 1 m<sup>2</sup> plots from which seed sources were removed (59 to 440, mean = 282.5, Table 4). These data collectively suggest that relationships among rates of survivorship, density of reproductive plants, and reproductive capacity contribute to short-term stability of established populations. However, the establishment and long-term stability of populations appear related to reduced competition and continued availability of open sites within the regional subalpine forest.

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# Demography and Monitoring of the Autumn Buttercup, *Ranunculus aestivalis* (Benson) Van Buren & Harper, South-Central Utah

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**Abstract:** The results are presented of a 4-year monitoring program on the autumn buttercup, *Ranunculus aestivalis* (L. Benson) Van Buren & Harper. The species is known from a single location in the wild, the Sevier Valley Preserve in south-central Utah, owned and managed by The Nature Conservancy. The autumn buttercup is a short-lived species that grows in a wet saline meadow that was regularly grazed by livestock until 1988. Several small patches of different ages have been mapped and observed since 1991. The size-age structure and demography of the largest flowering patch was followed from 1991 to 1994. Flowering plants in this patch declined steadily from a high of 39 in 1991 to 6 in 1994. Numbers of juveniles first increased, reaching a high of 104 in 1993, and then dropped slightly in 1994. Numbers of seedlings increased dramatically, from 94 in 1991 to 664 in 1992, but subsequently declined. Transitions of juveniles to flowering adults declined steadily throughout this period. After an initial large input of achenes into the soil in 1991 and 1992, reproductive output (achenes/plant) declined to a value of less than 1 percent of 1991 estimates. The species appears to be going extinct at the site. The most likely reason for the decline in fecundity and recruitment is the absence of disturbance, which the autumn buttercup apparently needs in order to flower. Deliberately introduced disturbances are probably required to maintain the species in the wild.

## Introduction

The autumn buttercup, *Ranunculus aestivalis* (Benson) Van Buren & Harper, is a relatively short-lived perennial. It has had a long and confused taxonomic history since it was first collected in 1894 in Garfield County, south-central Utah. The species was described in 1948 by Lyman Benson as a variety of the widespread *R. acriformis* L. (Benson 1948). In subsequent years, the species was neglected. During inventory work in the 1970s, following passage of the Endangered Species Act, the autumn buttercup could not be relocated at the type locality and was considered probably extinct. In 1982, a new population was discovered on private property about a kilometer distant from the type locality by U.S. Fish and Wildlife Service personnel. Counts done between 1983 and 1989 showed an alarming decline, from 471 individuals to 8 in 1985. Subsequent counts varied between 8 and 22 at the site (Spence et al. 1992). Because of the taxon's precarious status, it was listed as endangered on July 21, 1989 (Smith et al. 1989). In 1988, the property on which the autumn buttercup occurred was purchased by The Nature Conservancy and was named the Sevier Valley Preserve. In 1990, a second population of about 200 flowering individuals was discovered on the preserve (Callister and Schelz 1990). Subse-

quent genetic work on this population and related species indicated that the autumn buttercup was quite distinct from *R. acriformis* and should be treated as a distinct species (Van Buren et al. 1994).

The Great Basin Field Office of The Nature Conservancy obtained a grant from the Rodney Johnson and Katherine Ordway Stewardship Endowment in 1991 to establish a monitoring program on the autumn buttercup at the Sevier Valley Preserve. The principle goals of the autumn buttercup monitoring program were to (1) follow the fate of individuals over time, (2) determine reproductive capacity, (3) describe the vegetation and associated species, and (4) determine the habitat requirements of the species.

## Area Description

The Sevier Valley Preserve is located in the valley of the Sevier River about 15 km north of the town of Panguitch. The preserve consists of two geomorphic units with distinct vegetation communities: an old river terrace vegetated by big sagebrush (*Artemisia tridentata* Nutt.) and greasewood (*Sarcobatus vermiculatus* (Hook.) Torr.), and a saline wet meadow complex dominated by wiregrass (*Juncus arcticus* Willd.), sea milkwort (*Glaux maritima* L.), and Nebraska sedge (*Carex nebrascensis* Dewey). An ecotonal area between the two

communities is dominated by saltgrass (*Distichlis spicata* (L.) Greene), broom seepweed (*Suaeda calceoliformis* (Hook.) Moq.) and scattered greasewood. Patches of open water occur, and a small artificial pond fed by a spring is situated to the southeast of the river terrace. Directly east of this pond is the locality of the original population discovered in 1982.

The monitoring work was conducted at the location of the 1990 population, which was about 200 m northeast of the pond and original 1982 population. The elevation at this site was about 2025 m, and was gently sloping (5°) to the east towards the Sevier River. The study site was in the lower margins of the ecotonal area and the higher and drier parts of the wet meadow. The topography was highly irregular, with numerous scattered mounds. Adult buttercup individuals appeared to be concentrated on the mound tops rather than in the areas between mounds. The mound topography was probably the result of livestock grazing, as the meadow had been used as a pasture for horses and bulls. The duration of grazing at the Sevier Valley Preserve is unknown, but the Panguitch area was settled in the 1860s, and some grazing has probably occurred in the area since that time. When The Nature Conservancy purchased the property in 1988, livestock grazing was discontinued.

The climate of the area can be inferred from data for Panguitch, about 15 km to the south at an elevation of 2015 m. The climate is semi-arid, with cold dry winters and moderately warm summers. Mean annual temperature at Panguitch is 6.8°C (January mean -4.4°; July mean 18.7°), while mean annual precipitation is 262 mm, of which about 65 percent falls as summer thunderstorms during the period July–September. Mean annual snowfall is 480 mm (1948–1992 data; Ashcroft et al. 1992).

## Methods and Materials

Permanent plots were established around patches of the autumn buttercup. In the largest patch (1990 population), individuals were marked with flagged wooden stakes and mapped. Individuals were classified into three life stages: seedlings (current year), juveniles (older nonflowering plants), and adults (flowering plants). All observed adults either flowered or attempted to flower every year. Significant distinctions occurred between seedlings and juveniles in leaf size and shape. Occasionally seedlings that germinated early in the season put out juvenile-type foliage in

late summer; hence, counts of the three stages were made in the middle of summer (July and early August) to avoid over-counting juveniles. Plant variables recorded for juveniles and adults included number of leaves, length of longest leaf, diameter of leaf clump, height of tallest inflorescence, number of inflorescences and flowers, microsite position, signs of herbivory, and associated species. For seedlings the distance to the nearest adult and the microsite position were recorded. Microsite position was scored as mound top, side of mound, base of mound, between two mounds, or in flat areas away from mounds. Associated species were determined by recording the closest or most common species to each buttercup individual in each of the four main compass directions (N, S, E, W). In each year except 1993 fruit set was recorded from 10 different adults by counting the number of mature achenes and aborted or shriveled achenes. Reproductive output was recorded as achenes per adult by multiplying the mean yearly fruit set for the population by the number of flowers per adult. In 1993, inflorescences of all adult plants had been broken off or badly damaged by an unknown agent, possibly by either grazing rodents or lagomorphs, or exceptionally strong winds.

Three 25 m long permanent transects were located from the eastern margin of the ecotonal saltgrass vegetation east into the wet meadow. All transects occurred near buttercup individuals and two were directly adjacent to buttercup patches. Along each transect, a 50 cm x 25 cm quadrat was positioned directly over the tape measure at 1 m intervals from 0–24 m. Cover of all species rooted inside the plot, as well as overlying the plot, was estimated visually using the Daubenmire scale (Daubenmire 1968). Mean cover estimates for each species were determined from the midpoints of each Daubenmire class. Frequency was recorded for all species rooted in the quadrat in three nested subquadrats using the method of Smith et al. (1987). Transects were read in September of 1991 and August of 1995. Nomenclature of plant species follows Welsh et al. (1993).

Permanent photographic points were established at six locations, near the three largest patches of buttercups, and directly west of the beginning of each vegetation transect.

## Results

In a typical year, autumn buttercup individuals begin to produce leaves in spring, generally in

# FIGURE 1

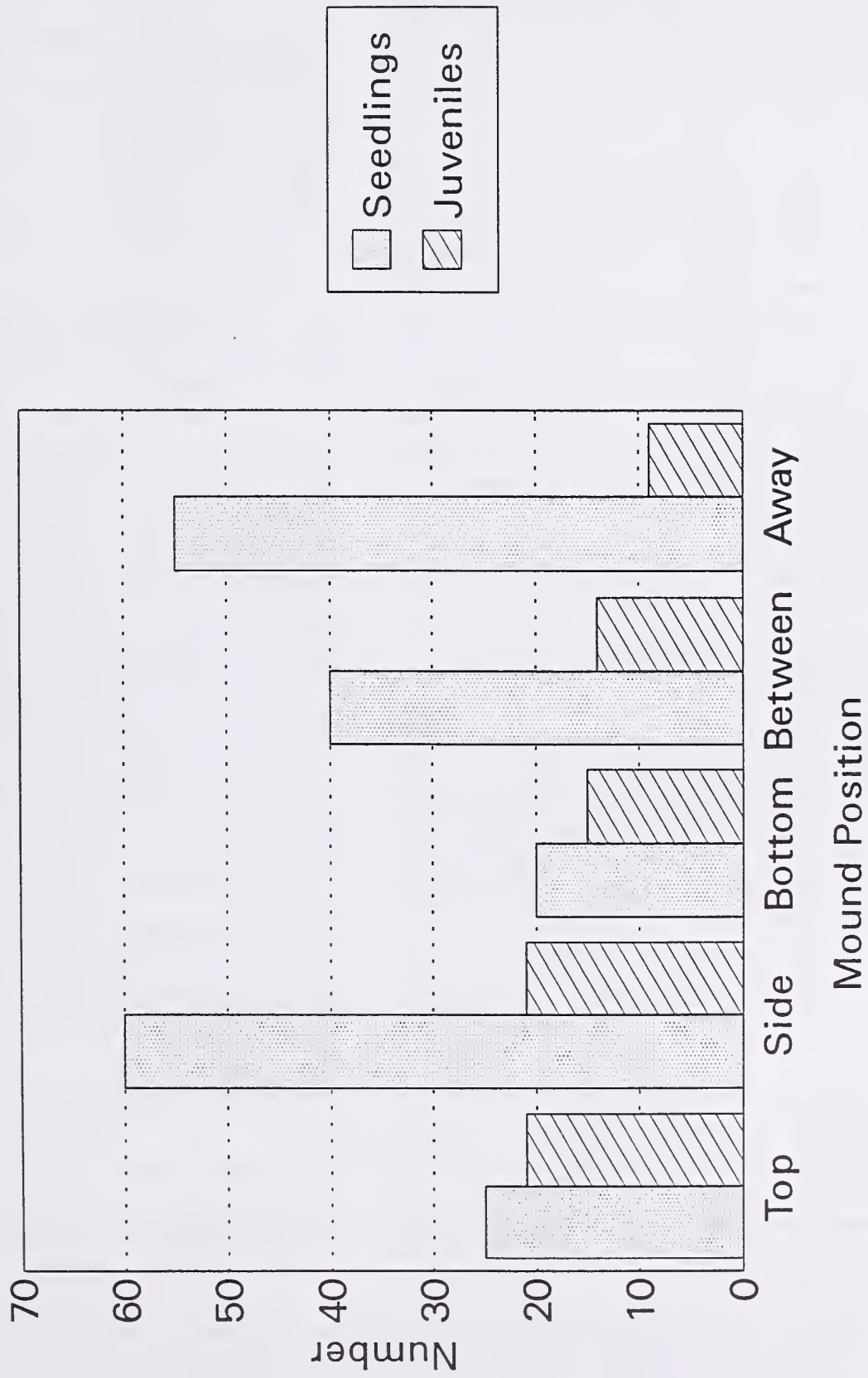


Figure 1. Autumn buttercup seedling and juvenile distribution among five microsites at the Sevier Valley Preserve.

early or middle May. The first flowers tend to open in late June, and flowering is heaviest in July and August. Achenes begin to mature in July and continue to mature through August and September. Dispersal of green achenes often occurs, with peak dispersal in August and early September. Leaf senescence first appears in September and all aboveground parts have died by late October or early November.

Initial searches in 1991 revealed that the autumn buttercup population occurred as a series of discrete patches within an area of about 1000 m<sup>2</sup>. Four patches were found, including the large group of flowering plants first found in 1990 (group A in Table 1). Table 1 summarizes size-age class numbers for 1991–1994 for these four patches, labeled A–D, estimated fruit set for patch A, and areal extent of patches. In addition to these four patches, a few scattered seedlings were observed within the 1000 m<sup>2</sup> area in 1991 and 1992. By 1994, all these plants had died.

The demographic work was done primarily in patch A. Initially, 149 plants were counted and marked in this patch in 1991. In 1992, a large number of seedlings appeared following heavy flowering in 1991, while numbers of juveniles and adults declined. In 1993, the number of juveniles increased, while numbers of adults declined slightly. By 1994, adults had declined to six, while numbers of seedlings and juveniles had also dropped (Figure 1). Table 2 summarizes overwintering survival of adults and transition states for seedlings to juveniles, and juveniles to adults, for the period 1991–1994. The seedling to juvenile transition was fairly similar over the three winters, but the juvenile to adult transition declined from 37 percent in 1991–92 to 3 percent in 1993–94.

Overwinter survival of adults varied from a low of 20 percent in 1993–94 to a high of 67 percent in 1992–93.

Patch B declined rapidly to 0 in 1993, while patch C was reduced to a few adults in 1994. Patch D, despite having large numbers of seedlings in 1991, and juveniles in 1992 and 1993, produced only two flowering plants over the 4 years. By 1994, only about 80 juveniles survived, with no signs of flowering. In 1995, patches C and D were checked again. Each had only a single adult present. By 1995, most of patch C had been overtopped by Scotch thistle, *Onopordum acanthium* L.

Adults in patch A were significantly larger than juveniles, indicating some threshold size before plants flower. Adults produced significantly more leaves than juveniles (Student's T = 4.8, df = 11, p = 0.0006), and had wider rosettes (Student's T = 4.7, df = 11, p = 0.0006). Reproductive output (estimated numbers of achenes) for patch A was initially high at 26,639 (Table 1). In 1992, this output declined to 10,674. Fruit set could not be estimated in 1993 because all inflorescences had been damaged or destroyed. In 1994, flowering and fruiting were very low compared to 1991–92. Average number of flowers per plant was only 3.5, with estimated achene production of only 395. For 1991, plant fecundity (number of flowers) was strongly correlated with number of leaves. A linear regression of number of flowers per plant against number of leaves per plant showed:

$$\text{Flower Number} = -15.8 + 2.7 * \text{Leaf Number}$$

with an adjusted R<sup>2</sup> of 0.582. The slope of the line was significantly different from zero (F = 69.3, df = 48, p < 0.0001). The only other variable that showed a strong correlation with flower number

Table 1. Numbers of autumn buttercup individuals in four patches at the Sevier Valley Preserve between 1991 and 1994 by size-age class (S = seedlings, J = juveniles, A = adults). See methods and materials for definitions of size-age classes. Reproductive output (RO) for patch A is estimated total achene production per year.

Patch*	1991			1992			1993			1994		
	S	J	A	S	J	A	S	J	A	S	J	A
A	94	16	39	664	13	18	575	104	15	113	76	6
RO	26,639			10,674			?			395		
B	1	3	2	0	1	0	-	-	-	-	-	-
C	27	7	3	12	11	1	0	12	1	0	2	5
D	200	79	1	100	200	0	0	127	1	0	80	0

\*Patch area sizes were: A = 42 m<sup>2</sup>, B = 1 m<sup>2</sup>, C = 4 m<sup>2</sup>, and D = 30 m<sup>2</sup>.

Table 2. Transition states and overwintering survivorship for three size-age classes of autumn buttercups in patch A. For seedlings and juveniles the proportion of individuals moving into the next size-age class is shown, while for adults overwintering survivorship is shown.

Size-Age Class	Period		
	1991-92	1992-93	1993-94
Adults	0.24	0.67	0.20
Juveniles	0.37	0.23	0.03
Seedlings	0.14	0.16	0.13

was rosette diameter, but it was weaker than leaf number and the two leaf variables were strongly correlated. An analysis of the residuals from the above equation showed no significant relationships with any other plant variable.

Microsite position was determined for the 280 plants in patch D, which displayed the most distinct mound topography. There was a significant interaction between microsite and juvenile and seedling size-age classes ( $\chi^2 = 17.2$ ,  $df = 4$ ,  $p < 0.001$ ). Juveniles were strongly associated with mounds, with 89 percent on mound tops, sides, or at the bases, or between two adjacent mounds. In contrast, seedlings showed a more even distribution among the five microsites, with 27.5 percent not associated with mounds at all (Figure 2).

The nearest neighbors of autumn buttercup individuals are shown in Table 3. All adults and juveniles in patches A and B were used (60 plants). Of the 240 nearest neighbors, *Sisyrinchium demissum* Greene was most common, with a count of 48. Other common species included *Juncus arcticus* (43), other autumn buttercups (39), *Muhlenbergia asperifolia* (Nees & Mey.) Parodi (38), *Plantago eriopoda* Torr. (16), and *Haplopappus lanceolatus* (Hook.) T. & G. (13). Ten other species also occurred next to buttercup plants.

Table 4 summarizes the transect vegetation cover data for 1991 and 1995. Significant changes in cover occurred for all species or groups noted in this table except *Juncus arcticus*. Declines were seen in bare ground, gopher activity (mounds), *Glaux maritima*, other native dicot forbs, *Distichlis spicata*, and *Carex nebrascensis*. Increases occurred in *Juncus arcticus*, other monocot species (mostly *Carex canescens* L.), other grasses, and especially exotics. Major increases occurred with most exotic species, especially in Scotch thistle, *Onopordum acanthium*. All species found in 1991 were still present along the transects in 1995, although *Iris missouriensis*

Table 3. Species associated as nearest neighbors in 1991 with each of 60 adult and juvenile autumn buttercups from patches A and B. The four nearest species (largest if two species were equally near) in each of the four cardinal compass bearings were recorded.

Species	Occurrences	Percent of Total
<i>Sisyrinchium demissum</i>	48	20
<i>Juncus arcticus</i>	43	18
<i>Ranunculus aestivalis</i>	39	16
<i>Muhlenbergia asperifolia</i>	38	16
<i>Plantago eriopoda</i>	16	7
<i>Haplopappus lanceolatus</i>	13	5
<i>Distichlis spicata</i>	9	4
<i>Glaux maritima</i>	7	3
<i>Carex canescens</i>	5	2
<i>Ranunculus cymbalaria</i>	5	2
<i>Dodecatheon pulchellum</i>	3	<1
<i>Onopordum acanthium</i>	2	<1
<i>Elymus trachycaulis</i>	2	<1
<i>Hordeum jubatum</i>	2	<1
<i>Carex nebrascensis</i>	1	<1
<i>Iris missouriensis</i>	1	<1

Table 4. Summary percent cover estimates for species or groups of species, bare ground, and pocket gopher activity at the Sevier Valley Preserve, based on three 25-meter transects and seventy-five 20 cm x 50 cm quadrats. For all species or groups except *Juncus arcticus* and total cover, the differences between years was significant at the 0.05 level (Student's T-test).

	Year	
	1991	1995
Bare Ground	24	0.6
Pocket Gopher Activity	14	0.2
<i>Juncus arcticus</i>	27.9	30.3
<i>Carex nebrascensis</i>	5.0	0.7
Other Native Sedges and Rushes	7.6	15.4
<i>Glaux maritima</i>	14.7	5.0
Other Native Dicot Forbs	14.0	5.1
<i>Distichlis spicata</i>	11.5	5.5
Other Grass Species	2.2	4.5
<i>Onopordum acanthium</i>	<0.1	5.4
Other Exotic Species	0.2	6.4
Total Mean Vegetation Cover	83.2	80.2
Total Native Species Along Transects	16	17
Total Exotic Species Along Transects	3	6
	<u>1991</u>	<u>1995</u>
Five Most	<i>Juncus arcticus</i>	<i>J. arcticus</i>
Common Species	<i>Glaux maritima</i>	<i>C. canescens</i>
Along Transects	<i>Distichlis spicata</i>	<i>D. spicata</i>
	<i>Haplopappus lanceolatus</i>	<i>Onopordum acanthium</i>
	<i>Carex canescens</i>	<i>G. maritima</i>

# FIGURE 2

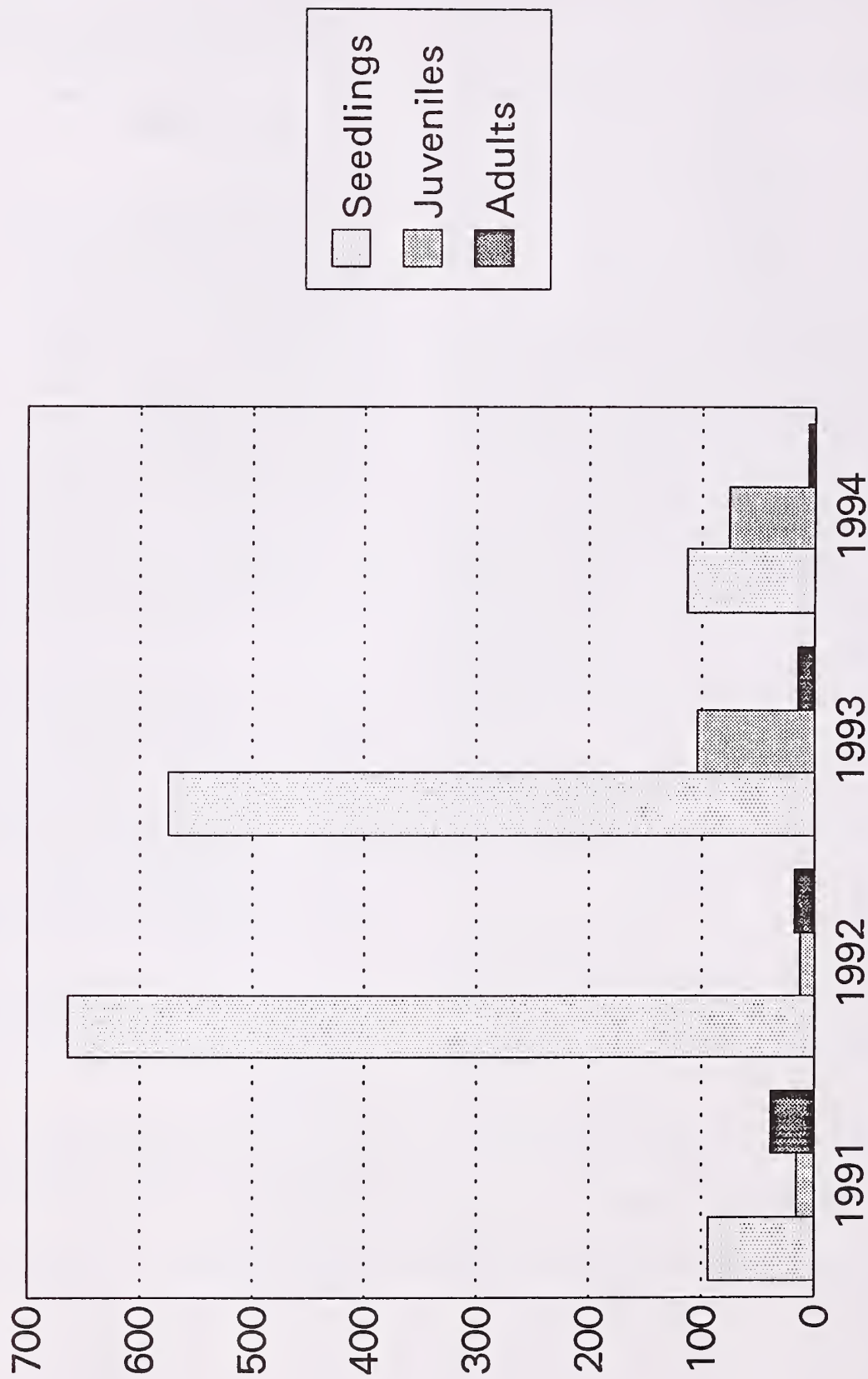


Figure 2. Counts of autumn buttercup seedlings, juveniles, and adults in patch A between 1991 and 1994 at the Sevier Valley Preserve.



Nutt. was not found in a quadrat in 1995. Several additional species appeared between 1991 and 1995, including *Epilobium hornemannii* Reichenb., *Poa secunda* Presl., *Descurainia sophia* (L.) Webb ex Prantl, *Chenopodium album* L., *Lactuca* sp., and *Scirpus pungens* Vahl. Total cover declined between 1991 and 1995 along transects 2 and 3, while it increased along transect 1.

Autumn buttercup flowers are visited by a variety of insects, including species of Diptera, Lepidoptera, and Hymenoptera. Members of seven families were observed visiting flowers in 1991. Fruit set was very high at 90 percent (Kimball Harper, personal communication 1991) and 88 percent (1992; N = 20 flowers), within the range for selfers. The species appears to be either self-compatible or apomictic, as caged plants had high fruit set (R. Fritts, personal communication 1991). Mean fruit set per flower (N = 20 flowers) for 3 years in which data were obtained was 23.8 (1991), 19.7 (1992), and 18.8 (1994).

Extensive disturbance of vegetation and soil occurred in 1991 and 1992 in the vicinity of patch A, most of which resulted from burrowing activities of pocket gophers (*Thomomys* sp.). Of the 39 adults in this patch in 1991, five (13%) had been buried and killed by gopher activity by the 1992 census. Gopher activity remained high through 1992, but declined sharply in 1993 and subsequent years. By 1995, gopher activity along the vegetation transects was only 25 percent of 1991 levels. In addition to burial, adult and juvenile buttercup plants were also grazed, with both leaves and inflorescences removed. Grazing was most intense on adults. The herbivores were not identified, but were probably either lagomorphs and/or rodents, such as pocket gophers. Overall grazing activity was low in 1991, but increased on individual plants in subsequent years as numbers of adults declined.

## Discussion

The autumn buttercup is in section *Chrysanthae* of the genus. Several species of the section that have been investigated are short lived and reproduce mostly by seed. For example, Harper (1977) noted that *R. acris* has a half-life of 65–196 weeks. High turnover of individuals in populations was noted, although mortality declined with increasing age (Sarukhan 1974). Heavy seedling mortality was characteristic of this species. Occasionally, *R. acris* populations lacked an above-ground component and persisted at the site via a seed bank. Most species investigated occur in meadows and other

habitats that are disturbed often.

The available evidence suggests that *R. aestivalis* is similar to other investigated species in section *Chrysanthae*. All adults tagged in 1991 had died by 1995, with most mortality in the 1991/92 winter. The species appears to favor disturbed sites, as it had persisted in the wet meadow complex despite the disturbance imposed by livestock activity. Seedling mortality was high at 84–86 percent. With the decline in flowering and seed production, decreases were also noted in the appearance of new seedlings, suggesting that the seed bank was probably transitory.

Two hypotheses can explain the decline in the population of autumn buttercups. The meadow appears to have dried out somewhat since 1991, as indicated by declines in wetland species like *Carex nebrascensis* along the transects. This may have increased overwintering mortality in adults and reduced growth rates of juveniles enough that they fail to reach flowering size. However, precipitation in the winter and spring of 1992–93 was well above normal, while the transition of juveniles to adults actually declined.

Another possible reason for the decline in the population is the absence of trampling and grazing disturbances by livestock. *Ranunculus aestivalis* may be a disturbance-adapted species and may require disturbances that break up vegetation or disturb the soil. In the absence of disturbance, the species may be out-competed by other species such as *Juncus arcticus*. In 1991, many individuals in patch A occurred in a part of the 42 m<sup>2</sup> area that became densely overgrown by *J. arcticus* in subsequent years. In the past, livestock grazing and trampling may have opened up the rush-sedge sward, allowing buttercup individuals to grow faster and become large enough to flower and fruit. Seedlings did not show any significant relationships with microsite topography caused by livestock trampling, suggesting that germination and establishment were not favored by these disturbances.

Between 1991 and 1995 a major increase in the cover of exotic species occurred on the Sevier Valley Preserve. In particular, *Onopordum acanthium* increased more than 10-fold and has completely occupied some areas where autumn buttercups originally occurred. This species poses a major threat to the continued survival of *R. aestivalis* and steps will need to be taken to control it before the species dominates the preserve. Other species have also shown major increases, including *Descurainia sophia* and *Hordeum jubatum* L. The absence

of livestock grazing may be one reason for the increase of many of these exotic species.

Because of the short life span of adults, and apparently short-lived seed bank, immediate steps will be needed to prevent the autumn buttercup from going extinct at the Sevier Valley Preserve. In the spring of 1995 Drs. Harper and Van Buren of Brigham Young University initiated a series of clipping and disturbance experiments designed to stimulate flowering and to determine the type of disturbance regime necessary to maintain a viable population of the species.

### Acknowledgments

The author would like to thank the Great Basin Field Office of The Nature Conservancy and the Rodney Johnson Stewardship Endowment for financial support during this project. Many thanks to Ben Franklin for help in the field, to Nick Van Pelt for his support and encouragement, and to Kimball Harper and Renee Van Buren for their interest in the project.

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# Results of an Eleven-Year Monitoring Study of the Endangered *Pediocactus peeblesianus* (Croizat) L.D. Benson var. *peeblesianus*

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**Abstract:** Peebles Navajo cactus is a narrow endemic restricted to specialized and localized soils in Navajo County, Arizona. It was listed as endangered in 1979. Four monitoring plots established in 1985 and 1986 have been read annually, tracing the life history and reproductive success of several hundred plants. Germination events have occurred only every few years and they are strongly correlated with rainfall. Growth rates are slow and plants do not reproduce until they are more than 8 years old. Heavy rodent predation was observed during dry years.

## Introduction

This paper summarizes studies undertaken on Peebles Navajo cactus (*Pediocactus peeblesianus* (Croizat) L. D. Benson var. *peeblesianus*) between 1985 and 1995. Also known as the Navajo plains cactus, this taxon was listed as endangered by the U.S. Fish and Wildlife Service (USFWS) under the Endangered Species Act of 1973 on November 28, 1979 (44 FR 61922). It is known from only a few small populations in the vicinity of Holbrook and Joseph City, Navajo County, Arizona. The cactus is apparently limited in its distribution by very narrow ecological requirements that are only partially understood. Impacts and threats to the populations include plant collection, loss and destruction of habitat due to road construction and gravel pits, trampling and indirect effects of grazing by livestock, rodent predation, and other biotic and abiotic stressors.

A recovery plan, prepared by the Arizona plant recovery team with Barbara G. Phillips, Arthur M. Phillips, III, and Mary Butterwick as primary authors, was approved by the USFWS and was published in early 1984 (U.S. Fish and Wildlife Service 1984). The plan listed actions necessary to prevent further reduction in numbers of the cactus and to stabilize and increase its population in nature to the point at which it is no longer in danger of extinction.

A monitoring program had been established by Mary Butterwick of the U.S. Bureau of Land Management (BLM) in 1980 at Joseph City, recording a total of 166 plants over 7 years. In the early 1980s, 77 individuals (44%) were removed or eliminated from her plots. Ten mature plants were eaten by rabbits or rodents in 1981; causes for other losses were unknown (Butterwick 1986, M.

Butterwick, personal communication). This dramatic decrease prompted the USFWS to fund establishment of an expanded monitoring program in 1985.

Initial work was conducted under U.S. Fish and Wildlife Service contracts with the Museum of Northern Arizona. Between 1990 and 1994, we continued monitoring at the sites without funding. Studies were conducted in 1995 with Section 6 funding administered by the Arizona Department of Agriculture. Part 2 of the Peebles Navajo cactus recovery plan called for studies of populations in their natural habitats at known existing sites. Our field studies, from 1985 to 1989, addressed the following recovery actions: (1) monitor populations on private land and expand the monitoring program on BLM lands by setting up additional permanent plots (Sections 119, 125, 131, 222), and re-read the plots in April in successive years; (2) study the ecological requirements and population biology of the cactus (Sections 21 and 22); (3) identify and inventory all potential habitat for the Peebles Navajo cactus and accurately determine distribution (Sec. 231, 24); and (4) measure microclimatic conditions at sites and correlate with National Weather Service information (Sec. 21).

## Methods

Three plots were established in 1985, two in the vicinity of the long-term BLM plot and one on private land. The first sites were chosen to provide independent observations of a healthy dense population near the site where a 5-year record had shown a serious decline in numbers and overturn in individuals. The other site (plot 4 in this paper) was chosen to provide data on a population on private land at a disjunct location. The plot was

established in a less favorable habitat, where the plants were less dense, to provide comparative data for assessment of the influence of habitat on population dynamics. An additional plot (called plot 3 in this paper) was established in 1986 in the same general vicinity of plots 1 and 2 but approximately 500 m away.

The following procedures were established in 1985: plants were located and flagged by examining 1-meter-wide parallel belt transects on hands and knees, starting at the perimeter of one side of the plot. Each plant was assigned coordinates within its square meter (e.g., 33, 21 represents distance up the meter tape, distance to the right of the tape, within each square meter). A galvanized roofing nail with two imprinted letters was placed in the soil 2–5 cm from the plant, to the upper left if possible. The locations of the plant and nail were accurately mapped on graph paper. Dimensions of plots, layout of transects, compass coordinates, and distinctive habitat features were also noted on the maps. Unmarked nails were placed at regular intervals under the transect lines and the ends of each line were marked and photographed to aid in exact relocation of plots. Overall views of the plots and their relationship to the habitat were also photographed.

Existing plots were re-read in April and May of successive years. Diameter and height of the plant above ground surface were measured to the nearest millimeter. In 1989, due to an unusually early flowering season (at least 3 weeks earlier than normal), most plants were past flowering and fruit development had begun when they were first visited. Therefore, only one spring visit per site was made in 1989. In most cases, fruit set was recorded and flower number was extrapolated.

In the late summer of 1987, we discovered the germination of many seedlings in the plots and did a complete re-inventory of the plots in November after all the fall germinants appeared to be present. The locations of isolated germinants were marked on the maps and drawings were made of their positions where they were close to the adult parent. An intensive re-survey for seedlings was conducted in spring 1988. Code names were assigned to the surviving seedlings of the fall 1987 cohort germinants.

The monitoring plots were re-read in the springs of 1990–1995. Plot 4 was not read in 1991 and 1994 due to time constraints.

Soil Conservation Service scientists conducted field analyses of soil profiles on April 30, 1985 from test pits located within plots 1–3 (without disturbing any plants), and provided information regarding the soil associations of the area in general. At each level the soil was characterized in terms of soil association, texture (gravel, sand, silt, clay, loam), cementing agents, permeability and water availability, gypsum content, and bedrock source. Four profiles were immediately adjacent to dense populations; three were in habitats where the cactus does not grow.

Four soil pits were dug in September 1985 to bedrock with a hand trowel to provide soil for microelement analysis. Each pit was 400 cm square. Rocks of the gravel lag surface were carefully removed and set aside on a plastic garbage bag. A pint of soil was removed at each level and placed in a paper bag to air dry. The bag was labeled with site name, pit designation, level of collection, and date. The profile in each pit was photographed and notations were made regarding the profile. Excess soil was placed on the garbage bag, then placed back in the pit and the surface rocks were carefully replaced so that disturbance to the site was minimal.

Analysis procedures were run at the Soils, Water and Plant Tissue Testing Laboratory, University of Arizona, Tucson. Routine analysis included pH, electrical conductivity (EC), soluble salts, Na, K, exchangeable sodium percentage (ESP), N, P, Ca, Mg; plus Mn, Fe, Zn, Cu, SO<sub>4</sub>, S, boron, and total gypsum followed McCreary and Pucker (1983). In 1986, additional soil samples were collected by the Transition Zone Horticultural Institute and analyses for organic matter, cation exchange capacity, available N, P, NH<sub>4</sub>NO<sub>3</sub>, exchangeable Ca, Mg, K, SO<sub>4</sub>, and chloride were performed at Northern Arizona University.

One Peebles Navajo cactus was collected under permit from USFWS to J. Milne. Care was taken to remove the plant with sufficient soil so that the fine feeder roots were not disturbed, and the plastic bag containing the specimen was placed in a plastic dishpan filled with moist peat moss to keep the roots alive during transport to Flagstaff. A procedure for clearing roots and staining for vesicular–arbuscular mycorrhizal fungi (Phillips and Hayman 1970) was modified slightly (Phillips et al. 1985). Specimens were mounted on microscope slides and observed.

### *Climate/Microclimate*

Weather conditions are important influences on the cacti during all seasons (influencing retraction of plants into the soil during dry periods, timing of exposure and hydration of plants, rotting during extreme wet spells, germination timing and success, and seedling establishment). We obtained preliminary microclimatic data in 1986 by establishing six ground-level microclimatic weather stations: one adjacent to plots 1, 3, and 4; two stations in nearby non-*Pediocactus* habitat (called plots 1-3-non; and 4-non); and one at the weather station in Winslow. Data were obtained on a weekly basis for the 11-week period between April 15 and July 6. Maximum temperature, precipitation, and gravimetric water content of the soils were measured by innovative techniques (Phillips et al. 1986). The date and time when weather readings were taken were recorded, as was air temperature.

We initiated procedures to obtain microclimatic data at the *Pediocacti* sites for one full year in 1987 (Phillips et al. 1988). The nearest long-term weather station (Winslow) is 40–48 km from the sites. Also, weather stations provide shelter-height air readings (137 cm above the ground). Within 5 cm of the ground the daily temperature swings are much greater than the general climatic figures of weather stations due to the thermal properties of the soil; thus there was a need for microhabitat information.

A weighing-type rain gauge and a recording thermograph were obtained from the Northern Arizona University School of Forestry and installed adjacent to plots 1–3 (sufficiently removed from and hidden in such a way as to not draw undue attention to the plots). Both the rain gauge and thermograph were securely locked to prevent tampering. The rain gauge was bolted on a wooden plate which was secured in the ground by 75-cm-long heavy steel fence posts. After field calibration, the rain gauge recorded between mid-April 1987 and mid-April 1988. A mixture of antifreeze and water was placed in the pail during the winter to prevent buckling of the pail.

The recording thermograph was placed inside a large upside-down ammunition can, with the air-recording cable suspended in a juniper and the soil thermometer 5 cm under the soil surface in an area where *Pediocacti* are growing. The thermograph charts were replaced every 2 weeks; the rain gauge charts were changed once per month.

We obtained additional microclimatic data

from four ground-level microclimatic weather stations that were installed in April 1987, following procedures established in 1986. Data were gathered every 2 weeks from mid-April, during the growing season, and monthly during the winter, from November 1987 to February 1988, until a full year of observations had been obtained.

The complete weather records of the Winslow weather station were obtained in 1989 on computer disc. The 22-year record from 1965 to 1986 was compared with the on-site weather record we had gathered in 1987 and 1988 at the *Pediocacti* sites. The data were ordered by month, and monthly means and departures were computed for the parameters of maximum temperature, minimum temperature, precipitation, and snow (melted). This information was then treated statistically using Excel and the statistical package Exstatix. Weather station data from 1987 to 1989 had not been processed by the National Weather Service into a computer-readable format, so we did not analyze those years statistically.

### *Surveys*

Field surveys for Peebles Navajo cactus, in 1985 and 1986, were oriented toward examining likely areas where additional populations might be found based on geologic and soils maps and aerial overflights of potential habitats. Also, surveys in remote potential habitat were conducted in the vicinity of Woodruff and east of Winslow for transplant sites.

### *Results*

The results of the first 5 years of monitoring (1985–1989) are encompassed in annual reports submitted to the U.S. Fish and Wildlife Service (Phillips et al. 1985, Phillips et al. 1986, Phillips et al. 1988, 1989, 1990). This paper briefly summarizes the results from those years and updates the results from continued monitoring through 1995.

It was easy to relocate plants each spring, because they had been identified with unique codes on nails and placed on an accurate map when the plots were established. This procedure also eliminated excessive compaction of the soil in the plots and inadvertent trampling of plants. By laying out a minimal number of transect lines and using the maps to go directly to the plants, known plant locations were avoided on successive samplings and only one person entered the plots. The density of plants in plots 1, 2, and 3 is similar. Plot 4 is about a third as dense, but is not as favorable a

site (steep slope, loose soil, grazed area).

The mean heights of plants fluctuated in a similar way in each size category throughout the year, responding to microhabitat conditions. Plants emerge from winter dormancy as temperatures rise in early spring, usually in late March. By early April, when flower buds are first visible, plants have doubled in height from an average of 6 to 14 mm above the soil surface. The plants become turgid, and vegetative growth occurs. Flowering generally occurs in late April. The plants reach maximum height during fruiting in mid-May. Then, during the arid foreshummer (late May–early July) with soil temperatures up to 44°C, the plants retract into the soil, losing approximately half their height above the ground. With the summer rains, the plants again emerge and become green and turgid. There is some variation in the amount and persistence of emergence of individual plants and variation in timing among the plots. In general, the plants remain emergent until early November when they again retract into the ground and many become flush with the soil surface or slightly covered with soil and pebbles.

Figure 1 shows the numbers of plants recorded in each of the plots from 1985 to 1995. Figure 2 illustrates the general trend for all plots, normalized to 1995 data. The numbers of plants in

plots 1 and 2 have tripled; the numbers in plots 3 and 4 have doubled. Although the proportion of seedling and juveniles to adult plants in the plots has increased due to germination events described below, the 1985–1987 cohorts are beginning to flower and thus contribute to the populations' reproductive efforts.

Plants were placed in the following size categories: 0–6 mm = seedlings, 7–13 mm = juveniles, 14–20 mm = young reproductive adults, 21–27 = adults, 28–maximum size = old adults. Figure 3 shows the distribution of these size classes in plot 1 in 1985, 1989, and 1995; this diagram is representative of the situation in all plots. The distribution was bi-modal in 1985, with plants in the seedling and mid-reproductive size classes. Many seedlings germinated in the plots in the late summer of 1987. In 1989, the results of the 1987 cohort of seedlings can be seen in the abundance of plants in the 0–6 mm size class. By 1995, no other large germination event had occurred and the seedlings were beginning to move into the juvenile and young reproductive size classes.

Figure 4 displays the yearly variation in numbers of flowers in each of the plots. Since there is very little mortality of adult plants in the plots (except as described below for plot 3), the variation represents phenologic response to climatic

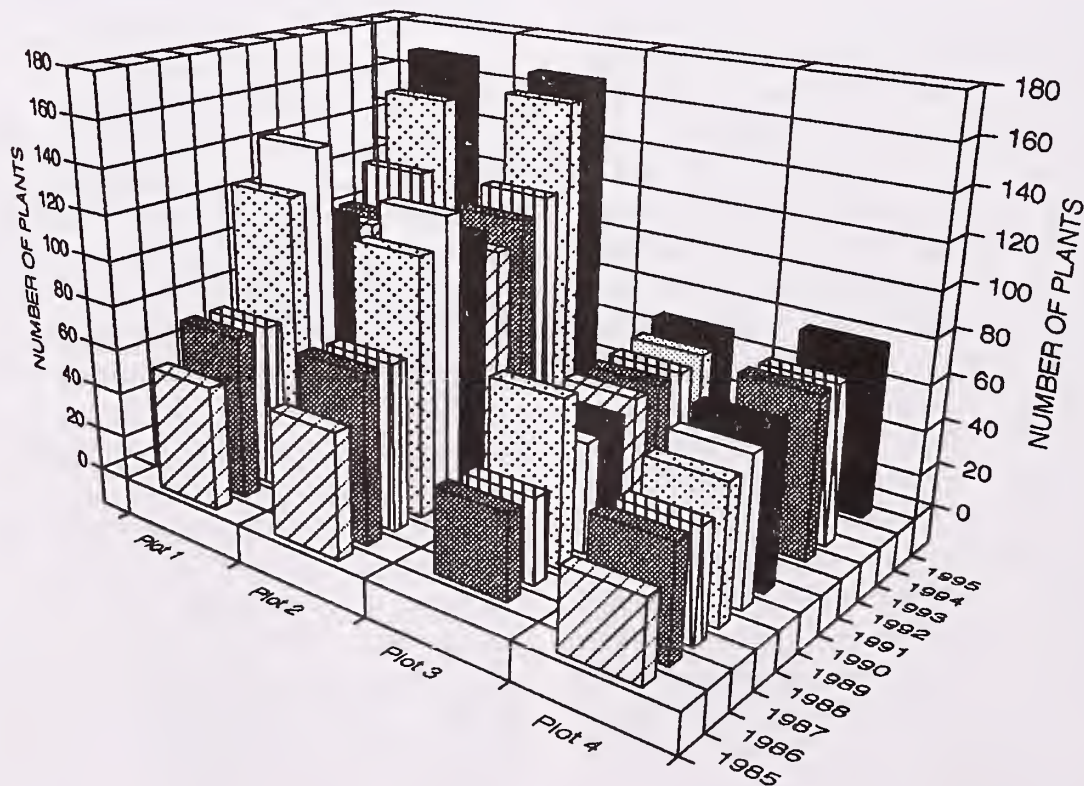


Figure 1. *Pediocactus peeblesianus* var. *peeblesianus* plots: numbers of plants 1985–1995.

(Note Plot 1 1985 and Plot 4 1991, 1994- data approximated from adjacent years.)

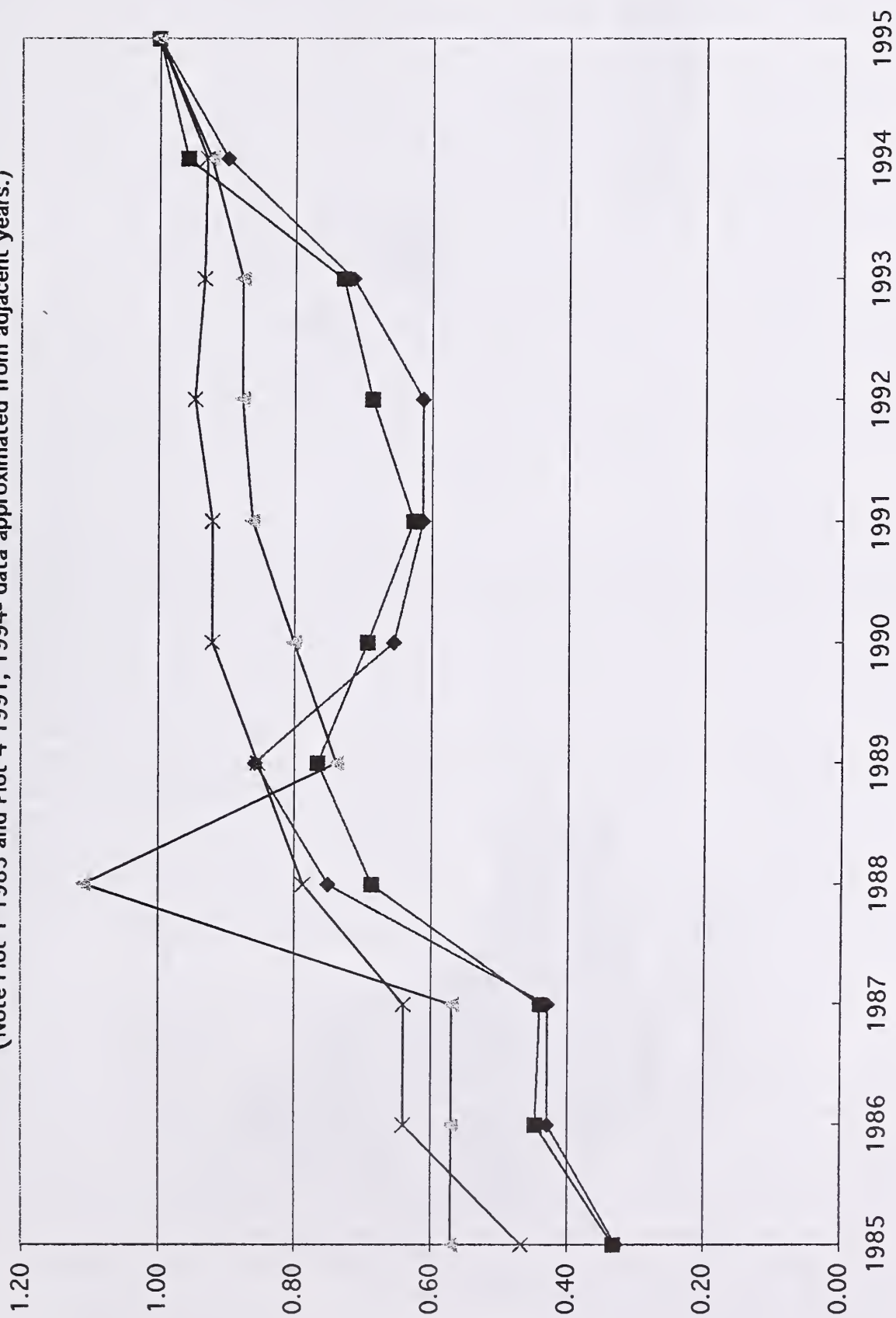


Figure 2. *Pediocactus peeblesianus* var. *peeblesianus* plot trends, 1985-1995.

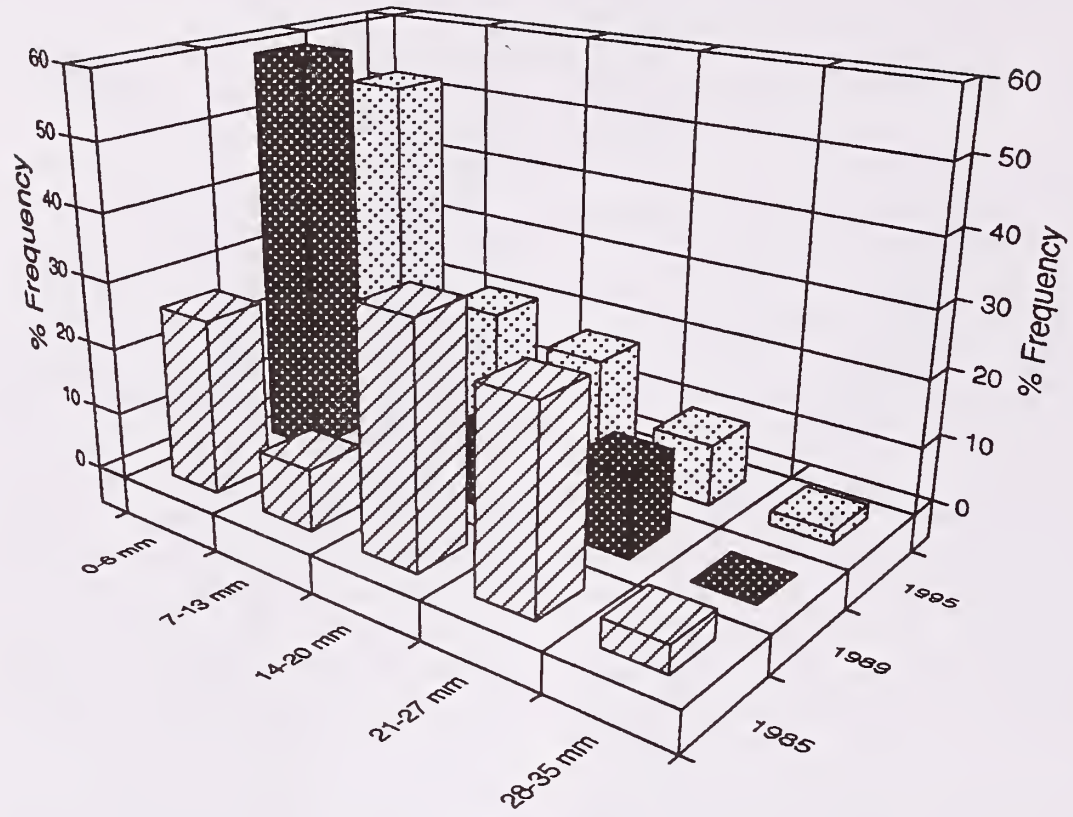


Figure 3. *Pediocactus peeblesianus* var. *peeblesianus* Plot 1: size class distribution 1985–1995.

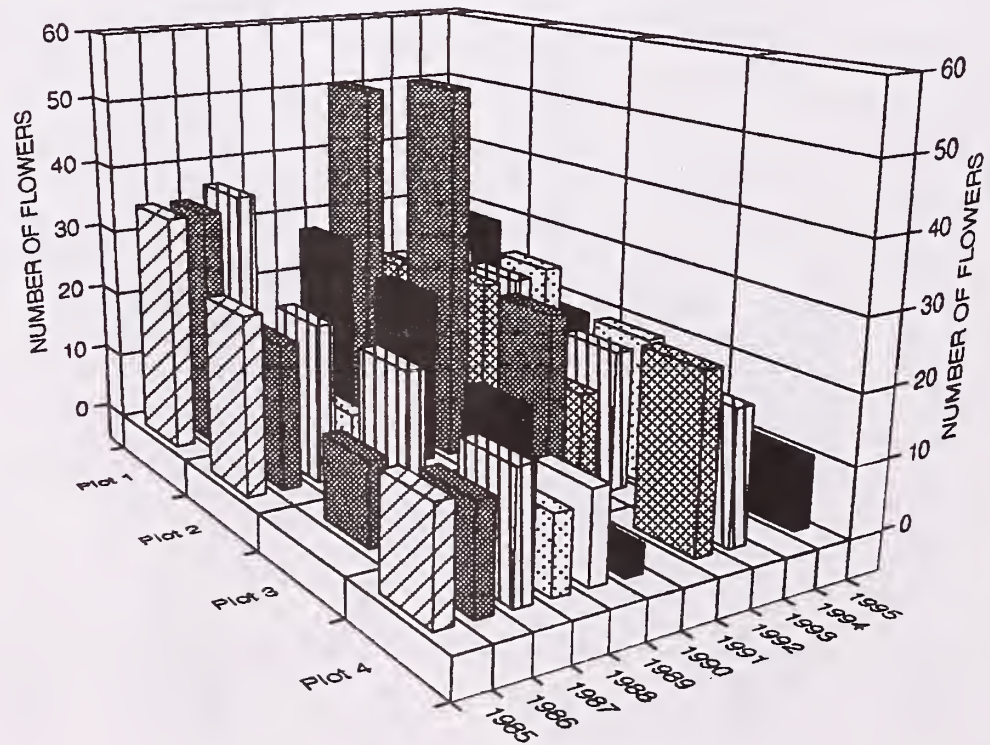


Figure 4. *Pediocactus peeblesianus* var. *peeblesianus* plot s: numbers of flowers 1985–1995.



variations. Site-to-site variability is also evident. Percent flowering was much lower in 1989 than in any of our previous readings, and fruit set was very low in plots 1–3. Many plants that normally flower did not do so in 1989, and by the time the plots were visited many buds and fruits had aborted. Plot 4, a more mesic site, showed only a slight decline in numbers of flowers that year. Flowering occurred much earlier than normal in 1989, probably due to the dry winter and early spring. In 1990 and 1995, the numbers of flowers were fewer in plot 4 compared to the other plots. Figure 5 graphically shows the year-to-year variability in numbers of flowers in plot 1.

Since none of the caged plants set fruit in 1985, nor did a hand-selfed plant, we concluded that the cactus requires cross-pollination. We have recorded 1–3 fruits per reproductive adult. Fruiting success also varies considerably from year to year, depending upon climatic conditions. Reproductive plants that produce up to three fruits in good years produce one or none in poor years. Young reproductive adults produce no fruits in poor years.

The most important impact to the plots we recorded was the loss of 23 plants (including six large reproductive adults) from plot 3 prior to our 1989 surveys. (In Figure 2, see the decrease in number of plants in plot 3 in 1989 compared to the other three plots, which show increases in plants due to the 1987 germinants.) Apparently the losses were due to rodent or rabbit predation as holes and spine clusters were found where most of the plants had been. Many seedlings that were closely associated with the adults were also lost, probably inadvertently uprooted along with the larger plants. We attribute the increased levels of predation over previous years to the exceptionally dry and warm weather during the late winter and spring of 1989.

In general, one to two seedlings from each of the 1985–1988 cohorts died each year in the plots between 1985 and 1989. Survivorship of the 1987 cohort was high initially (Phillips and Phillips 1988) and 33–42 percent of the plants are still alive in 1995 (Figure 6). Although the exact age of reproductive adults could not be correlated to size at the initiation of this study, following the 1985–1987 cohorts we now know that plants reach reproductive maturity at 8–12 years of age.

#### *Soils/Mycorrhizae*

*Pediocactus peeblesianus* var. *peeblesianus* is a narrow endemic restricted to specialized and

localized soils. Edaphic studies carried out in 1985 indicated that the soils of the habitat are shallow to deep, well-drained to excessively well-drained soils formed in mixed alluvium belonging to the Gypsiorthids–Torriorthents–Haplargids association. Trace element and mycorrhizal analyses on the soils at two Peebles Navajo cactus sites were carried out by Phillips et al. (1986). Most of the microelement values reported are low to very low, but typical for desert soils that have free lime (calcium carbonate) (T. Doerge, personal communication).

The soils are very droughty (A. DeWall, personal communication) in an area that receives very little annual precipitation and has long dry periods (Sellers and Hill 1974). Roots of the Peebles Navajo cactus are heavily colonized with vesicular–arbuscular endomycorrhizae, including *Glomus deserticola*. The low phosphorus in the soils at the sites is important for the growth of the endomycorrhizae. It is typical to find mycorrhizae in very rocky, droughty soil, where non-mycorrhizal plants have difficulty surviving.

#### *Climate/Microclimate*

At the *Pediocacti* sites during the flowering seasons in April 1987–1988, the air temperature was 27–32°C between 10 AM and 6 PM almost every day. By May 10, soil minima and maxima were 13°C and 44°C, respectively, and soil temperatures continued to occur above 44 °C from June until mid-October. During a rainy period in August, the soil maxima were about 38°C, with minima about 21°C. By mid-September, soil and air temperatures were closer together with night-time temperatures about 4 degrees apart. During a late-September storm, soil and air temperatures were essentially the same for 4 days (making the chart too difficult to decipher). By mid-November, night-time air temperatures were -7 to -1°C every night; daytime highs were 4–15°C.

Rainfall was somewhat correlated in time between Winslow and the plots, reflecting the fact that weather patterns affect a fairly widespread area. However, the variations in timing and amount of precipitation recorded correspond to our field observations that showers are very localized in extent. The smaller amounts of precipitation recorded at the sites correspond to our observations that "it rarely rains on *Pediocacti* sites" even though it is raining across the plains.

The total precipitation at plots 1–3 was about 17 cm from May to December 1987. About 24 cm of precipitation fell between May and the end of

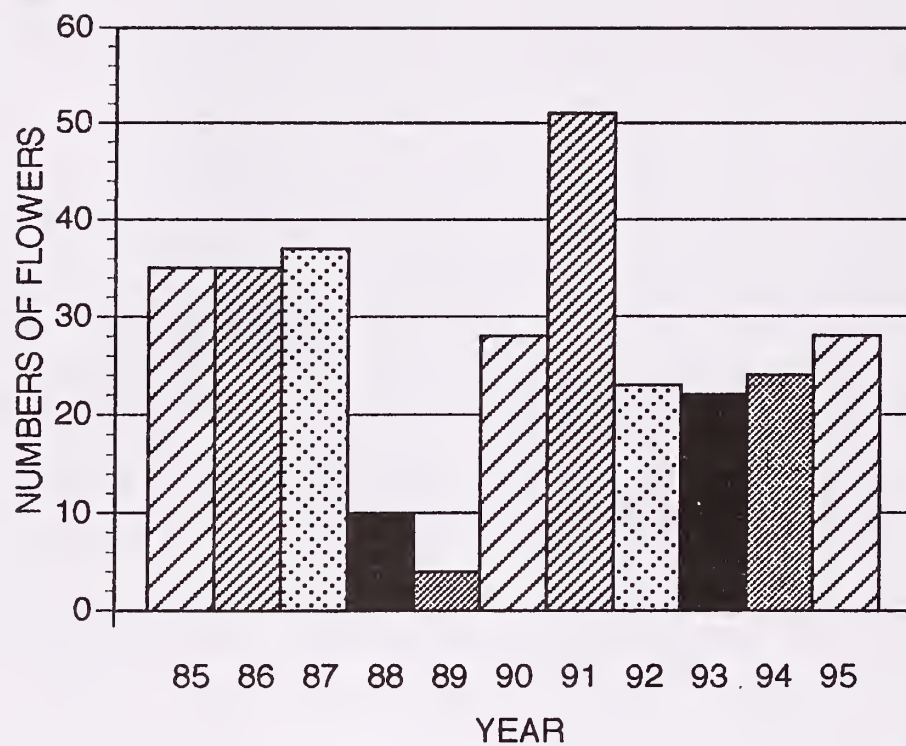


Figure 5. *Pediocactus peeblesianus* var. *peeblesianus* Plot 1 flowers, 1985–1995.

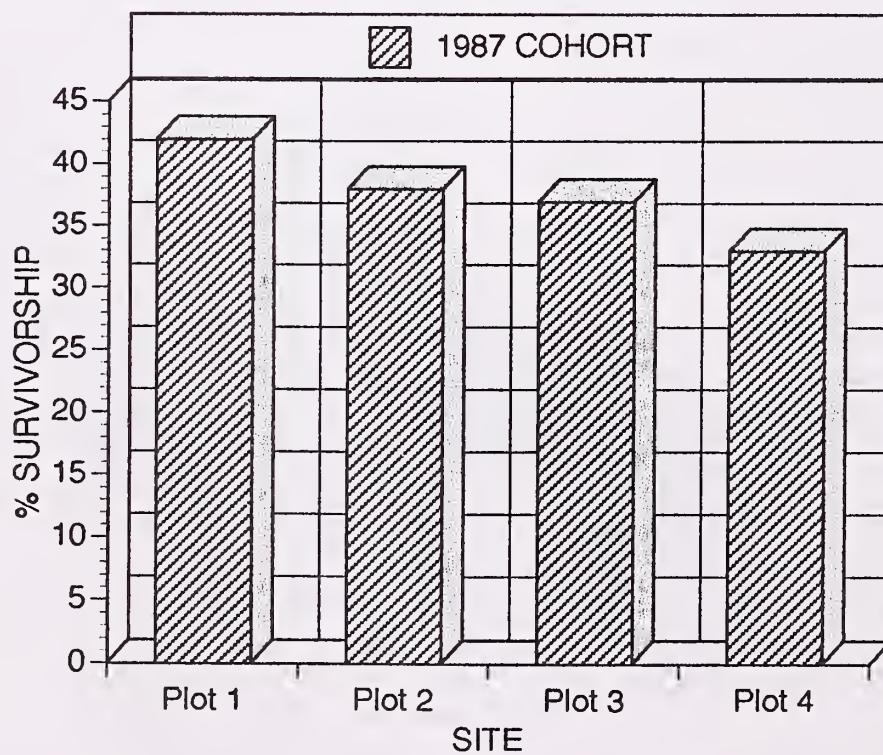


Figure 6. *Pediocactus peeblesianus* var. *peeblesianus* plots: survivorship of 1987 cohort in 1996.

December at plot 4. Major storms occurred in May (3.45 cm), August (4.05 cm), September (3.70 cm), and late October (5.13 cm) at plot 4. Near plots 1–3, these storms yielded the following amounts: May (1.34 cm), August (4.22 cm), September (1.05 cm), and late October (2.90 cm). Essentially no rain fell from June to mid-July. During December, precipitation in the form of snow fell at all sites and remained on the ground for long periods at some sites.

The soil moisture content at plot 3 ranged from 2.05 percent (July 23) to 16.72 percent (Jan 3, 1988). Following heavy rains soil moisture content rose to 12.83 percent (May), 15.19 percent (Aug), and 12.57 percent (Sept). November, December, and January readings were all high (13.65–16.72%). The pattern at plot 4 was similar to plot 3 although the mean during non-rainy periods was a bit less.

In contrast, plot 1–3-non had high percentages after the August rain (11.31%), but then quickly dropped and most of the growing season was very low (mean about 1.30%) except after rains. The mean for the non-rainy periods for plot 4-non was 3.16 percent, only slightly below plot 4 and greater than for plot 3-non. November, December, and January 1988 soil moisture readings were all high (13.65–16.72%). However, the plants were retracted and dormant during this period and the soil temperature is probably so low most of the time that the water is unavailable.

By using growing-degree days on a monthly scale, a relationship between the plots and Winslow temperature regimes became apparent in 1988 (Phillips et al. 1989). The trend of warming in the early spring, plateau of high summer temperatures, and cooling in the fall was revealed, along with the dry foresummer and dry fall precipitation regime (Figure 7).

The 22-year record at Winslow (1965–1986) reveals a high degree of correlation of each month's number of growing-degree days per month with the mean, with December 1967 and January 1968 standing out as having unusually cold minima. December 1967 mean monthly temperature was more normal (Phillips et al. 1990).

The precipitation showed a bi-modal weather pattern, with wet winters or wet summers (Phillips et al. 1990). In several cases successive months showed record amounts. The minimum precipitation recorded in the 22-year period was zero and the maximum amount was 3.6 cm in December 1967. February and March 1973, July and August 1977, July–September 1983, and July and August

1984 were wet months. As noted previously, July and August 1987 also had very favorable amounts of precipitation.

Wind plays a significant role in this region. Winds along an exposed (windward) edge of the sites may well register higher velocities and 65–80 km/hr winds are fairly common especially preceding the passage of frontal air masses (C. Avery, personal communication, B. Phillips and A. Phillips, observations).

### Surveys

Intensive surveys of potential habitat in 1985 and 1986 did not reveal additional populations of *Pediocactus peeblesianus* var. *peeblesianus* and the known distribution remains confined to the Joseph City–Holbrook area defined in the recovery plan of 1984. In 1986, surveys on previously unsurveyed private land immediately adjacent to the known populations revealed a very low density of plants in one area, and several larger colonies (totaling 50 counted plants). The thoroughness of the surveys in areas that produced negative results for *Pediocacti* is indicated by the fact that other species of cacti as small as seedling *Pediocacti* (3 mm diameter) were observed. In most cases, it was possible to evaluate that the habitat seemed to lack certain attributes that are characteristic of the cactus sites. Several search areas on Arizona state land had been heavily impacted by being scraped and turned into gravel pits. If, as seems probable, that land had been habitat for Peebles Navajo cactus, it is no longer. In 1989, surveys in remote sites for potential transplant locations also verified the known range.

### Discussion

The work reported in this paper addresses the concerns of sections of the recovery plan that called for field monitoring. Soil profiles and microelement analyses provided insight into localized edaphic parameters. The low values for manganese, iron, and zinc might prevent some potential competitors from establishing in *Pediocactus* habitat. The gypsum levels in the range of 6–16 percent are typical of desert soils and do not cause adverse effects if the salinity is not high. Mycorrhizae could provide a competitive edge for the cactus by greatly facilitating the absorption of water and nutrients. In less droughty areas other plants could more easily compete with the cacti because of more favorable conditions. *Glomus deserticola* is also abundant on *P. peeblesianus* (Croizat) L.D. Benson var. *fickeiseniae* L.D. Benson, and *P.*

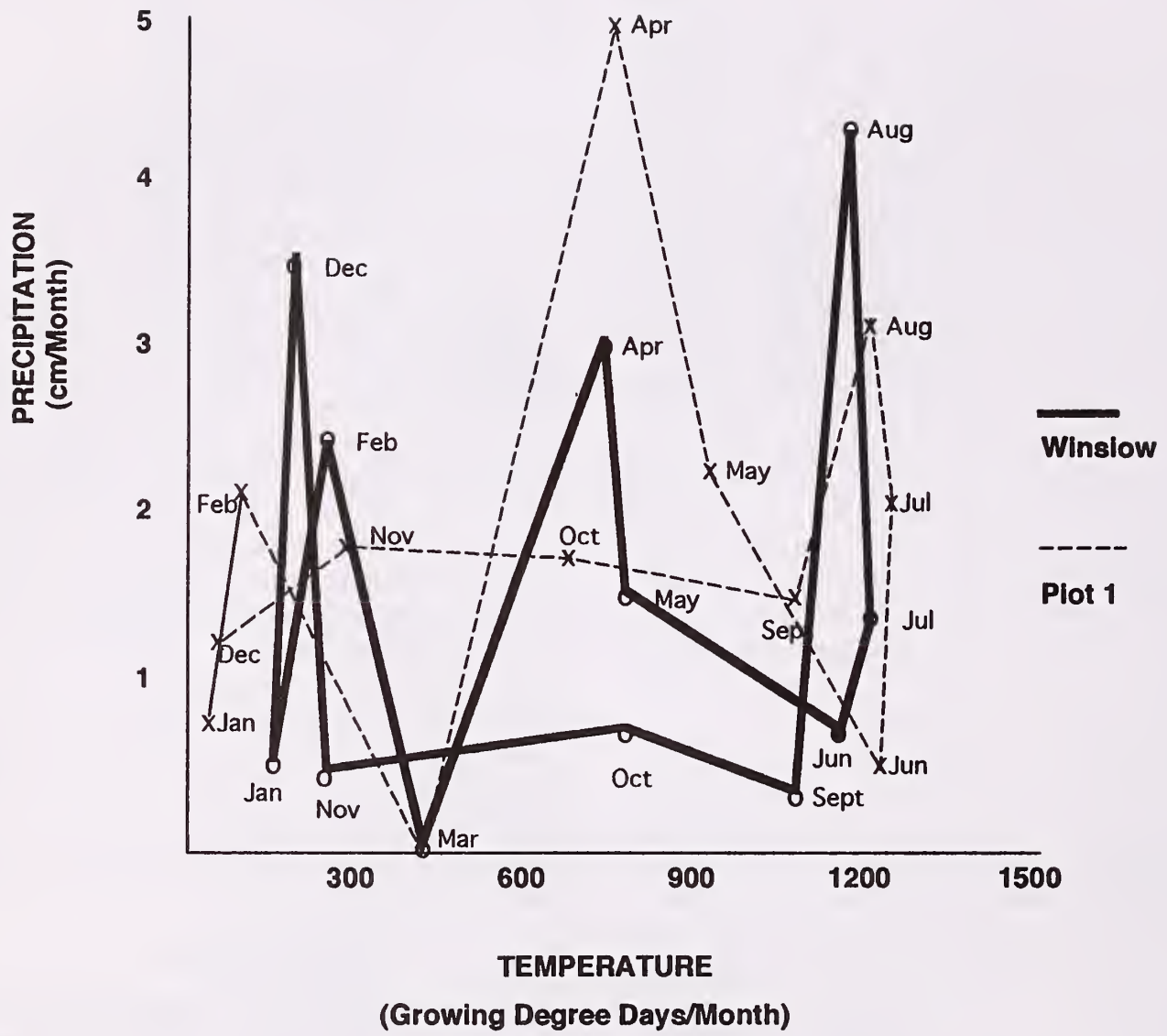


Figure 7. Climatograph for Winslow and Plot 1, 1987-1988.

*knowltonii* L.D. Benson. It is also present (but not abundant) on *P. bradyi* L.D. Benson and *P. paradynei* B.W. Benson and is not present on *P. sileri* (Engelm.) L.D. Benson (J. Milne, personal communication).

The climate/microclimate study has given us something to use to interpret the long-term weather record of Winslow for the *Pediocacti* sites. Major weather events occur over the whole area between Winslow and Holbrook, although individual storms are quite spotty in distribution, especially in the summer, resulting in differences in precipitation and temperatures in local areas. We can calculate monthly growing-degree days for Winslow for given years and calculate with fair accuracy the type of climate the plots would have for those years. Combined with precipitation records, this gives us ideas about what years might have had extreme anomalies as far as growing conditions are concerned.

Soil temperatures are much lower and moisture content of soil increases in response to rainfall events more readily in April and early May than later in the spring. *Pediocacti* take advantage of these favorable environmental conditions by timing their germination, seedling establishment, and flowering to correspond with them. By early June, fruit set has occurred and the cacti begin to retract into the soil. The cacti emerge in the fall for another period of time. Significant seedling germination can occur after summer rains, also.

Within the last 25 years, four summer periods and one late-winter period appear to have had favorable precipitation amounts for germination bursts. Many seedlings were recorded in spring 1985, spring 1986, and fall 1987, which do correlate with favorable summer 1984 and summer 1987 precipitation amounts. It is probable that there were favorable germinations in the spring 1973, fall 1977, and summer 1984 also.

*Pediocacti* seeds survive in the soil for a few years (S. Brack, personal communication). However, successful synchronicity of good seed crops with appropriate weather conditions for germination and seedling survival may require many years to achieve. The flexibility of the species to germinate in either spring or fall allows the plants to take advantage of rare combinations of favorable conditions whenever they occur. Fall germination may actually result in a higher level of survival if soil moisture conditions remain favorable for a long period of time during the ensuing winter season. The plants will be 6–8 months old before enduring their first foreshummer

drought stress rather than 1–2 months old. In most years, there are significant dry periods in May–June, and September–October in the areas where the cactus grows. The winter of 1988–89 was very warm and dry. The survival of the fall 1987 germinants through this period was probably helped by the fact that they were over a year old by then. It has been shown that cactus seedlings can tolerate much longer periods of drought when they are larger. Like other cactus, Peebles Navajo cactus seedlings first have a cylindrical shape, which is not as efficient for water economy as a spherical shape (Gibson and Nobel 1986). Only after several years do the seedlings develop a more spherical shape.

Seedlings may or may not survive to adulthood. Survivorship depends upon them not encountering a long dry period before they have reached sufficient size to withstand the drought. Severe droughts are probably occasionally responsible for the death of mature plants.

Although positive conclusions concerning the cause of the decline in number of plants previously noted in the BLM plots still cannot be made, some packrat predation was proven from sorting of middens. Only two *Pediocacti* remains were found, but the small succulent plants lack real spine protection and could easily be totally eaten so that no remains would be evident. Packrats are inveterate collectors, and in the course of their foragings they gather most of the important plant species in the vicinity of their dens (Finley 1958, Van Devender 1977, Wells 1976, Phillips 1984). The normal foraging range is probably less than 100 m from the den, but availability of food materials and population density of packrats undoubtedly affect the distance. The loss of many seedlings and adult plants in plot 3 in just one season due to predation reveals just how radically the situation for a population can change in a very short period of time.

The 11-year record now available for Peebles Navajo cactus provides a good perspective on the life history of this obscure, rare cactus. Mature plants change little in size and flowering, and the age of individuals may be measured in decades rather than years. Germination events may occur once in several years, and survival of seedlings is fairly good. It appears that plants pass from a juvenile to a reproductive state at 8–12 years of age. The fencing erected around the BLM study area in 1984 has resulted in removal of trampling and indirect impacts of cattle grazing in that area and the trend toward dramatic decreases in plants

in that area appears to be reversed. In the future, by correlating weather data with changes in the populations, it will be possible to analyze trends in seedling establishment, age-classes, and mortality of plants in each monitoring plot due to weather, and project demographic trends for the total population of Peebles Navajo cactus.

### Acknowledgments

We wish to thank the many assistants who worked diligently over the years on this project for what may have seemed innumerable hours, many times under very adverse weather conditions. Thanks also to Ruth Phillips and Dede Weage, mother and wife respectively of Art, who cheerfully accompanied us in the field in recent years. We greatly appreciate their help and company. We also thank Bob Wilson for his help compiling raw plot data into spreadsheets and preparing summary statistics. We, the authors, take sole responsibility for the content of this paper.

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# Population Viability Analysis of *Pediocactus paradinei*

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**Abstract:** Detailed population monitoring of *Pediocactus paradinei* has been performed on 14 permanent plots since 1987. Projections of future population growth were made using both deterministic and stochastic models. Of the 14 plots, only one consistently demonstrated a high probability of persistence in the future. The fate of the populations on the other 13 plots is uncertain at this time. The methods, limitations, and uncertainties associated with rare plant population modeling will be discussed in relation to the projections of the fate of this species.

## Introduction

Warren et al. (1993) reported the results of 4 years of detailed population monitoring of *Pediocactus paradinei* on 14 permanent plots located on five study areas distributed throughout the range of the species. Their work described the demography of the populations studied and discussed the future status of the species. Here this work is extended by calculating reproductive value, and stage class transition probabilities, and projecting the future population trajectories on the monitored plots.

## Methods

Five diameter size classes were defined based on the work of Warren et al. (1993):

Diameter Size (mm)	Size (Stage) Class
0-10	1
11-20	2
21-30	3
31-40	4
> 41	5

Diameter data for all years were converted into size classes from which transition probabilities were calculated. Individual cacti that had complete records of occurrence during the 4 years of continuous census periods (1987-1990) or that disappeared and included a definite cause of death were used in this analysis. Individuals with missing data were not included in the calculation of transition probabilities for that transition period as it was not possible to determine the stage class of the individuals during the missed census(es). This provides a conservative estimate of transition probabilities.

Transition probabilities and reproductive value were calculated for each plot, each site, and for all sites combined. The mean and variance of

transition probabilities for each plot were calculated across years for transitions between 1987 and 1990. Transition probabilities were calculated for each year and plot separately, and also for each site by determining the number of individuals that moved from one stage (size) class to another and dividing each of these numbers by the total number of individuals in the stage class. For example, if there was a total of 20 individuals in stage class one on a plot in 1987 and 8 remained in stage class 1 in 1988, the probability that an individual in stage class 1 did not change stage class was  $8/20 = 0.40$ . For this example, if we assume that four individuals grew to size class 2, then the transition probability for movement from class 1 to 2 would be  $4/20 = 0.2$ . If we then hypothetically found that two had grown into stage class 3 between 1987 and 1988, the probability of transition from class 1 to 3 would be  $2/20 = 0.10$ . The probability of death for an individual in this hypothetical example would then be  $6/20 = 0.30$ . The probability of death was only calculated for individuals known to be dead; individuals that disappeared from the records, but had no recorded year or cause of death, were not included. Individuals that appeared as post-seedlings were not counted as recruits, because they were not deemed to be new plants, but were simply not seen the year before. Subsequent year censuses of these individuals were included for transition changes.

Estimation of the reproductive contribution of an individual in a stage class from one year to the next was calculated with the following formula:

$$\frac{\left(\frac{R_i}{\Sigma R_i}\right)(TR)}{D_i}$$

where  $R_i$  is the total number of flowers and fruits produced by the individuals of age class 1,  $\Sigma R_i$  is the total number of flowers and fruits for all age

classes at that site or on the plot, TR is the total number of recruits (at the site or on the particular plot), and  $D_i$  is the total number of individuals in the stage class at the site or on the plot. For example, on site 3 (Valley) during the 1988–1989 censuses a total of 12 flowers and fruits were produced by four individuals in the fifth age class. All told, 83 flowers and fruits were produced by individuals on site 3 during the 1988 census and 38 recruits were noted in 1989 on the plots in site 3. Thus,

$$\frac{\left(\frac{12}{83}\right)(38)}{4} = 1.374,$$

so 1.374 new individuals were produced by each member of the fifth age class on site 3 between 1988 and 1989. As with transition probabilities, this statistic was calculated separately for each transition period and for each plot. Means and variances were calculated across years. As with the transition probabilities, data were also lumped over the plots for each site to calculate reproductive value on an overall site basis.

Simulations of potential changes in population growth were calculated using Ferson's RAMAS/STAGE simulation system (1994). This commercially available software provides a convenient platform for performing the matrix algebra necessary for calculating population growth using stage class models. Both deterministic and stochastic simulations can be performed with the software. In the simulation results presented here, future population size of the species was examined over a 100-year period. In all cases the starting number of individuals was from the 1990 survey. That was the last year in which all plots were surveyed.

The software allows the incorporation of density-dependent effects in the model of population growth. Density dependence was not included in the models used here, however, as no information on density dependence is available for the species.

Deterministic simulations were performed for all the sites combined and for each site for each transition year between 1987 and 1990. The deterministic simulations assume that transition probabilities and reproductive value are constant and invariable from year to year. The models thus show exponential growth or decline. The deterministic simulations provide an estimate of the fate of the population based on the assumption that the transition probabilities and reproduction coefficients do not change from year to year. This is not a realistic assumption.

A more realistic approach to simulating future population growth is to assume that transition probabilities and reproductive value will vary from year to year. If a relationship exists between transition probability and some variable such as precipitation, an equation describing the relationship may be used in the simulation. If no functional relationships are known, it is nevertheless possible to simulate year-to-year variability using stochastic simulation techniques. RAMAS/STAGE facilitates stochastic simulations by allowing the input of means and variances for transition probabilities and the choice of an underlying distribution of probabilities. In all stochastic simulations reported here a uniform distribution was assumed to give the maximum possible variation in the simulations. Given sparse data and no a priori knowledge of the way demographic variables change, this assumption is the most conservative. In addition, my experience with these types of stochastic simulations has been that the specific distribution chosen does not influence the results significantly. Subsequent census data will be used to determine if this and other assumptions made in these simulations are reasonable approximations of the population behavior.

RAMAS/STAGE calculates a stochastic simulation by choosing a value for the transition probability or reproductive value from the chosen distribution specified by the mean and variance input. The program then calculates the population size of the stage class in the next year using the randomly chosen transition probabilities. A new value is chosen for the transition probability (or reproductive value) at each time step in the simulation. RAMAS/STAGE allows up to 1000 such simulations to be made. All stochastic simulations reported here were made using 1000 replicate simulations. The only value retained during these simulations was the total population size at the end of each time step, although the program will retain the numbers of each stage class if desired. Simulation results were saved as a file and imported into either LOTUS or SIGMA-PLOT spreadsheets. Standard deviations were converted into standard errors for graphing. Graphs of the stochastic simulations show the mean and the standard error about the mean.

## Results and Discussion

Histograms of the total frequency of diameter sizes for all plots, sites, and years were inspected for any unusual structure. None was detected.



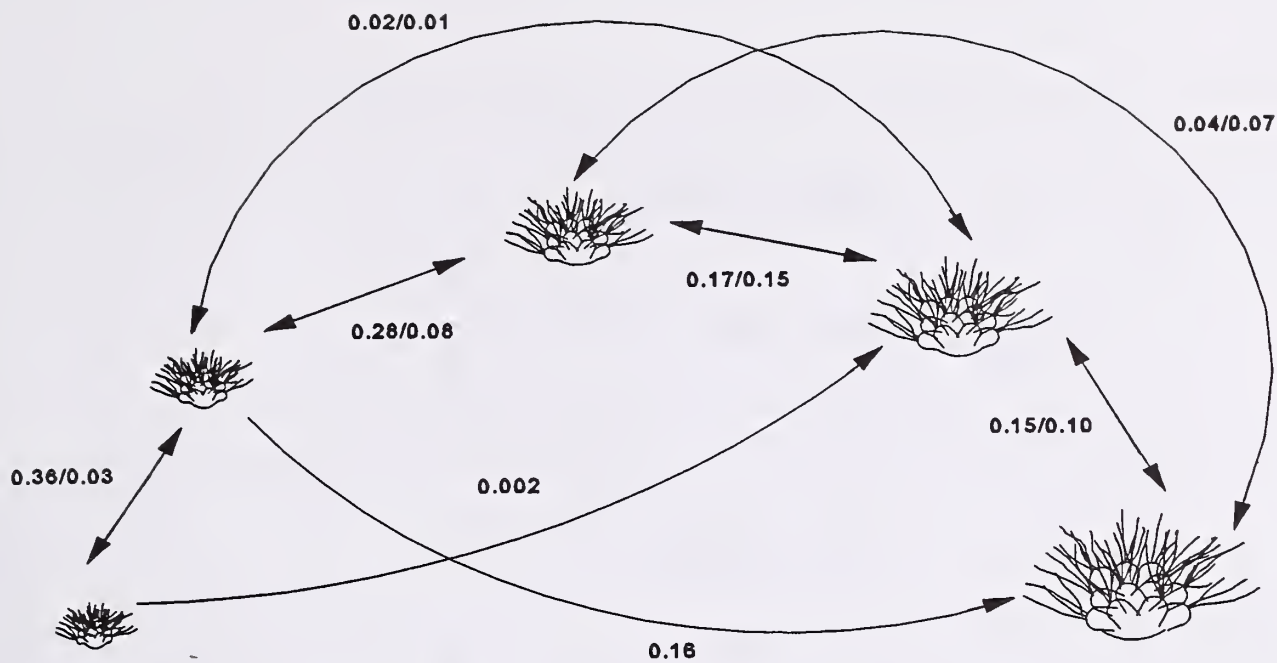


Figure 1. Average life cycle of *Pediocactus paradinei* based upon five size classes. The numbers left of the line are the transition probabilities of an individual growing from the smaller stage class to the larger. The number to the right of the line is the probability of transitioning from the larger to the smaller stage class.

Figure 1 is a diagram of the life cycle of the species used in this study. The numbers to the left of the line are the mean probabilities for transition from the smaller to the larger stage class; numbers to the right of the line are the mean probabilities for transition from the larger to the smaller. Figure 2 shows the change in the population sizes of each plot and for each site between 1987 and 1994. Between 1987 and 1990 plots on the Valley, Trail, and Kane sites show increasing population sizes while the Pasture plots show declining numbers and those on the House Rock site appear stable. Partial surveys conducted during the period of 1992–1994 show a large decline on the Valley and Pasture plots with no change in the Trail plots and what might be an increase in the House Rock and Kane plots. Figure 2 indicates relatively stable populations (with the exception of the Valley site) between 1987 and 1994 particularly on the plots with the highest densities (plot 10 and plot 3). Based on the size histograms and the population numbers noted between 1987 and 1994, one could conclude that this population is relatively stable and is not currently at risk. The large decline in numbers noted on the Valley plots (nos. 1 and 2) however does raise some concerns. We can explore some of these concerns through an examination of the simulations of future population changes.

Figure 3 shows the results of four deterministic studies of future population sizes of the entire population of the cactus based on transition probabilities and reproductive value calculated between the years 1987–1990. In interpreting these figures and the others presented, the actual population sizes should not be considered of any great importance; rather, the qualitative future increase or decrease in the population should be noted. Thus, using the data on reproduction and survival between 1987 and 1988, the future for the population looks good since during the 100 years of the simulation, the population shows positive growth. On the other hand, survival and recruitment were so poor between 1988 and 1989 that the population would become extinct if no change occurred in the demographic variables of the population. The conditions between 1989 and 1990 were also not conducive to population expansion and a relatively slower decline would be evident under these demographic conditions. However, even after 100 years the population would not be extinct. When aggregated over all years the demographic conditions, if unchanging, would lead to the eventual extinction of the population (within 80 years).

Deterministic simulations for each of the five sites using 1987–88 transition data showed all sites

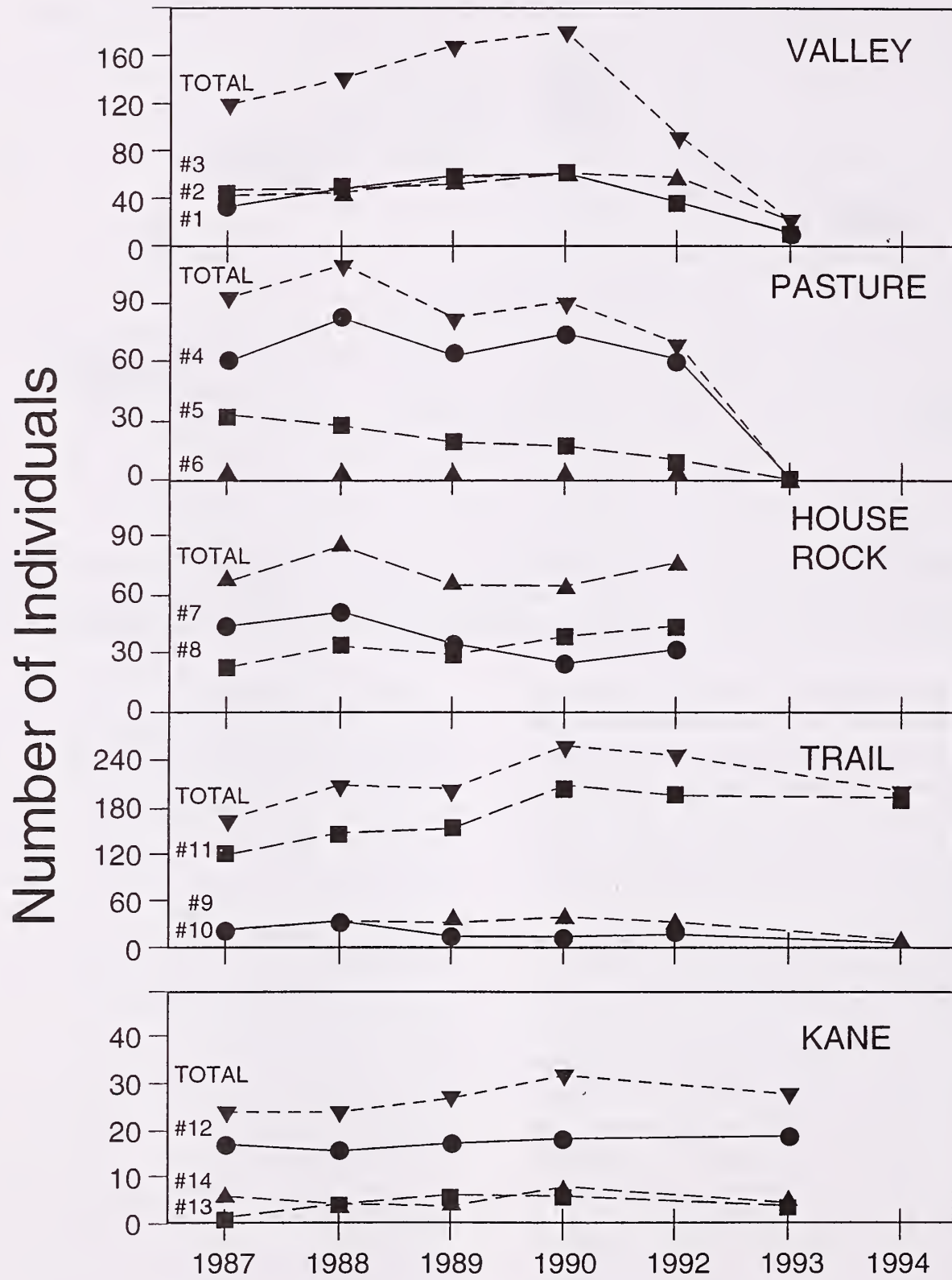


Figure 2. Number of individual *Pediocactus paradinei* by plot and site.

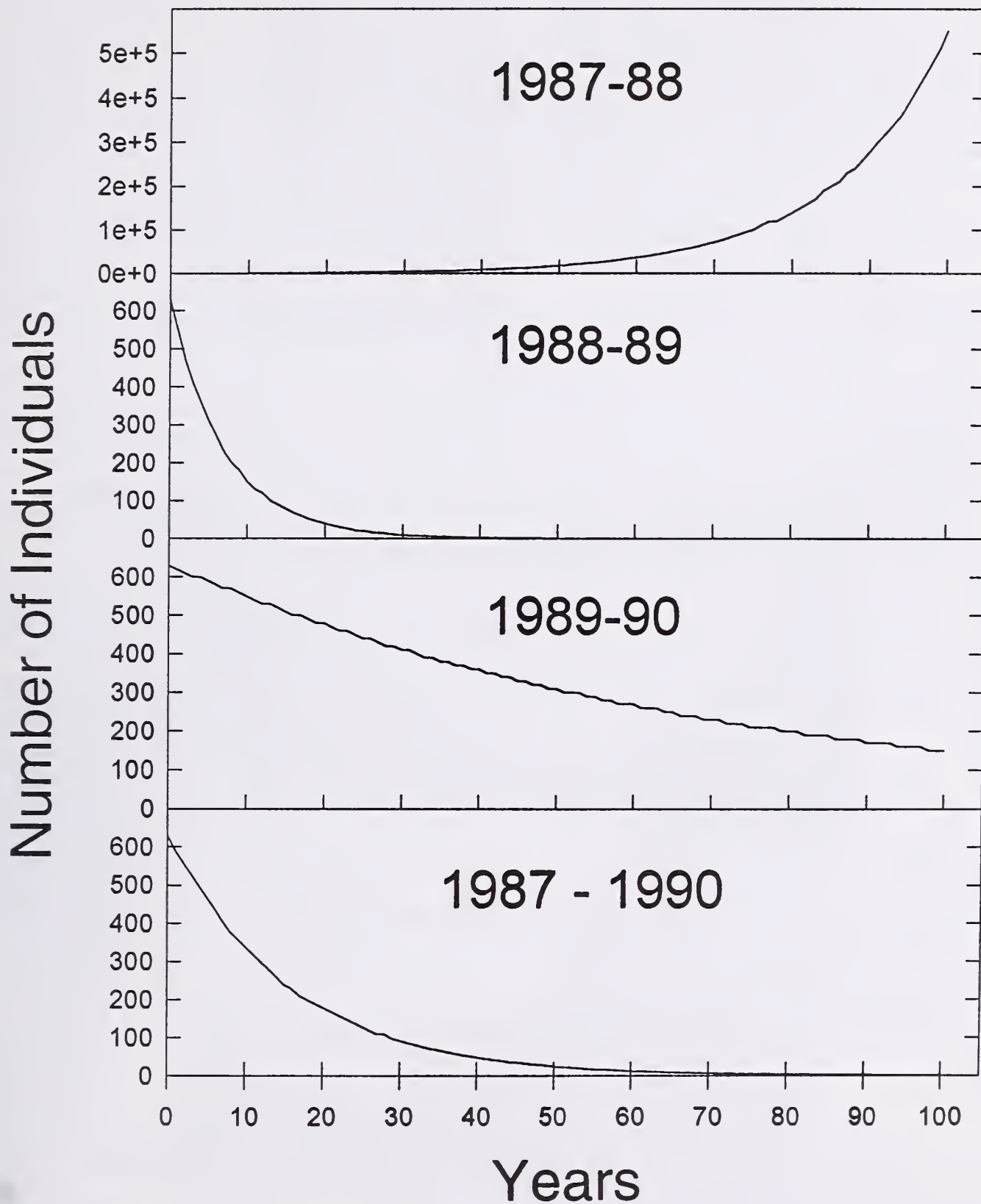


Figure 3. Deterministic simulation of all plots by year.

but Kane increasing. Simulations using 1988–1989 data indicate increasing populations at the Trail and Valley sites, but decreasing populations at the other three sites. Simulations based on the 1989–1990 data were positive for all of the sites except Pasture. It is apparent from these calculations that the Kane and Pasture sites seem to be the most vulnerable of the populations under constant demographic conditions while the Trail, Valley, and House Rock sites seem to have a brighter prognosis. Obviously though, demographic conditions change with changes in the physical and biological environment. To examine the impact of demographic variability, I calculated a series of stochastic simulations.

Figure 4 shows the results of a stochastic simulation of the entire population of the cactus over 100 years. For this simulation, aggregated data on survivorship and reproduction across all plots was used to estimate each year's demographic condition. The three transition years were then used to estimate the mean transition probabilities and reproduction and the variation about the means. The results of 1000 simulations is shown in Figure 4. The line represents the mean population size for that year over 1000 simulated population trials. The error bars around the means are the standard errors of the means. It should be noted that the standard error of the mean simulated population size overlaps with zero after only 9 years of simu-

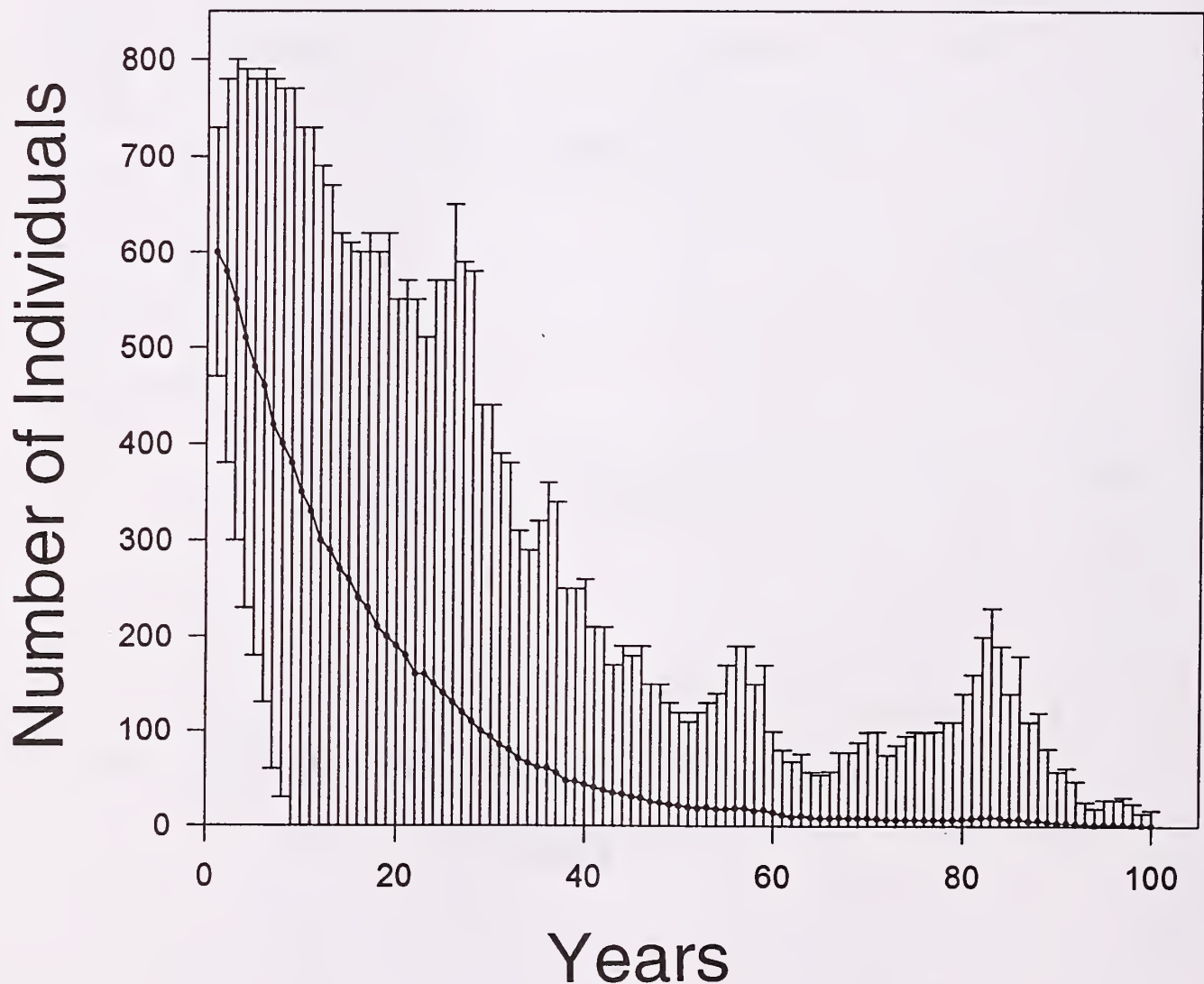


Figure 4. Stochastic simulation of all plots for all years, means, and standard errors.

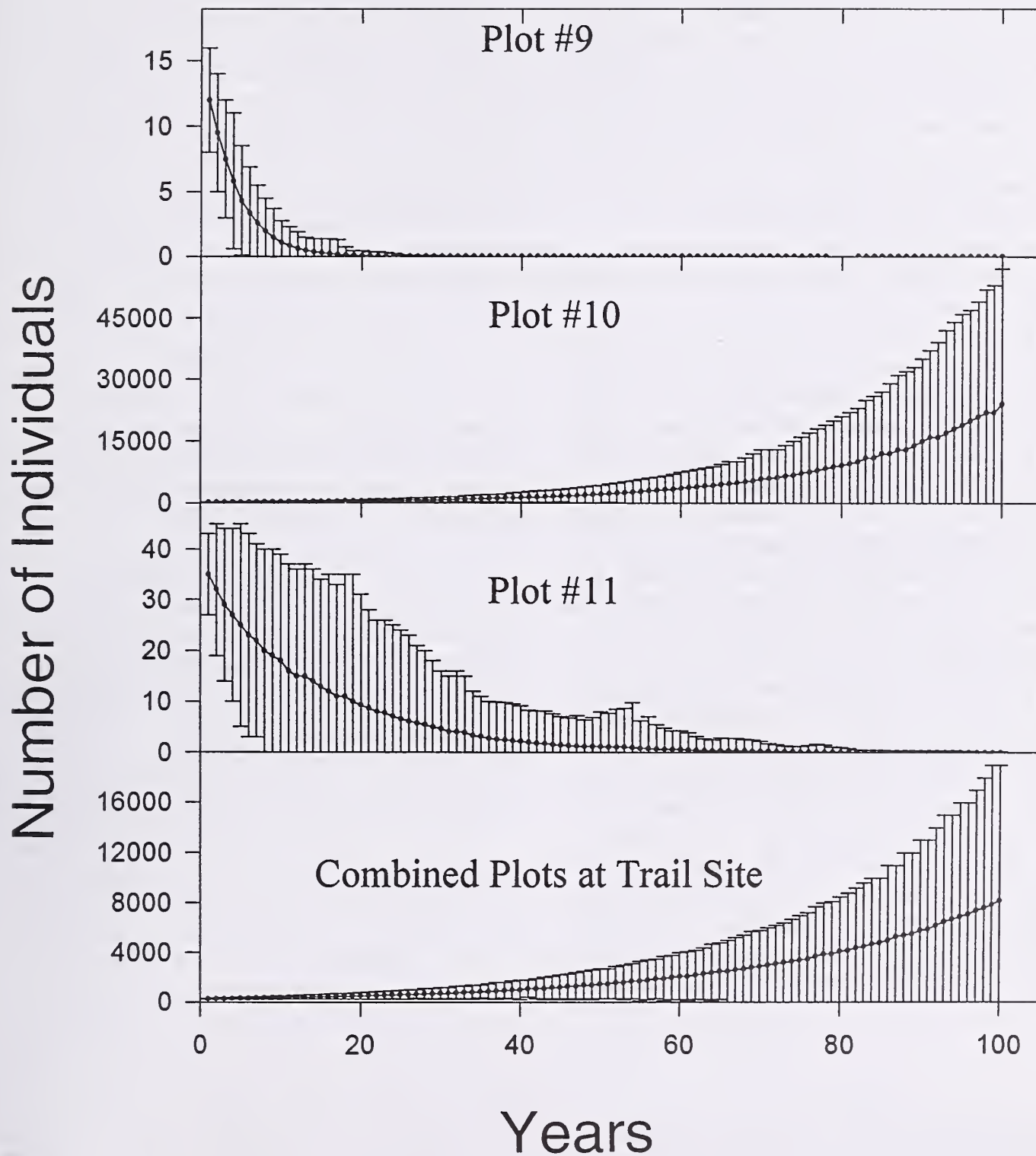


Figure 5. Stochastic simulation of trail plots for all years, means, and standard errors.

lated time. Clearly the risk of extinction under these demographic conditions is high.

Simulations for each plot and for the aggregated data on each plot were calculated for each site. The simulations for all the sites and plots within sites indicated declining populations with the exception of the Trail site. Figure 5 shows that at the Trail site, plot 10 has a net positive growth while plots 9 and 11 show a decline over time. Notice, however, that even for plot 10, the standard error of the mean population size still overlaps zero, implying the possibility of extinction. It is interesting to note that this is the plot with the largest number of individual plants.

One obvious difference in the examination of the extant population and the population viability analysis as calculated here is that the relatively stable behavior we see in the population between 1987 and 1994 is not evident in the simulations. The reason for this can be found in the reproduction tables. Inspection of these tables and the tables of transition probabilities could lead to the conclusion that due to relatively low death rates of the individuals, a short-term stability in population size should be expected (and seen, Figure 2). However, all plots show a relatively low level of recruitment. Even with very low mortality rates populations with little or no recruitment must inevitably decline.

Does this portend extinction of the species? This question is hard to answer. The relatively long life of individual *Pediocactus paradinei*, combined with a variable but occasionally high recruitment rate, could lead to a persistent population with relatively stable numbers. A population of organisms that lives for 10–20 years may need only one or two good years of reproduction out of 10 or 20 poor years to replace itself. A short-term sampling of the population might be likely to miss the good year of reproduction and thus overesti-

mate the risk of extinction. That is entirely possible with *Pediocactus paradinei*. It is interesting to note that during the period of study of this population, the highest levels of precipitation recorded at the North Rim of the Grand Canyon were not correlated with higher recruitment on the plots. This may imply that we have yet to see a truly good year for this population. An inspection of the precipitation records over the last 50 years shows that while the precipitation that has occurred since 1987 is not noticeably high, it has been higher in the past. The only sure way to answer the question is to continue to acquire data on the populations.

The management implications from this work are not obvious. The Trail site, subjected to a low-intensity burn in 1987, shows positive growth in the simulations. The Pasture site, subjected to a hot fire in 1988, shows declining populations and an uncertain future in the simulations. Clearly, the use of prescribed fire must be undertaken with some caution and prescribed fires should be designed to be cool burns. These recommendations do not differ from previous management recommendations (Warren et al. 1993).

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# Demographic Monitoring of *Pediocactus peeblesianus* var. *fickeiseniae* on the Arizona Strip

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**Abstract:** Fickeisen plains cactus is a rare, narrow endemic restricted to Kaibab Limestone derived soils. The populations are disjunct. The plant is rare and is vulnerable to population decline with damage to a small area of habitat. It is a category 1 candidate for listing as threatened or endangered. Man-induced threats include off-highway vehicles and livestock trampling. The Bureau of Land Management established two monitoring plots and two transects at four places across the Arizona Strip to measure size, mortality, fruiting, and recruitment, and to map the cacti in the plots. The data have been gathered since 1986. Plants in the 10–20 mm and 20–30 mm wide size categories are predominant. The populations seem stable. Herbivory was the largest cause of mortality in dry years. Fruiting has been best in wetter than average seasons.

## Introduction

Fickeisen plains cactus, *Pediocactus peeblesianus* (Croizat) Benson var. *fickeiseniae* Benson (= *Navajoa fickeisenii* Backeberg, *Toumeyia fickeisenii* Earle, and *Pediocactus fickeisenii* Benson) is a category 1 species. It is an endemic that grows on Kaibab Limestone derived soils; however, the soils are far more widespread than is the cactus. Fickeisen plains cactus is a very small, unbranched cactus that shrinks into the gravelly soils after flowering and fruiting. Mature plants are 3 inches tall and 1.5 inches in diameter, but the majority of the stem is underground. Stems have tubercles that form a spiral pattern around the plant. The spines are corky with the central spine being 3/8-inch long, ashy white, and pointed upwards. Flowers are cream colored to yellow.

The geographic range of this cactus occurs from Mainstreet Valley on the west to Marble Canyon on the east, Mohave and Coconino counties, Arizona. It occurs in some abundance in Mainstreet Valley where a dense population occurs, but is also found in scattered populations in the general areas of Hurricane Valley, Hurricane clifftops, Clayhole Ridge, Sunshine Ridge, and Houserock Valley. It grows in shallow soils near to exposed layers of Kaibab Limestone on the margins of canyons or well-drained hills in Navajoan desert or Great Plains grassland at 4000–5000 feet (Gierisch 1987, Phillips et al. 1979).

The predominant vegetation in its habitat includes desert grassland, sagebrush, and shadscale vegetation types. Associated species include blue grama (*Bouteloua gracilis*), black grama (*Bouteloua eriopoda*), galleta (*Hilaria jamesii*), fourwing saltbush

(*Atriplex canescens*), snakeweed (*Gutierrezia sarothrae*), and sagebrush (*Artemisia tridentata*). Brady pincushion cactus (*Pediocactus bradyi*) also occurs close to this species.

Current Bureau of Land Management (BLM) policy is to conserve this cactus to preclude it being listed. Under the Arizona Native Plant Law it is classified as highly safeguarded—no collection is allowed. Under the State Rank by Arizona Heritage data management system, this plant is S-2, which signifies that it is rare. It contributes diversity to the plant communities in which it occurs. During drought, it and other succulents become food for small mammals, thus contributing to the food chain. As with rare items generally, some individuals wish to possess it. Collection pressure to date appears light on the Arizona Strip—studies and observation have not demonstrated any collection (see Table 3). The value of this taxon to humans is its rareness, its potential contributions to the human race, and the ecosystems in which it occurs.

This paper describes results from 9 years of demographic monitoring studies.

## Methods

Annual measures on size/age structure of the cactus were recorded from 1986 to 1994 at the four localities across the Arizona Strip on lands managed by the BLM. The size structure trend is measured in two plots—Dutchman and North Canyon. There are also two transects that are read annually at two locations—Clayhole and Sunshine. These transects give less accurate data, but are nevertheless accurate enough to detect changes in size

structure similar to that shown in plots. The transect search method is less accurate because smaller sized cactus go undetected. Other small populations in outlying habitat are marked with rebar. In general, this is a cluster of cactus around a rebar. Sites with rebar are visited once every 5 to 10 years to determine whether the cactus are still present at the sites.

The cacti were tagged, mapped, and measured at all plots and transects. Mortality was noted, and if possible, the cause of mortality was recorded. Fruiting was noted if monitoring occurred at the proper time of the year.

### Results

For the Dutchman Plot (Table 1, Figure 1), where the densest population occurs, the data demonstrated fluctuations in numbers. The first-year numbers of 'Fick' are much lower than the remainder of the years (Table 1, Figure 1), because many were missed when plots were first established. Good winter precipitation seems to influence the population growth positively.

The first 3 years show no very small cacti (0–4.9 mm), but then from 1989 through 1992, good recruitment of very small cacti occurred. In all years, the plot was dominated by the size class of 10–20 mm, which is the size when fruiting begins to occur. All size classes except 0–4.9 mm are well represented in the plot. The site is covered with grass, and the 0–4.9 mm size may have been difficult to observe. In years when it was dry for a few months before monitoring, the small cacti usually retracted and may have been missed during monitoring (Table 1).

Mortality data for the Dutchman Plot demonstrated that natural causes—such as rodent depredation, old age, drought, and rot—were responsible for most deaths of this cactus in the plots. There was one death since 1988 that could be attributed to cow hoof placement (see Mortality, Table 2).

The Clayhole transect size structure (Table 1) showed many larger, and thus older, individuals (20–30+ mm). Only a single plant in the 0–20 mm size class was seen in 8 years, but searches were not intensive for small cactus. This population is scattered over a mile. Few cacti died in the transect (Table 2) and most mortality was due to depredation and rot. Three cacti were killed by livestock trampling in 1988.

The North Canyon plot size structure (Table 1, Figure 2) was dominated by the 10–30 mm size class. Although the very small cacti (0–4.9 mm)

Table 1. Size structure (measure of width in millimeters) (USDI 1994).

Year	0–4.9 mm	5–10 mm	10.1–20 mm	20.1–30 mm	30.1+
<b>Dutchman Plot (33 ft wide x 66 ft long)</b>					
1986	0	9	4	6	2
1987	0	22	42	30	13
1988	0	15	36	35	16
1989	10	53	72	40	10
1990	7	37	76	40	20
1991	23	51	69	35	16
1992	19	45	95	49	11
1993	0	27	80	50	11
<b>Clayhole Transect (follows Clayhole Ridge for .25 mile)</b>					
1986	0	1	1	15	5
1987	0	0	4	15	4
1988	1	6	4	19	6
1989	0	0	3	16	12
1990	0	0	3	15	14
1991	0	3	4	25	5
1992	0	7	5	23	9
1993	0	8	6	11	9
<b>North Canyon Plot (27 feet x 27 feet)</b>					
1986	0	5	3	2	4
1987	0	1	3	10	2
1988	0	6	8	10	3
1989	0	2	13	10	3
1990	0	2	8	10	13
1991	0	7	10	14	5
1992	0	0	0	4	3
1993	0	4	2	2	5
1994	0	1	7	4	4
<b>Sunshine (cluster around rebars)</b>					
1986	0	0	2	4	0
1987	0	1	2	6	3
1988	Data not taken				
1989	0	0	0	6	2
1990	0	2	9	18	4
1991	0	0	19	14	10
1992	0	6	15	17	6
1993	0	6	6	9	11
1994	0	12	10	9	4

were sought, they were not found. But because timing is crucial for finding these very small cacti, they may be visible only rarely. There are quite a few in the 5–10 mm size. This plot's population took a beating in 1992, when depredation occurred to 26 cacti. The cacti are just beginning to recover.

The Sunshine population transect showed a similar size structure pattern as at Clayhole. Two



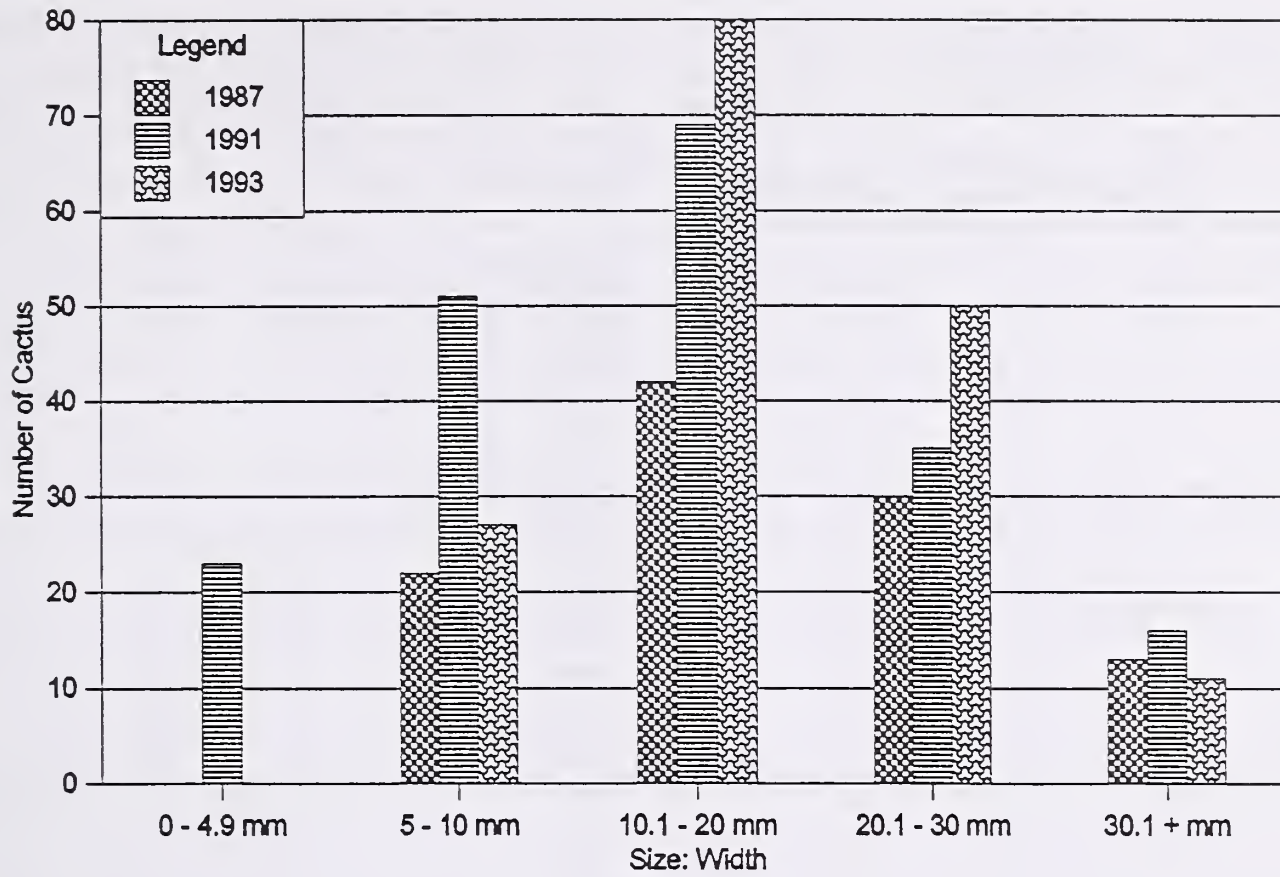


Figure 1. Dutchman Plot size structure.

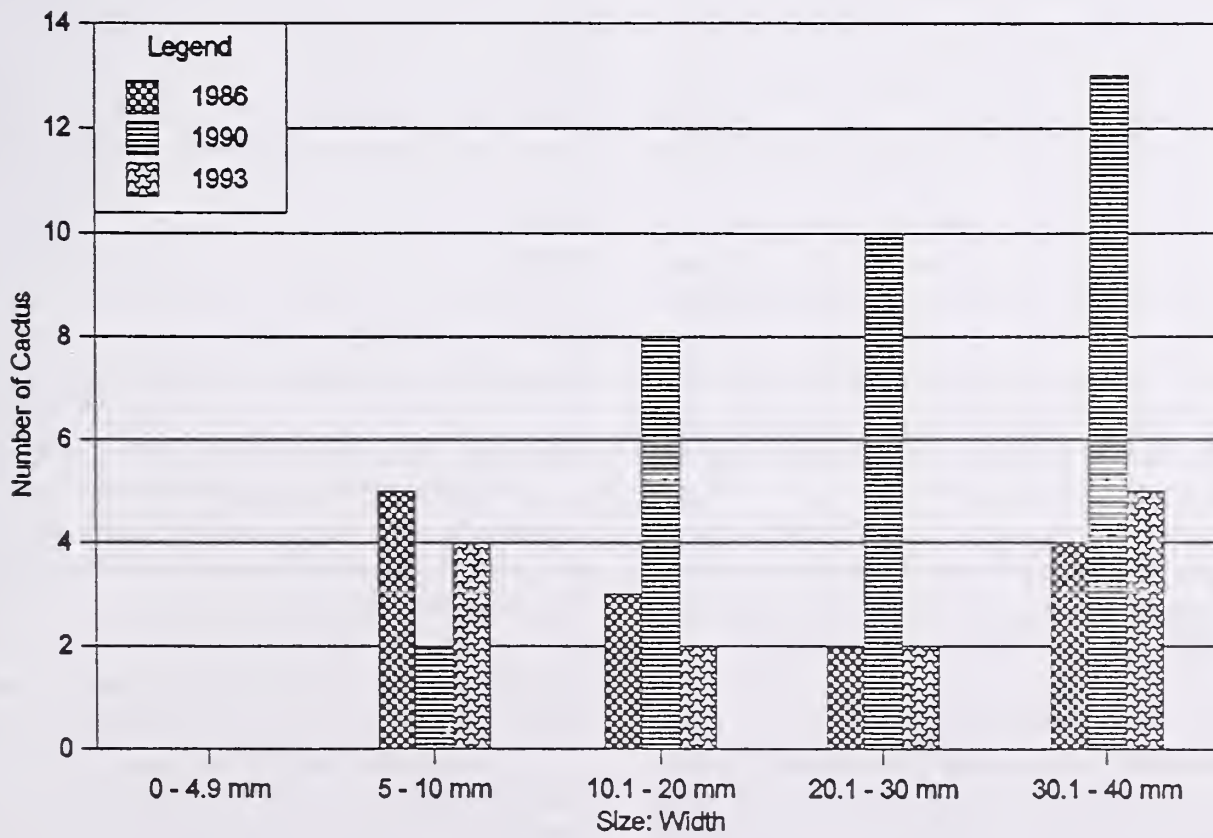


Figure 2. North Canyon Plot size structure.

Table 2. Mortality, on each study area, was noted starting in the year shown (USDI 1994).

Year	Off-Highway Vehicles	Livestock Trampling	Collections	Natural
<b>Dutchman Plot</b>				
1988	0	0	0	7
1989	0	0	0	0
1990	0	0	0	4
1991	0	0	0	26
1992	0	0	0	5
1993	0	1	0	0
<b>Clayhole Transect</b>				
1988	0	3	0	5
1989	0	0	0	0
1990	0	0	0	4
1991	Data not taken			
1992	0	0	0	2
1993	0	0	0	3
1994	0	0	0	3
<b>North Canyon Plot</b>				
1986	0	0	0	0
1987	0	0	0	1
1988	0	0	0	0
1989	0	0	0	1
1990	0	0	0	1
1991	0	0	0	1
1992	0	0	0	26
1993	0	0	0	0
1994	0	0	0	1
<b>Sunshine</b>				
1989	0	1	0	3
1990	0	0	0	1
1991	0	0	0	3
1992	0	0	0	13
1993	0	0	0	5
1994	0	0	0	5

plots were established in 1993 and 1994, and the number of 5–10 mm cacti made more of a showing. In fact, there were more in the 5–10 mm size group than the other categories as more intensive searches in small areas enabled the location of the small cacti. While mortality in this transect was mainly from natural causes, one cactus was killed in 1989 by a hoof placement.

Fruit production ranged from 18 to 80 percent of the tagged cactus (see Table 3). The amount of seed production seems to be controlled by the weather (see Figures 1 and 2, Tables 4 and 5).

### Discussion

*Pediocactus peeblesianus* var. *fickeiseniae* is a rare, narrow endemic, restricted to Kaibab Limestone derived soils. The disjunct populations occur in clusters with miles between clusters. Because of this rare and disjunct status, it is vulnerable to depopulation if damage occurs in a small area

Table 3. Percent of tagged plants flowering and fruiting.

Year	Flowering	Fruiting
<b>Dutchman</b>		
1989	60%	55%
<b>Clayhole</b>		
1990	29%	–
1991	–	49%
1992	–	34%
1993	–	18%
1994	–	29%
<b>North Canyon</b>		
1989	–	40%
1990	18%	–
1991	–	61%
1992	0	0
1993	–	50%
<b>Sunshine</b>		
1989	–	62%
1990	Retracted	All Retracted
1991	79%	79%
1992	–	–
1993	–	28%
1994	–	17%

The sizes of the cactus and corresponding amounts of fruit produced from all plots and transects are reported below from years 1989 through 1994 (USDI 1994).

Width Class (mm)	Average Fruit Produced	Range of Fruit Produced	Total Cactus Fruiting
0–9.9	0	0	0
10–19.9	1	1	8
20–20.9	1.37	1–3	77
30–30.9	1.69	1–4	107
40–40.9	2.47	1–5	52
50+	3.60	2–5	3

where the plant grows. The plant seems to have a small reproductive capacity (low seed production), so that any great increase in number is prevented even during favorable weather. In the study plots or transects, a big increase in numbers occurred 2 to 3 times within the last 9 years. So with a small population, low reproduction potential, and its environment, it appears this plant will remain rare.

This species is maintaining its numbers on the Strip. The size structure at the Dutchman and North Canyon plots showed dynamic changes in numbers. At Dutchman, the very small (0–4.9 mm) plants had peaks in 1991 and 1993. In all plots and transects, the larger cacti are predominant and are

Table 4. Rainfall 1 year previous to monitoring of the Dutchman Plot.

	Fall	Fall Avg.	Winter	Winter Avg.	Spring	Spring Avg.	Summer	Summer Avg.	Total	Total Avg.
1987	1.84	1.75	2.32	2.91	2.35	2.43	3.06	3.53	9.57	10.61
1991	.37	1.53	2.81	2.19	.90	2.01	1.53	3.29	5.61	9.02
1993	1.91	1.52	5.84	2.38	3.45	2.16	2.02	3.24	13.22	9.30

Table 5. Rainfall 1 year previous to monitoring of the North Canyon Plot.

	Fall	Fall Avg.	Winter	Winter Avg.	Spring	Spring Avg.	Summer	Summer Avg.	Total	Total Avg.
1986	1.60	1.46	2.12	1.71	.53	1.44	2.34	3.00	6.59	7.61
1990	.12	1.46	2.30	1.71	*	1.44	5.35	3.00	7.77	7.61
1993	.63	1.46	4.37	1.71	.93	1.44	1.20	3.00	7.13	7.61

\*Vandalized

more stable. The dominance of the 10–20 mm size class at Dutchman seems to indicate a younger population. Most mortality was by natural means with a small number killed by man-induced impacts—livestock. The lack of the very small plants (0–4.9 mm) may or may not be a problem, as this size is hard to find. The other size classes have had recruitment through the years from somewhere, so the small size classes are present, but difficult to locate. The fluctuation in number appears to be weather related (see Figures 1 and 2, Tables 4 and 5). The larger plants (30+ mm) retract underground less frequently, but some retract in long dry spells. Overall, this cactus appears to be maintaining itself in all its original (since 1978) range and most mortality is natural with a small impact from livestock.

Most or all of this species' range, since the 1970s, has not been disturbed. Populations are located next to the Mainstreet road and the Mt. Trumbull road. Construction of these roads could have disturbed long-past populations.

The major potential threats to this cactus from man-induced activities are off-highway vehicles and livestock trampling. However, the resource management plan of 1992 restricting vehicle activity to existing roads and trails should reduce the threat from off-highway activity (USDI 1992). There are also ranger patrols to help reduce off-road vehicle activity. Predation by rodents and old age are the largest causes of death for this cactus.

Most of these mortality factors are probably natural for any population of cacti. Whether the occasional high numbers of cacti eaten by herbivores is caused by livestock grazing, rodents foraging during drought periods, or the drought reducing the supply of preferred forage of herbivores, cannot be ascertained. Although the mortality data demonstrates some disturbances from livestock trampling, backcountry plots (North Canyon), which have little livestock grazing around them, also have experienced heavy depredation. The healthiest and densest population of this cactus (Dutchman) occurs within a quarter of a mile of a reliable livestock water tank. The livestock use the area in the spring and fall of most years. This population has also experienced heavy depredation in dry years and the cactus thrived in other years. No preponderance of evidence demonstrates that man-induced depredation is an imminent threat to this species.

Currently, through state law and policies and BLM policy, there are adequate regulatory mechanisms in place to conserve the species. BLM conserves this species under a policy stated in Manual 6840: *The BLM will carry out management, consistent with the principles of multiple use, for the conservation of candidate species and their habitats and will ensure that actions authorized, funded, or carried out do not contribute to the need to list any species as T/E.* The will to carry out the mechanism also exists. Monitoring of this species is in place and we are increas-

ing our ability to detect trends in size structure, mortality, fruiting, and recruitment of the species. Projects that could injure the species' population are precluded.

This species needs management as a sensitive and rare plant. The population study of this plant shows stability with years of increase and years of decrease. It is recommended that a conservation agreement be prepared to develop consensus on practices to maintain and improve this species' population to prevent listing.

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# Morphological Variation Within and Among Four *Cimicifuga arizonica* Populations

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**Abstract:** Arizona bugbane (*Cimicifuga arizonica* Wats.), a rare plant with narrow habitat requirements, exists only in four small population areas in Arizona. Development of a successful conservation program for this species is restricted by a lack of biological, ecological, and genetic information. Maintenance of genetic diversity is important for successful species conservation, yet Arizona bugbane populations have likely suffered genetic diversity losses due to repeated bottlenecks resulting from flood-caused mortality in their riparian habitats. Results of research comparing coarse plant morphology within and among populations at four sites in the Oak Creek population area show little significant variation within or between populations. This apparent lack of variation at the phenotypic level will be further evaluated at the genetic level through the use of electrophoretic isozyme analysis.

## Introduction

Arizona bugbane (*Cimicifuga arizonica* Wats.) is a rare, herbaceous, perennial plant in the *Ranunculaceae*. It is the only species of *Cimicifuga* in Arizona. Four population areas of Arizona bugbane have been located in Arizona and these are found only in the Mogollon Rim area of central Arizona, all on Forest Service land (Phillips and Popowski 1995).

Arizona bugbane has very large compound leaves, and flowering plants can reach heights of 2 meters. The stem of the plant terminates in long slender racemes. The flowers on these racemes are small and white with no petals and up to 35 can be found in bloom on any one raceme (Figure 1) from July to mid-September. The flowers are pollinated by three species of bumblebees, with the peak flowering in early August coinciding with the peak population abundance of the bumble bee (Pellmyr 1985). The flowers develop into short pedicellate dehiscent follicles in August and September (Figure 1).

Arizona bugbane grows in small, scattered clumps or as a continuous understory in the ecotone between coniferous forests and riparian areas. It occurs near perennial or intermittent streams, usually between 1829 and 2529 meters in elevation. The habitat requirements for this species appear to include sites of high humidity with moist, rich, fertile soils that are high in humus (Phillips and Popowski 1995). In Arizona, these conditions are typically created under the closed canopies of mixed coniferous forests (*Pseudotsuga menziesii* Mirb. Franco var. *glauca* Beissn. and *Abies concolor* Lindl. & Gord. overstory; *Cornus*, *Alnus*, and *Acer*

mid-canopy) with deciduous shrub understories and are most often found in the riparian areas of steep, narrow canyons. These habitat conditions tend to isolate populations of Arizona bugbane from each other.

As a result of Arizona bugbane's limited geographic range, combined with the very narrow habitat restrictions within its range, it is listed as a Forest Service sensitive species and is currently a category 1 candidate for federal listing as threatened or endangered under the Endangered Species Act (Federal Register 50, 6195, 1990). In addition, a conservation assessment and strategy plan has been prepared for the Coconino and Kaibab national forests by Phillips and Popowski (1995). Successful conservation programs require a thorough understanding of a species' biology and distribution and should include a genetic management program as well (Meffe and Carroll 1994). Unfortunately, little is known about Arizona bugbane other than general information regarding habitat descriptions and where the populations are located, along with limited information on pollination ecology (Pellmyr 1985) and germination (Marschinski, personal communication 1994).

Effective genetic management programs include protection and monitoring of between as well as within-population genetic diversity (Meffe and Carroll 1994). The greatest number of options for species' conservation can be created by retaining genetic diversity through the maintenance of as many populations as possible, while still preserving natural patterns of genetic diversity within and between these populations. This genetic diversity provides the flexibility needed to evolve



Figure 1. *Cimicifuga arizonica* (from Phillips and Popowski 1995).

solutions to problems such as outbreeding depression (decreased fertility and/or survival of offspring of hybrids between wild and captive populations) or environmental change (Echelle 1991). Small, isolated populations such as Arizona bugbane tend to lose genetic variation over time due to founder effects (few individuals establish a new population), demographic bottlenecks (severe temporary reduction in population size), genetic drift (random loss of alleles [alternative forms of genes]), and the effects of inbreeding (mating of individuals related by common ancestry) (Hartl and Clark 1990). Bottlenecks intensify genetic drift and are considered a major cause of loss of genetic variation in populations (Brown and Moran 1981, Echelle 1991). The genetic diversity of Arizona bugbane has likely been influenced by these factors due to catastrophic population loss from flooding in this species' canyon-bottom habitat. The 1993 floods decimated three of five populations that had been located prior to the floods and left other populations with significantly fewer plants. Other threats such as heavy spring rain, snowmelt, road and trail construction, grazing, and recreation also put entire populations at risk (Phillips and Popowski 1995).

### Purpose and Objectives

The purpose of the project reported here was to initiate research that will contribute to the conservation of Arizona bugbane through improved understanding of the genetic structure of the species. The specific objectives were to:

1. Quantify coarse morphological variation between and within populations of Arizona bugbane.
2. Use this phenotypic information to develop a sampling scheme for subsequent isozyme-based quantification of genetic diversity within and between populations of Arizona bugbane.
3. Use the phenotypic and genotypic information in the development of conservation management strategies.

Only the first objective outlined above has been completed thus far. Therefore, the results reported below relate only to morphological variation found within and between four populations in the Oak Creek population area.

### Methods

Four sites within the Oak Creek drainage population in the Coconino National Forest were selected for this initial study. Figure 2 illustrates

the general location of each site. Populations were sampled at two locations along the West Fork of Oak Creek and populations were also sampled in Pumphouse Wash and in Fry Canyon. Populations were measured in Lower West Fork on July 20, in Fry Canyon on August 3, in Pumphouse Wash on August 9, and in Upper West Fork on August 23. The sampling locations were all relatively isolated from each other by distance or by geography. Populations were inventoried within each sampling area and individuals from the largest continuous populations at each site were selected for inclusion in the morphological analyses. Plants from two large continuous populations were randomly selected and measured at each of the three sites within the Upper and Lower West Fork and Pumphouse Wash locations. Fry Canyon had three large continuous populations from which plants were randomly selected for measurement.

Data collected from each plant included (a) number of basal leaves originating from the base of the stem at the ground line, (b) stem caliper (mm) measured at 10 cm above the ground surface, (c) height (cm) measured from the ground surface to the leaves furthest up on the stem, and (d) number of racemes. Attempts to quantify the number of flowers per raceme were abandoned due to excessive handling of the reproductive structures.

The data were analyzed within a site (e.g., within Fry Canyon) by comparing individuals from the continuous populations (three populations in the case of Fry Canyon) to each other. Variation between sites was analyzed using within-site population means (e.g., means from the three Fry Canyon populations were compared to population means from the other locations). Analyses of variance and mean separation procedures were utilized to evaluate within and between-site morphological variation. In addition, all analyses were conducted separately for flowering versus nonflowering plants and all analyses used an alpha level of 0.05.

## Results

### *Within-Site Variation*

Results of the within-population analyses of variance are summarized in Table 1. In general, most of the analyses indicated no significant differences between populations within a site for both flowering and nonflowering plants. With the exception of the Upper West Fork flowering plants, Chi square analyses of the distribution of the significant differences indicated that they were

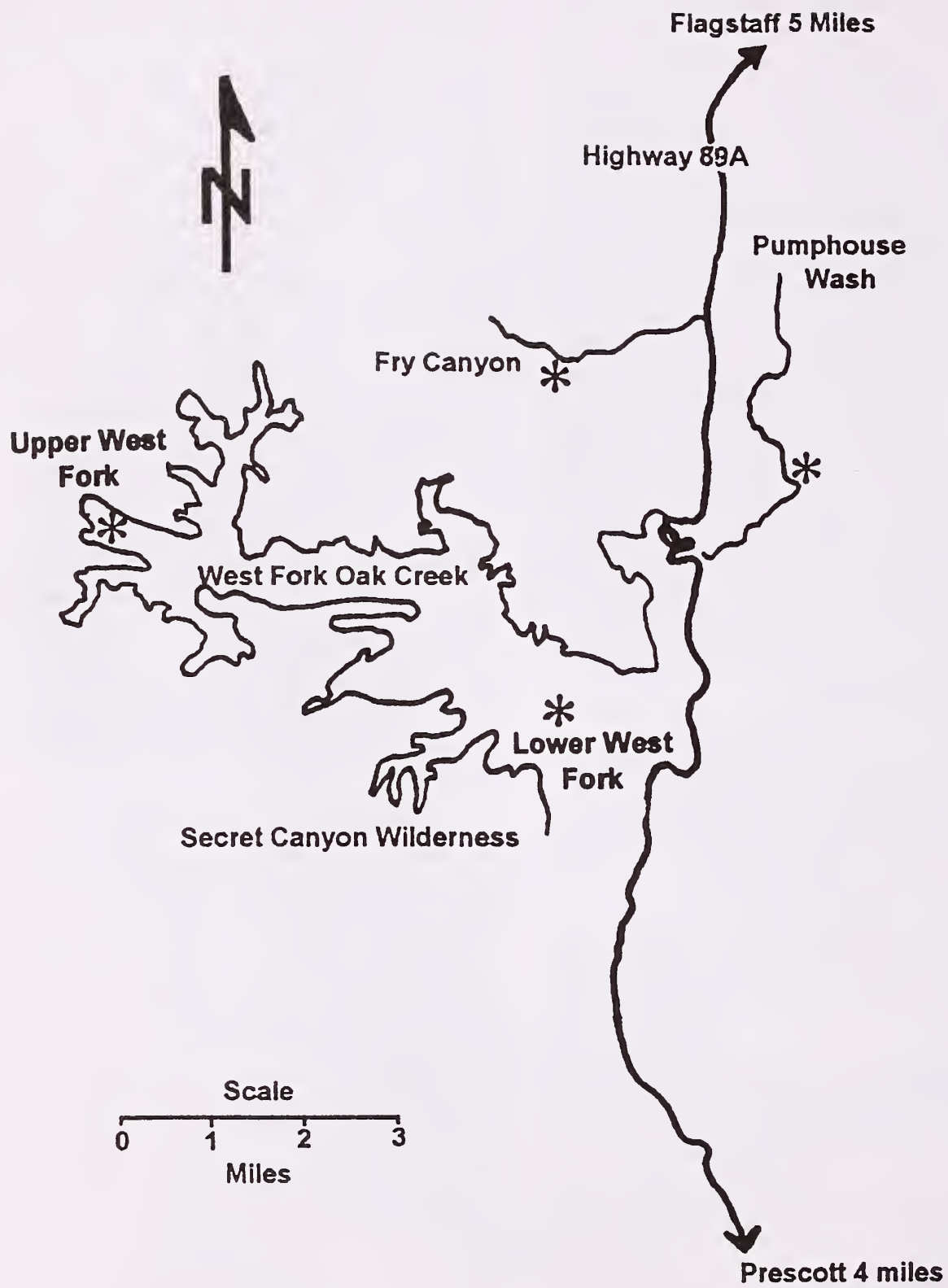


Figure 2. Approximate Oak Creek Canyon locations of *Cimicifuga arizonica* populations sampled in this study (locations are indicated by \*).



Table 1. Summary of analyses of variance of morphology data for within-site variation for four *Cimicifuga arizonica* locations in the Oak Creek, Arizona drainage system.\*

Trait	Lower West Fork	Fry Canyon	Pumphouse Wash	Upper West Fork
Flowering plants				
Height (cm)	ns	0.05	ns	0.01
Caliper (mm)	ns	ns	0.05	0.01
Number basal leaves	ns	ns	ns	0.01
Number racemes	ns	0.01	ns	0.01
Nonflowering plants				
Height (cm)	0.01	ns	ns	ns
Caliper (mm)	ns	0.05	ns	ns
Number basal leaves	ns	ns	0.01	ns

\*ns = Within-site variation not significant; 0.05 and 0.01 = within-site variation significant at alpha = 0.05 and 0.01, respectively.

randomly distributed among sites and traits. The exception to this random distribution occurred with the Upper West Fork flowering plants. These two populations differed significantly from each other for all traits measured.

Analyses of flowering versus nonflowering plants within a site indicated that flowering plants had larger stem calipers and were taller than the nonflowering plants. These differences were significant at all sites. In addition, flowering plants had significantly more basal leaves than nonflowering plants at the Lower and Upper West Fork sites. The number of basal leaves did not differ significantly among flowering and nonflowering plants in the Pumphouse Wash and Fry Canyon drainages (see Figure 3).

#### *Between-Site Variation*

Number of basal leaves ranged from a low of 1–8 for flowering plants and up to 10 for nonflowering plants. The overall average number of basal leaves was 2.4 ( $\pm 0.7$ ) for flowering plants and 2.0 ( $\pm 0.8$ ) for nonflowering plants. The mean number of basal leaves at each of the four sites, illustrated in Figure 3a, did not differ significantly between sites for flowering plants and nonflowering plants.

Basal caliper ranged from 2 to 15 mm for flowering plants and 1 to 11 mm for nonflowering plants. The average flowering plant caliper was 8.0 ( $\pm 1.5$ ) mm and differences between sites illustrated in Figure 3b were not significant. The average caliper for nonflowering plants was 4.0 ( $\pm 1.2$ ) mm. Differences between the Lower West

Fork, Fry Canyon, and Pumphouse Wash populations illustrated in Figure 3b were not significant. However, the Upper West Fork nonflowering plants had significantly larger stem calipers than populations at the other three sites.

Flowering plant heights ranged from 26 to 186 cm and 2 to 55 cm for nonflowering plants. The average flowering plant height was 108 ( $\pm 15$ ) cm and differences among sites illustrated in Figure 3c were not significant. The average height of nonflowering plants was 36 ( $\pm 10$ ) cm and differences between the Lower West Fork, Fry Canyon, and Pumphouse Wash populations illustrated in Figure 3c were not significant. However, the Upper West Fork nonflowering plants were significantly taller than populations at the other three sites.

The number of racemes per plant ranged from 1 to 16 with an overall average of 3.5 ( $\pm 1.3$ ). The differences between sites illustrated in Figure 3d were not significant.

#### Discussion

Flowering plants were larger than nonflowering plants. Most of the nonflowering plants appeared to be vegetative in origin, sprouting from the rhizome of the main flowering stem and likely younger than the flowering stem. It is possible that the nonflowering plants lacked the maturity to develop floral structures, or they may represent nonreproductive structures throughout their life cycle. The lack of significant coarse morphological differences between and within sites indicates low phenotypic variation, at least at the level measured

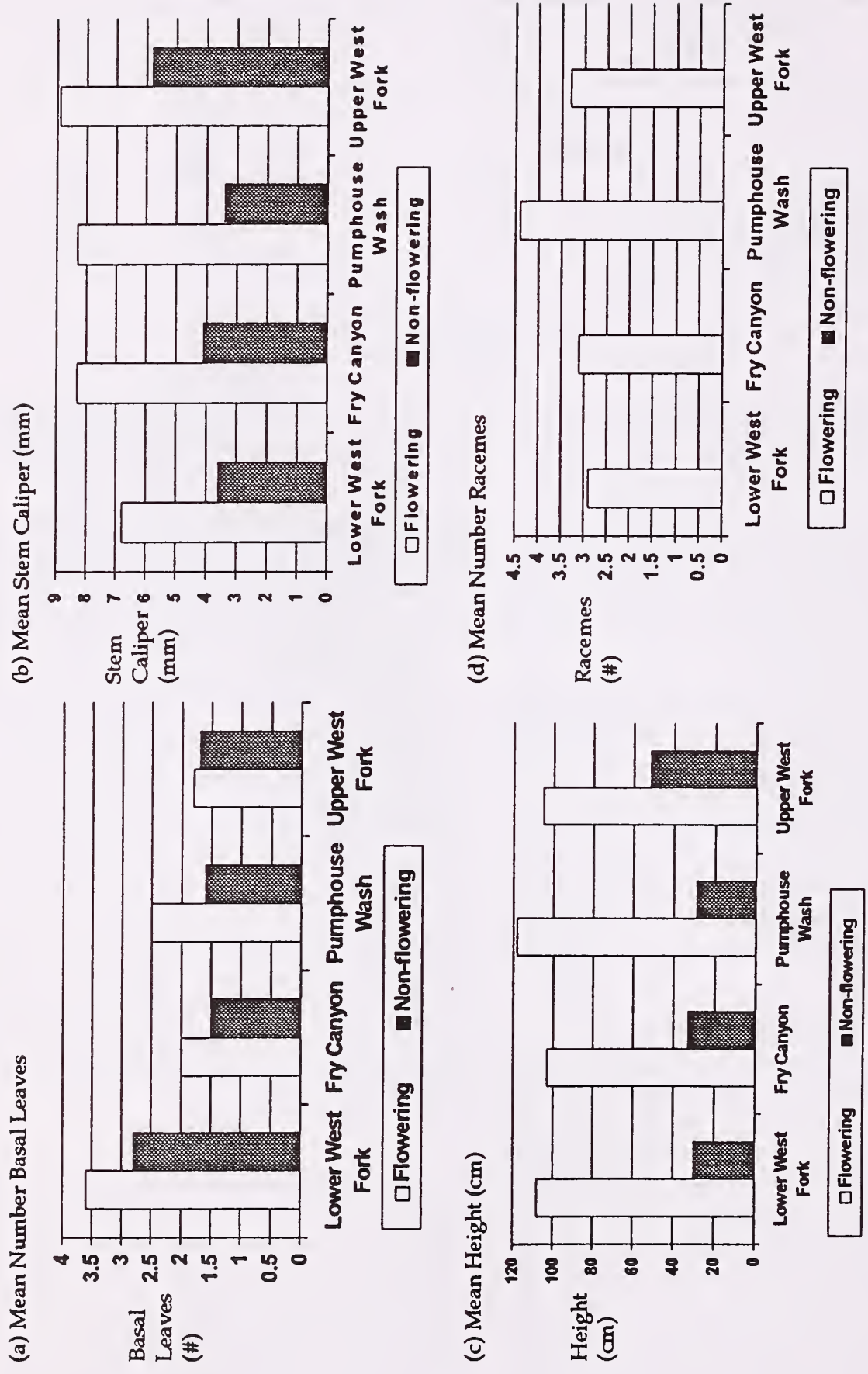


Figure 3. Population mean number of (a) basal leaves, (b) caliper, (c) height, and (d) number of racemes for flowering and nonflowering *Cimicifuga arizonica* at four sites in Oak Creek Canyon, Arizona.

in this study. It is somewhat surprising that genetic drift and/or bottlenecks have not resulted in greater differences between sites. There may not have been enough time to allow for drift, or the lack of differences may be due to pollen exchange between the four sites sampled in this study. In addition, there may be variation that is occurring at a finer morphological level than was surveyed in this study.

Another likely cause for the lack of differences found between sites is related to the habitat in which Arizona bugbane was found. The micro-environments of the habitats of all four sites studied were very comparable and since the traits examined were related to biomass, similar environmental conditions resulted in plants of similar size. The only consistent significant differences were associated with the Upper West Fork site and this was the only site where the habitats of the two populations differed. One population was on a north-facing slope, while the other population was on a south-facing slope. The south-facing site was protected enough to maintain high soil moisture levels, but the plants receive more sunlight and were able to accumulate more biomass than those on the north-facing slope, resulting in within-site differences. In addition, the overall mean caliper and height of the Upper West Fork nonflowering plants was strongly influenced by the larger-sized plants in the population on the south-facing slope. When the population on the south-facing slope was omitted from the between-site analyses, the significant differences in caliper and height disappear.

Whether or not the low phenotypic diversity is reflected in the genetic makeup of these plants will be examined in the next phase of this research.

Isozyme analyses will be used to quantify levels of heterozygosity between and within these four sites. In addition, further morphological research will include the West Clear Creek area as well as the populations in the Bill Williams area. It would be very unlikely that pollen exchange is occurring between these sites and the Oak Creek population area, and the results should yield further clues regarding the phenotypic and genetic variability of *Cimicifuga arizonica* populations in Arizona. If there is as little genetic diversity as there is phenotypic variation in the Oak Creek populations, conservation strategies to improve diversity should be considered.

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# Analysis of *Rumex orthoneurus*, a Rare Species in Arizona, Using RAPD Markers and Polymorphisms in *rbcL*

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**Abstract:** We examined genetic variation in five populations of the rare *Rumex orthoneurus* and one population of a widespread sister taxon, *R. occidentalis*. Populations were analyzed using random amplified polymorphic DNA (RAPD) markers amplified from total cellular DNA extracts to determine the genetic variation within and among populations. Restriction endonuclease digestions of chloroplast DNA (cpDNA) amplification products were also performed in an attempt to identify distinctive restriction site or fragment length differences. Thus far, these molecular data suggest that *R. orthoneurus* and *R. occidentalis* are genetically distinct and variation occurs among the different Sky-Island populations. A lack of variation in RAPD banding patterns within populations of *R. orthoneurus* suggests long-term isolation, a conclusion that is corroborated by cpDNA data.

## Introduction

Studies of the Chiricahua or Blumer's Dock, *Rumex orthoneurus* Rech. f. (Polygonaceae), exemplify how problems with taxonomic delineation can be critical for the status and preservation of a rare plant. In addition, southern Arizona presents a unique opportunity to study the population genetics of taxa whose habitats have become fragmented through geologic time. The Sky-Islands of the Madrean Archipelago represent geographically separated but comparable habitats that contain reproductively isolated populations of rare plants, such as the Chiricahua Dock, *R. orthoneurus*, and its commonly occurring close relative, *R. occidentalis* Wats. This species manifests two common issues in conservation biology: monophyly and taxonomic ranking, and the consequences of rarity.

## Rarity in Plants

As a species becomes rare, several problems that are associated with small population sizes can arise. Genetic drift, the random loss of variation resulting from erratic mating patterns in small populations, can reduce genetic variation and therefore environmental adaptability in the affected populations. Instability due to stochastic events may lead to an increase in the probability of extinction. Inbreeding, as a result of limited numbers of individuals remaining in the population, may lead to inbreeding depression effects such as decreased fitness and/or fecundity. Managing agencies, such as the U.S. Fish and Wildlife Service, historically advocate protection by isolation or captive propagation, which may increase the

genetic consequences of rarity. Captive breeding often *lowers* the effective population size by increasing the number of individuals derived from only a few sets of parents. Translocation to new habitats (especially in the case of plants) may compound the decline of genetic variability and population structure by introducing *new* selective environmental pressures, as organisms may become locally adapted to a particular environment. Selection in combination with a reduction in population size (by human or stochastic influences) could eliminate adaptive variability; introducing endangered plants to an unnatural environment might disrupt evolutionary processes. Another way that responsible agencies may try to increase levels of genetic variability is by combining plants from different populations. If this species is adapted to low genetic variability, the population may suffer what is known as outbreeding depression, which results from crossing organisms that are too distantly related (Templeton 1986, Purdy et al. 1994).

The field of conservation biology must take into consideration knowledge about the genetic distinctiveness of the endangered species or taxon. A way to elaborate on this issue is to examine the genetic structure of the population(s) and to also include a systematics approach involving the relationship or phylogenetics of that taxon and its close relatives. Information regarding close relatives can establish the significance of the degree of genetic diversity and distinctiveness of the populations at stake. This can also allow us to determine whether they are recent derivatives from common species or simply relict populations.

## Description of *Rumex*

*Rumex orthoneurus* is a monocious perennial that is capable of clonal propagation. It is restricted to montane riparian habitats along stable streams. Each geographic population or "metapopulation" (Levins 1970) is further divided along perennial streams. Understanding the degree to which populations are subdivided may help determine the overall health of each metapopulation. The habitats of *R. orthoneurus* are threatened by numerous human activities, such as recreation, logging, cattle, development, or watershed mismanagement (Rutman 1992), each of which by interrupting gene flow may lead to further isolation. In addition, this taxon closely resembles the widespread species *R. occidentalis*, which raises a question about the taxonomic significance of the five populations of *R. orthoneurus*.

The morphological characters of many species of *Rumex* overlap (e.g., *R. orthoneurus*, *R. hymenosepalus*, *R. nematopodus*, *R. densiflorus*, *R. conglomeratus*, *R. crispus*, *R. obtusifolius*, *R. violascens* and *R. fueginus*), such that botanists have difficulty identifying specimens without knowledge of the locality of the plants. The best characters for identifying a specimen are those of the valves, but even these are often confusing and require the plants to be both mature and flowering. Molecular characters could be of great utility in determining the true relations among the species. However, when using molecular data for phylogenetic analyses, outgroups must be included to provide an evolutionary perspective on the data. We use *R. obtusifolius*, a European dock that is distantly related to *R. orthoneurus* and *occidentalis*, collected from the Huachuca Mountains (Cochise County), as our outgroup.

## Why Use Molecular Data?

The application of DNA technology is a fairly recent innovation in the area of population and conservation biology. Some techniques, such as random amplified polymorphic DNA (RAPD), microsatellites, sequencing, and restriction fragment length polymorphism, have the advantage of being able to detect greater levels of polymorphism than isozymes (different allelic forms of enzymes) (Avice and Nelson 1988, Riesberg and Ellstrand 1993) and provide levels of resolution unavailable by morphometric or other protein analyses. For instance, an isozyme analysis of *Lactoris fernandeziana* Phil. (Lactoridaceae) revealed no variation among organisms (Crawford et al.

1991), whereas significant levels of variation were found with the RAPD technique (Brauner et al. 1992). The polymerase chain reaction (PCR) is a powerful tool that allows an investigator to obtain large quantities of DNA with very little source material (Welsh and McClelland 1990, Williams et al. 1990). Using a primer of a certain sequence, a specific gene or region may be targeted for amplification by *Taq* polymerase, an enzyme that recognizes these primers.

The RAPD technique is a variation of PCR. A 10 base pair primer of random sequence is used to amplify many different DNA fragments from throughout the target genome. The amplified fragments are subsequently separated via gel electrophoresis to produce a particular banding pattern. Patterns and specific bands can become markers for individuals, populations, or species. The RAPD technique provides a great number of markers to assess close relationships. For RAPD markers, the percent of bands shared provides an estimate of genetic relatedness (Marsolais et al. 1993). Genetic distances can be calculated to create a dendrogram by using the neighbor-joining algorithm (Saitou and Nei 1987). Genetic diversity that occurs within and between these populations can be determined by calculating the percent of polymorphic loci that occur as well as unbiased heterogeneity per polymorphic locus (Nei 1972, Nei and Li 1979). This technique has been used to elucidate genetic relationships and diversity in plants (Welsh and McClelland 1990, Williams et al. 1990), including rare plant species (Brauner et al. 1992, Dawson et al. 1993, Reisberg and Ellstrand 1993). RAPD markers have even been applied to detecting genetic variations that were correlated with geographical and ecological variables (Dawson et al. 1993).

Chloroplast markers have been used extensively to assess relationships among plant species (Palmer et al. 1988). Chloroplast markers are generally useful for investigating putative hybridization events, where conflicting nuclear and chloroplast phylogenies may result from the maternal inheritance of the chloroplast (Delgado et al. 1993, Bremmer 1991, Jansen et al. 1991, Ishii and Tsuniwaki 1991). Restriction site mapping of chloroplast DNA (cpDNA) offers good resolution for studying systematics below the family level (Bremmer 1991, Palmer et al. 1988). The ribulose 1,5 bisphosphate carboxylase/oxygenase gene (*rbcL*) has been exploited extensively to study phylogenetic relationships, as it is highly conserved. However, we have learned that *rbcL* is

uninformative in the study of relationships within *R. orthoneurus* populations. The intergenic spacer region (IGS) between the *rbcL* gene and ORF106 of the chloroplast genome is an area of greater variation than *rbcL* (Arnold et al. 1991). These nontranscribed regions of DNA are often found to have more variability because of their lack of coding importance. Chloroplast markers, like any other genes, are easily amplified by PCR as many primers are well conserved among taxa (Demesure et al. 1995).

As both methods of study rely on PCR-based techniques, the need for plant material is minimized. This is particularly advantageous when limited occurrences of endangered or rare species restricts the collection of tissue. Our laboratory uses a molecular approach to work on problems facing rare plants. We use PCR to study species delineation in a taxon where the specific classification of a certain population whose identity is in doubt will affect the implementation of management programs.

To address these issues, we investigate the geographic distribution of genetic variation within and among populations of both the rare and widespread species. We examine the genetic structure of the widespread congener as a model to predict the appropriate measures for conservation (Hamrick et al. 1991). Preservation strategies may be improved by an understanding of the genetic variability present within the species, *R. orthoneurus* in the present case. Likewise, an understanding of the uniqueness of its genetic constitution in comparison to close congeners, such as *R. occidentalis*, will be useful to managers and systematists concerned with phylogenetic relationships.

## Methods

### *Plant Collections*

Plants were collected from five populations within Arizona. Plants were sampled at 1 km intervals along any drainage. Voucher specimens were made from root cuttings that were propagated in a greenhouse to avoid sacrificing an entire plant, or from individual leaves. At the conclusion of these studies, voucher specimens will be deposited at the University of Arizona herbarium (UA). Leaf material was collected and stored on ice until transferred to a -70°C freezer in our laboratory at the University of Arizona in Tucson. *Rumex occidentalis* is represented from a population near Ferncliff, Boulder County, Colorado. *Rumex obtusifolius* was sampled from Ramsey Canyon in

the Huachuca Mountains, Cochise County, Arizona. *Rumex crispus* was sampled from the Rillito River drainage in Tucson, Pima County, Arizona. *Rumex orthoneurus* was sampled from the type locality at Rustler Park in the Chiricahua Mountains, Cochise County, as well as from Workman Creek in the Sierra Ancha Wilderness, Gila County, Mount Graham in the Pinaleno Mountains, Graham County, and the Phelps Botanical area in the White Mountains, Apache County. DNA was isolated from plant leaves using the procedure of Doyle and Doyle (1990), except that the CTAB extraction step was repeated.

### *RAPD PCR*

Seventeen decanucleotide primers, purchased from Operon Technologies (Alameda CA), were used to amplify fragments from genomic DNA (Table 1). PCR reaction mixtures contained 25 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01% w/v gelatin, 50 μM each dATP, dCTP, dGTP, dTTP, one unit *Taq* polymerase (Stratagene) and 0.5 mM primer, with a total reaction volume of 30 μL. Reactions were carried out in a Cetus/Perkin-Elmer thermocycler under the following reaction conditions: 94°C for 3 minutes, followed by 45 cycles of 94°C for 1 minute, 36°C for 1 minute, and 72°C for 2 minutes, ending with 1 cycle of 72°C for 5 minutes as described by Williams et al. 1993. The amplified products were then size-fractionated on a 1.4% agarose gel containing ethidium bromide and visualized under UV light.

Table 1. List of RAPD primers used.

Primer	Sequence 5' → 3'	Primer	Sequence 5' → 3'
OPF-03	CCTGATCACC	OPG-14	GGATGAGACC
OPF-05	CCGAATTCCC	OPG-15	ACTGGGACTC
OPF-06	GGAATTCCGG	OPG-16	AGCGTCCTCC
OPF-13	GGCTGCAGGT	OPG-17	ACGACCGACA
OPG-07	GAACCTGCGG	OPH-01	GGTCGGAGAA
OPG-08	TCACGTCCAC	OPH-09	TGTAGCTGGG
OPG-09	CTGACGTCAC	OPH-11	CTTCCGCAGT
OPG-12	CAGCTCACGA	OPH-17	CACTCTCCTC
OPG-13	CTCTCCGCCA		

### *cpDNA Analysis*

Identification of cpDNA markers for *R. orthoneurus*, *R. occidentalis*, and *R. obtusifolius* involved the use of oligonucleotide primers homologous to the region containing the intergenic spacer between *rbcL* and ORF106 (*rbcL*, Z1264, 5'-GTAGCTT TAGAAGCCTGTGTACAAGCTCGTAA; ORF106, 5'-ACTACAGATCTCATACTACCCC-3', Arnold et

al. 1991). The Z1264 primer is identical to positions 1264–1296 of the *Zea mays rbcL* gene. The ORF106 primer is identical to the *Oryza sativa* conserved open reading frame ORF106 (Arnold et al. 1991). PCR reaction mixtures (100  $\mu$ L) contained 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.001% gelatin, 0.1 mM each dATP, dCTP, dGTP, and dTTP, and 2.5 units of *Taq* polymerase (Stratagene). Reactions were carried out in a Cetus/Perkin-Elmer thermocycler under the following reaction conditions: 94°C for 1 minute, followed by 35 cycles of 94°C for 1 minute, 56°C for 1 minute, and 72°C for 4 minutes, ending with 1 cycle of 72°C for 7 minutes. Amplified DNA products from six plants from each of the five populations of *R. orthoneurus* listed above and six *R. occidentalis* and *R. obtusifolius* plants were digested by 23 restriction endonuclease enzymes.

#### Calculating Genetic Distance

The RAPD banding patterns were used to calculate genetic distances between all pairs of plants, using the formula  $D_{xy} = 1 - (n_{XY} / n_X + n_Y - n_{XY})$ , where  $n_{XY}$  is the number of shared bands between plants X and Y,  $n_X$  is the number of bands present in plant X, and  $n_Y$  is the number of bands present in plant Y (Marsolais et al. 1993). The pairwise genetic distances were then used to create a dendrogram showing the relationship between individual plants using the neighbor-joining algorithm from the Phylip suite (Felsenstein 1993). *Rumex obtusifolius*, collected from the Huachuca Mountains in southern Arizona, was used as an outgroup. Figure 1 is a dendrogram suggesting the historical relationships among the populations of *R. orthoneurus* to each other, *R. occidentalis*, and *R. obtusifolius*. Banding patterns were almost uniform among plants from individual populations (Bellsey and Mount, in preparation).

## Results and Discussion

#### RAPD Analysis

The RAPD data suggest variability among the different populations and species of *Rumex* tested, but very little variation within the populations. Four plants from a closely and a distantly related species of *Rumex* were included in this RAPD analysis (e.g., *R. occidentalis* and *R. obtusifolius* respectively). The dendrogram (Figure 1) suggesting the relationships among various groups indicates that the plants from Arizona (other than the alien *R. obtusifolius*) are significantly different

from *R. occidentalis*. Each of the three species share less than 15 percent of their marker loci. The more distantly related two species are, the less probable that marker loci are identical by descent. Therefore, it stands to reason that beyond a certain genetic distance individuals will share a certain fraction of their markers by chance (i.e., identical in state, not by descent). These data would indicate a significant degree of divergence between similar species; however we conclude that RAPDs are not suitable for determining the phylogenetic relationships among species of *Rumex*. By comparison, a study by van Buren and colleagues (1994) demonstrated the relationship between the endangered autumn buttercup (*Ranunculus acrifolius* var. *aestivalis*) and close congeners using RAPDs. Based on the results of their analyses and on a review of ecological and morphological characters, they elevated the autumn buttercup to the species rank as *R. aestivalis*, which has direct implications for its protection and management.

RAPD analysis also demonstrates that plants from the White Mountain population are related to other plants from Arizona and not *R. occidentalis*, which they resemble morphologically. Analysis using the neighbor-joining algorithm (Saitou and Nei 1987) indicates 10–20 percent differentiation among populations. The greatest amount of RAPD variation resides among the metapopulations; 46 of 203 RAPD loci surveyed are polymorphic. The analysis indicates that the closest relationships among populations of *R. orthoneurus* are between Workman Creek (Sierra Ancha Wilderness Area) and Mount Graham (Pinaleno Mountains), and the Huachuca and Chiricahua mountains (see Figure 1). Genetically speaking, the White Mountain population was the most distantly related population.

To determine the amount of genetic variation within populations, between 140 and 180 loci were surveyed from 8–15 plants per population of *R. orthoneurus*. Less than 15 percent of the loci are polymorphic (Table 2). The limited variation observed within each population of *R. orthoneurus* may be attributed to extensive inbreeding or clonal propagation (Brauner et al. 1992, Dawson et al. 1993, Reisberg and Ellstrand 1993, Welsh and McClelland 1991). An alternate hypothesis is that the plants are outcrossing but a low level of genetic variation within the populations is common for this species of *Rumex*. The divergence between populations of *R. orthoneurus* may be due to genetic drift or selection in the context of adaptation to local environments.

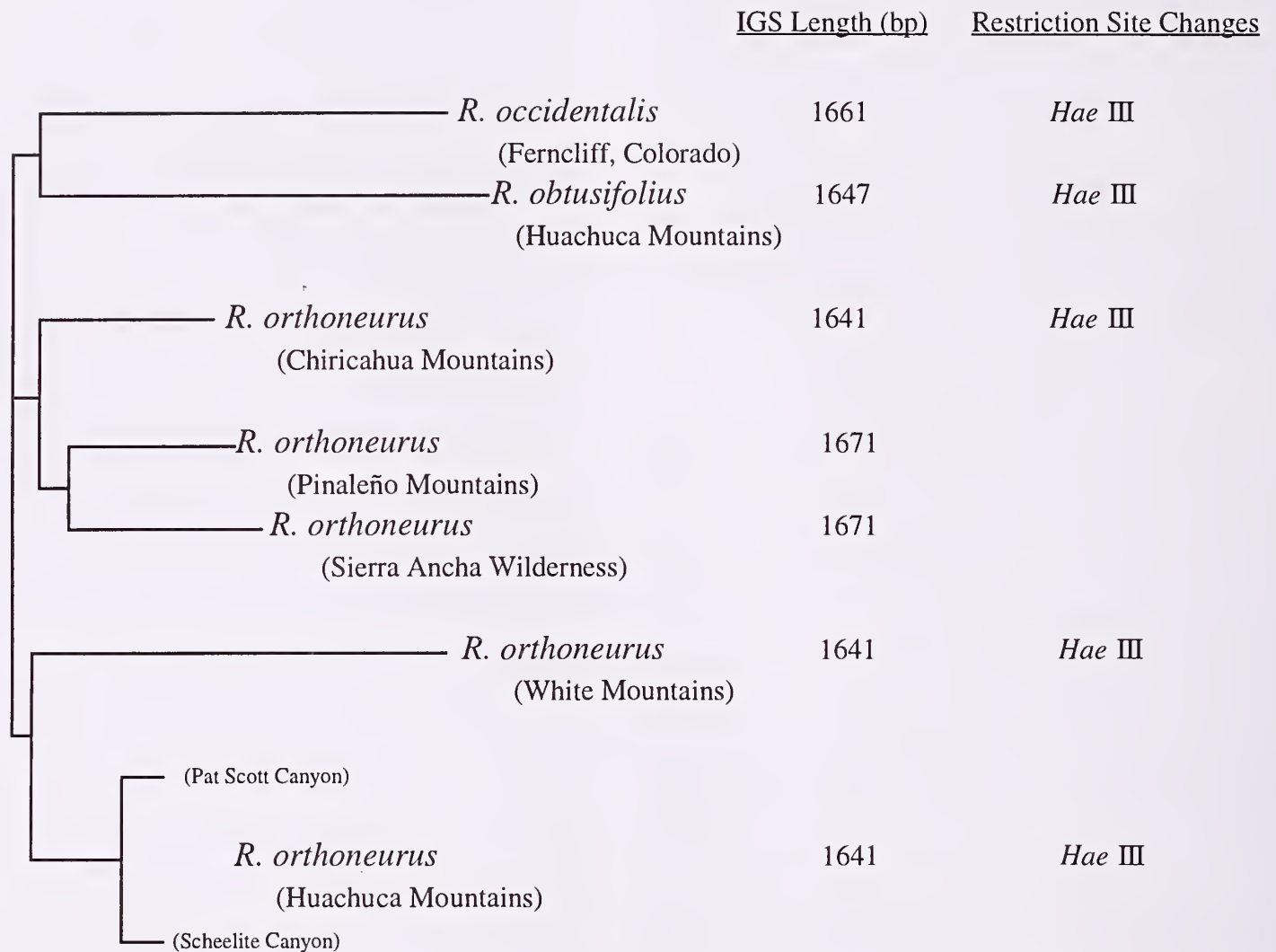


Figure 1. Using 17 RAPD primers we generated approximately 200 markers. Percentage of bands shared provides a rough estimate of genetic relatedness (Marsolais *et. al.* 1993) which was used to calculate the genetic distance between individuals (Nei 1972; Nei and Li 1979) which we used to create this dendrogram using the Neighbor Joining algorithm (Saitou and Nei 1987). Individuals are nested within their respective populations. The congener *R. occidentalis* shares less than 15% of it's RAPD markers with *R. orthoneurus*. The length of the 3' spacer of *rbcL*(IGS) and restriction site changes are listed next to their respective populations.



Table 2. RAPD variation within populations of *Rumex orthoneurus* (percent variable bands).

Population	Number of Plants	Number of Loci	Percent Variable Loci
Chiricahua	11	121	3.6
Huachuca	8	120	5.8
Pinaleño	5	129	0.6
Sierra Ancha	10	144	4.5
White Mountains	15	137	16.6

#### *cpDNA Analysis*

The amplification products of IGS from the different species of *Rumex* were  $\approx$  1600 bp long. Twenty-three restriction enzymes were used to digest the IGS from three species of *Rumex*. Most enzymes produced identical restriction patterns, except for five that produced patterns whose smaller fragments were of varying lengths between the taxa. The lengths of the spacer region from each group was determined. One restriction site difference was found in *R. orthoneurus* from one geographic region (see Figure 1).

The cpDNA analyses of *R. obtusifolius* and *R. occidentalis*, as well as the various populations of *R. orthoneurus*, provide length and site polymorphisms that are consistent within each group. The varying lengths of the spacer region, as well as the one *Hae* III site polymorphism, are not enough to indicate a phylogenetic relationship among these groups, but can corroborate certain associations suggested by the RAPD data. It is interesting to note that the plants from the White Mountains population shared the same profile as the plants from the Huachuca and Chiricahua mountains and not that of *R. occidentalis*, suggesting that these plants are historically related to *R. orthoneurus*. This classification had previously been in doubt. Plants from Mount Graham and Sierra Anchas share the same IGS fragment length and the only site polymorphism, which concurs with the relationship suggested by the RAPD data. The loss or gain of a restriction site may be caused by as little as a single mutation of one nucleotide. Changes in the length of a sequence, such as IGS, may be due to multiple mutations over time. This uncertainty makes it difficult to assess the significance of such length variations; however, since the polymorphisms are consistent for all plants examined within a population, the differences between populations (and species) corroborate the RAPD data.

#### *Conclusions and Suggestions*

RAPD and cpDNA data would suggest that the plants from southeastern Arizona share a common ancestry or have previously undergone genetic exchange. These techniques have provided an enhanced understanding of the relationships among these plants. Several points should be considered as a result of these data. It may be important to maintain the genetic integrity of the different populations of *R. orthoneurus*. If populations are differentiating genetically, then trying to enhance the genetic variability within populations by transplantation (1) will homogenize the species and (2) might interrupt evolutionary processes (speciation) and potentially disrupt co-adapted gene complexes. On the other hand, if it becomes evident that population size and health is waning, new populations, created in extirpated habitats by combining plants from various subpopulations within a single geographic region, might be a suitable alternative. A consideration is that once plants from different populations are combined, several generations would have to pass for the genetics to be sorted out. Unfortunately, little is known about the longevity or phenology of this species.

Populations of *R. orthoneurus* such as the Black River population in the White Mountains with thousands of plants distributed along the main stem and tributaries (personal observation) or SA Creek in the Gila National Forest with less than 100 plants (personal observation), harbor varying amounts of genetic variation. It would be impossible to carry out investigations similar to the present one on each population and subpopulation. It may prove more useful to understand the genetic properties of a species whose habitat is rare and highly structured. An investigation of whether geographic populations are genetically structured along their regional habitats may provide an understanding of the importance of interaction or isolation of extant populations and of maintaining the continuity of these habitats, which understanding can be subsequently applied to the management and preservation of the ecosystems and natural processes.

It is important to understand the population genetic structure of rare and threatened species, because genetic diversity is considered to correlate with adaptive capacity (Les et al. 1991). It is true that the relationships between molecular, genetic, and ecological data are poorly understood (Learn and Schaal 1987, Schemske et al. 1994), as is the

adaptive significance of molecular polymorphisms (Lewontin 1974, Koehn and Hilbish 1987). However, as we begin to comprehend these relationships, we develop better ways to cope with the problems associated with conserving endangered species and biodiversity in general. Correlating information from these analyses with ecological data is useful in developing a strategy for conservation and will serve as an important model for other taxa whose habitats are similarly threatened.

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# A Genetic Evaluation of Three Potential Races of the Rare Kachina Daisy

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**Abstract:** As originally described, *Erigeron kachinensis* (Asteraceae) was a category 2 species endemic to hanging garden communities in the Colorado Plateau region of southeastern Utah. Two new races of *E. kachinensis* have recently been discovered. One grows in hanging gardens along the Dolores River in Colorado and is morphologically differentiated from typical materials collected at Natural Bridges National Monument. Another grows on exposed substrates at high elevations on Elk Ridge in southeastern Utah. It is morphologically similar to type materials but occupies a different habitat. Genetic evaluation of this complex was performed using allozymes from 14 enzyme loci to determine if these races represent distinct taxa worthy of separate management. Allozyme data show that the races are genetically distinct. Phylogenetic analysis demonstrates that the morphologically distinct race (Dolores River) is more closely related to type materials than the ecologically distinct race (high elevation) and both may merit varietal status based on allozyme divergence.

## Introduction

The Kachina daisy (*Erigeron kachinensis* Welsh & Moore) was discovered and named in 1968 (Welsh and Moore 1968). It is a rare perennial composite restricted to southeastern Utah and adjacent Colorado. The species grows in small, isolated populations at seeps and alcoves in canyons on the Colorado Plateau. The Kachina daisy was proposed as "endangered" by the U.S. Fish and Wildlife Service, June 16, 1976 (U.S. Department of Interior 1975, 1976). Later proposals downgraded the original recommendation to "threatened" status for the species (U.S. Department of Interior 1988). Currently, the Kachina daisy is listed by the U.S. Fish and Wildlife Service as a category 2 species.

Because this species is often restricted to seeps and alcoves, typically along a single seep line within a canyon, there is concern for its preservation. If drought caused seep lines occupied by this species to dry up, many populations could be eliminated. As tourism increases in the canyon regions of southeastern Utah, so does the threat of human impacts on rare species. Tourists hiking in canyon bottoms look to shady alcoves as refuge from the hot summer sun. Many of the seeps and alcoves contain small, prehistoric Anasazi ruins, which also attract tourists.

The Kachina daisy was originally known only from Natural Bridges National Monument (NBNM) and an isolated population in Montrose County, Colorado. With only a few known popu-

lations confined to uncommon sites, resource managers were concerned that the species might be vulnerable to extinction. In 1990, a survey of canyons surrounding NBNM was initiated under a contract with the Bureau of Land Management in hopes of finding additional populations of *E. kachinensis*. As a result of this effort, the Kachina daisy has now been found on lands administered by the Bureau of Land Management (Allphin 1992). Populations are known in Fish, Arch, White, and Birch canyons; Elk Ridge, San Juan County, Utah; and from the lower portion of Coyote Wash, Montrose County, Colorado (Allphin 1992, Welsh et al. 1987).

These new collections have significantly increased the number of known populations of the Kachina daisy and have raised the possibility that it should be deleted from the list of candidates for federal protection. Land managers from Utah recently met and discussed this very proposal. Before final status can be designated, however, it must be determined if *E. kachinensis* represents a single taxon.

Three potential races or clusters of populations can be recognized for this species (Allphin and Harper 1994, Welsh et al. 1993). The type population is located in Natural Bridges National Monument (Welsh and Moore 1968). It can be found growing in hanging garden communities in Cedar Mesa Sandstone substrates of the Colorado Plateau region of Southeastern Utah. A second form of this taxon is found along the Dolores River in

Montrose County, Colorado. These congeners are morphologically distinct from the type specimens of *E. kachinensis* with respect to several characters: ray flower color, flower head size, leaf size, and leaf shape. A third group of populations from rock crevices on Elk Ridge, San Juan County, Utah are morphologically similar to typical materials, yet grow in an entirely different ecological regime. The habitats associated with specimens from Elk Ridge rock faces and those associated with alcove daisies of the deep canyons of NBNM are very different with respect to elevation, soil moisture, solar radiation, soil salinity, and associated species (Allphin and Harper 1994).

To assess whether these three groups of populations represent a single taxon, it is necessary to determine how closely allied they are genetically. Therefore, our objectives for this study were to evaluate genetic differences among the three groups or potential races of *E. kachinensis*, and to assess whether genetic differences are correlated with any other characters such as ecology, morphology, and geology.

### Materials and Methods

Genetic differences among the three potential races were assessed using enzyme electrophoresis. Populations of each potential race were sampled from the localities shown in Figure 1. We sampled 20–40 individuals for each population (Figure 1). Young leaves collected from each individual were ground in a phosphate–PVP grinding buffer (Soltis et al. 1983) using a mortar and pestle. Ground material was absorbed into wicks made of Whatman 3MM filter paper and stored in an ultra-cold freezer (-70°C) until analysis (up to 4 months later).

Electrophoresis was performed using a variety

of gel and electrode buffers (Table 1) and samples were analyzed using 12% starch gels that were sliced and stained following standard protocols (Soltis et al. 1983). The allozyme markers from 14 enzyme loci that provided consistent interpretable results were analyzed (Table 1). We recorded the presence or absence of each allele detected for all populations. These data were analyzed phylogenetically using PAUP 3.1 (Phylogenetic Analysis Using Parsimony) (Swofford 1990). Both heuristic and exhaustive searches for the most parsimonious trees were conducted and statistical indices were computed.

In addition to the allozyme study, all herbarium collections of *E. kachinensis* at Brigham Young University (BYU) and the University of Utah (UT) were examined for evidence of any morphological differentiation among the three potential races. Collection records were also assessed for any differences in ecology, geology, or physiology that might be correlated with the allozyme data.

### Results

The individual allozymes detected at the 14 analyzed loci are listed in Table 2. Given the low level of genetic divergence among closely related taxa within *Erigeron* (Noyes et al. 1995, Windham, unpublished data), the allozyme differences observed between “races” of *E. kachinensis* is surprising. For example, an entire locus is missing from the high-elevation race which is present in the other races (Table 2). For several of the enzyme loci, the hanging garden races appear to have only a subset of all the alleles present in the species. This can be observed at PGI-2 and PGM-2 (Figures 2 and 3). At PGI-2, the Causeway population carries a second allele that is absent in other

Table 1. Enzymes used in allozyme analysis of three potential races of *Erigeron kachinensis*, gel and electrode buffer systems used, and number of loci scored.

Enzyme	Gel/Electrode Buffers (Soltis et al. 1983)	Acronym	Number of Loci Scored
Esterase	8	EST	1
Isocitrate dehydrogenase	11 & M	IDH	1
Leucine aminopeptidase	8	LAP	1
Malate dehydrogenase	M	MDH	1
NADH-diaphorase	8	NADH-DP	3
Phosphoglucoisomerase	6 & 8	PGI	2
Phosphoglucomutase	6	PGM	2
6-Phosphogluconate dehydrogenase	11 & M	6-PGD	2
Triosephosphate isomerase	6 & 8	TPI	1
Total number of loci analyzed to date:			14

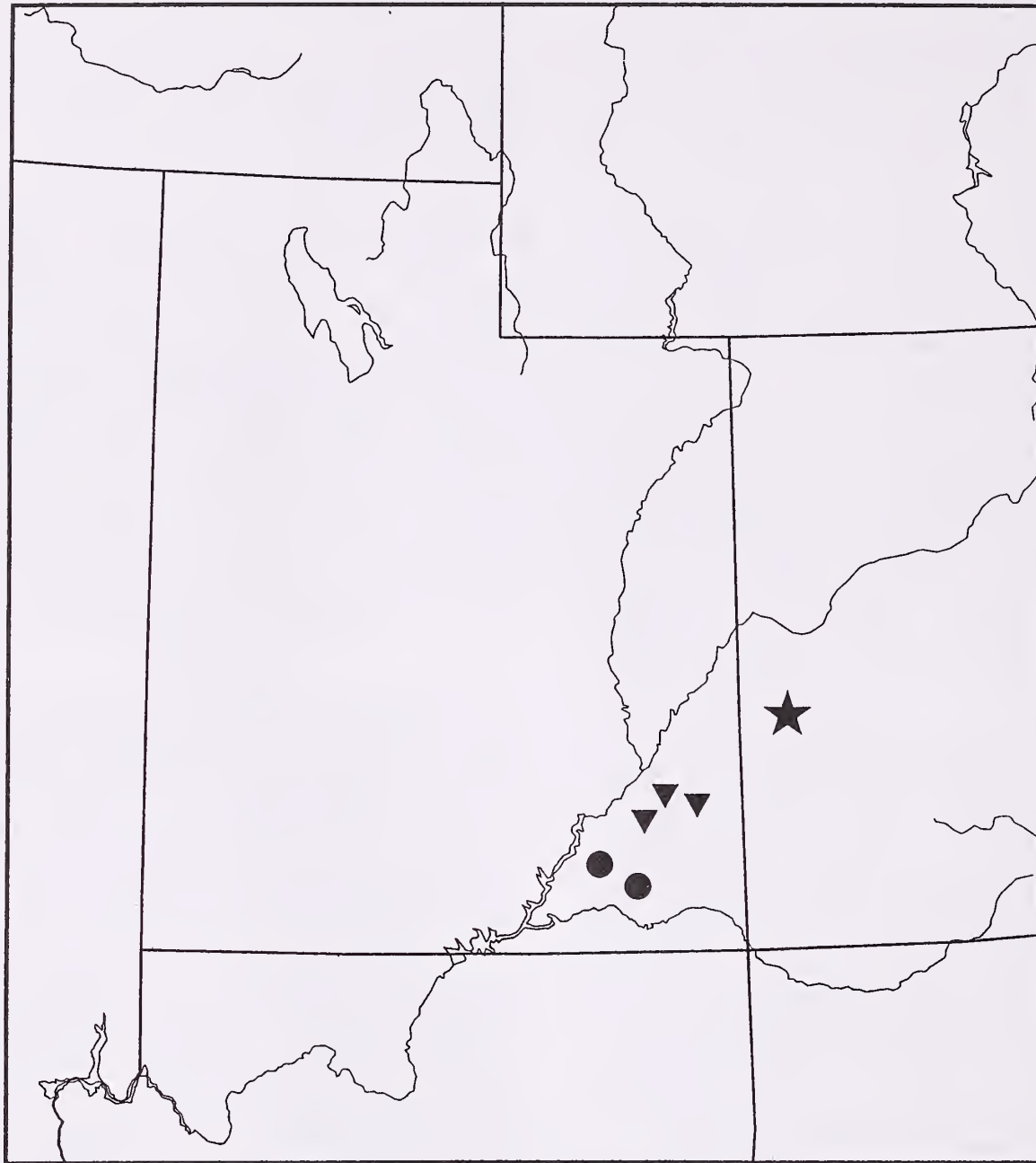


Figure 1. Locations of sampled populations of *E. kachinensis* for enzyme electrophoresis. The star represents a population along the Dolores River (Montrose County, Colorado) where 25 individuals were sampled. The inverted triangles represent three populations located on the Elk Ridge, Manti La Sal National Forest, San Juan Co., Utah (Causeway, Chippean Rocks, and Elk Ridge) where approximately 24 individuals were sampled per population. The circles represent populations of typical Kachina daisy. The most western circle represents a total of 40 individuals taken from six populations in Natural Bridges National Monument (San Juan Co., Utah). The eastern circle represents a population in Fish Canyon (Grand Gulch Primitive Area, San Juan, Co., Utah) where 20 individuals were sampled.

Table 2. Results of isozyme electrophoresis of three races of *Erigeron kachinensis*. The presence of an allele at a particular locus is indicated by a plus sign (+) and the absence of the allele at that same locus is indicated by a negative sign (-). A question mark (?) indicates missing data. An asterisk (\*) indicates that the locus is absent for the entire high-elevation race.

Alleles	Causeway	Chippean	Elk Ridge	Dolores	Nat. Br.	Fish
PGI1a	+	+	+	+	+	+
PGI2a	+	+	+	+	+	+
PGI2b	+	-	-	-	-	-
PGM1a	+	+	+	+	+	+
PGM1b	+	-	-	-	-	-
PGM2a	-	+	+	-	-	-
PGM2b	+	+	+	+	+	+
PGM2c	+	+	-	-	-	-
LAP1a	+	+	+	+	-	-
LAP1b	+	+	+	+	+	-
LAP1c	-	-	-	+	+	+
TPI1a	+	+	+	+	+	+
TPI1b	-	-	-	-	+	-
MDH1a	+	+	-	-	-	-
MDH1b	+	+	+	+	+	+
6PGD1a	+	+	+	-	+	-
6PGD1b	+	+	+	+	+	+
6PGD1c	-	+	-	-	+	-
6PGD2a	+	+	+	+	+	+
6PGD2b	+	-	-	-	-	-
IDH1a	-	+	-	-	+	-
IDH1b	-	-	-	+	-	-
IDH1c	+	+	+	+	+	+
IDH1d	-	+	-	-	-	-
EST1a	-	-	-	+	+	+
EST1b	+	+	+	+	+	+
EST1c	+	-	-	+	+	+
EST1d	+	+	-	+	-	-
NDP1a	-	-	-	-	-	+
NDP1b	+	+	+	+	+	+
NDP1c	+	+	+	+	+	+
NDP2a	+	+	+	+	+	?
NDP2b	+	+	+	+	+	?
NDP2c	+	+	-	-	-	?
NDP2d	+	-	-	-	-	?
NDP3a*	-	-	-	-	+	-
NDP3b*	-	-	-	+	+	+
NDP3c*	-	-	-	-	+	+

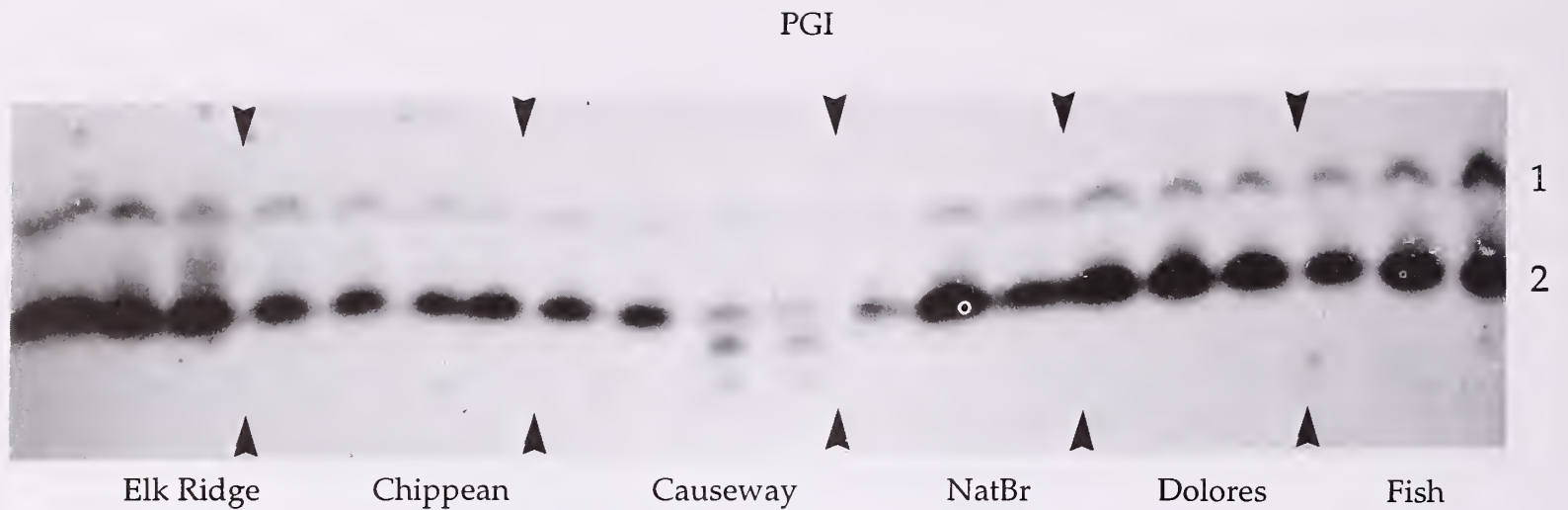


Figure 2. Photo of allozyme variability at both loci of PGI for all six study populations of *E. kachinensis*. Number 1 represents PGI-1 locus (monomorphic) and number 2 represents the PGI-2 locus.

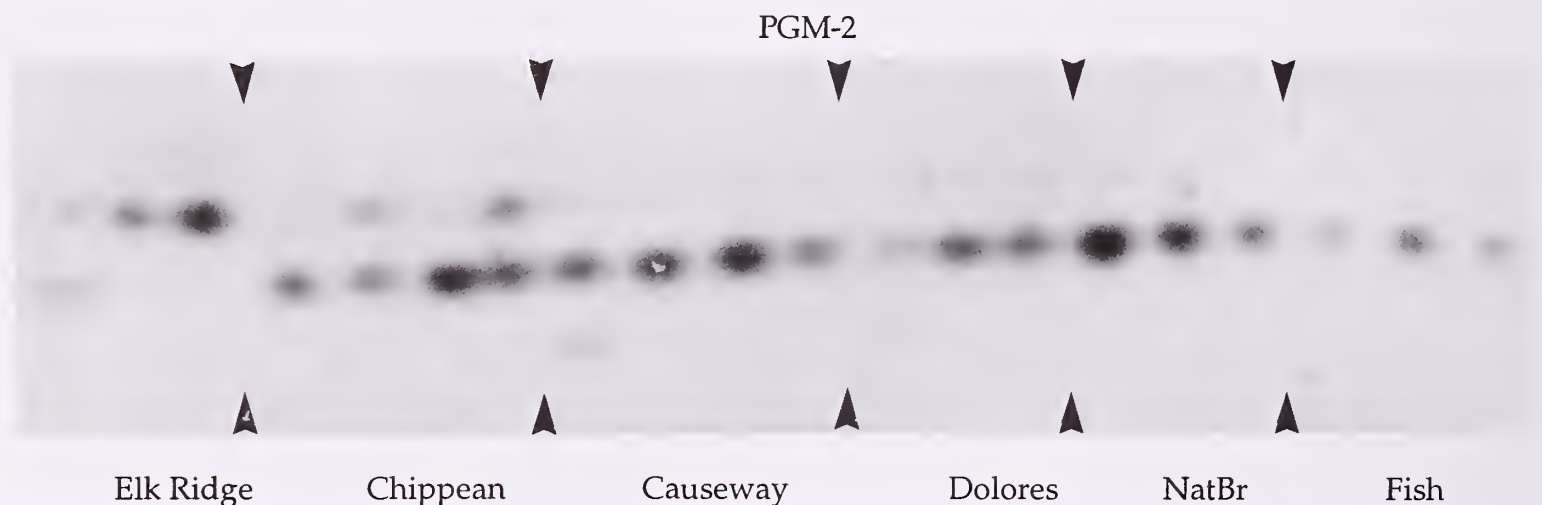


Figure 3. Photograph of allozyme variability at the PGM-2 locus for all study populations of *E. kachinensis*.

populations (Figure 2). At PGM-2, all three of the high-elevation populations (Causeway, Chippean, and Elk Ridge) possess alleles (a and c) that are absent from the other populations (Figure 3).

Phylogenetic analysis resulted in two equally parsimonious trees, both with relatively high consistency indices (CI = .721). A strict consensus of these two trees (Figure 4) demonstrates two potential clades within *E. kachinensis*. One clade representing all three populations of the high-elevation races, forms an unresolved polytomy at the base of the tree. A well-defined clade representing all hanging garden populations branches from within this polytomy. The morphologically distinctive Dolores River population occupies the basal branch of the hanging garden clade and

might prove to be a distinct evolutionary lineage. These same groupings are supported by data on genetic distances and character distances (Table 3). High genetic distances can be seen between populations of the hanging garden race and populations of the high-elevation race. Populations of the hanging garden race appear to be more closely related to the morphologically distinct race (Dolores River) than to the morphologically similar race at high elevations.

A reassessment of morphological trends among the three races supports molecular findings of three distinct races among populations of *E. kachinensis* (Table 4). The high-elevation race is distinguished from typical materials by having a thickened, branching caudex and >8 cauline



Table 3. Mean genetic distances and character distances among study populations of *Erigeron kachinensis*.

	Mean Genetic Distances					
	Causeway	Chippean	Elk Ridge	Dolores R.	Nat. Br.	Fish Canyon
Causeway		.333	.400	.533	.867	.733
Chippean Rocks	6		.333	.600	.667	.800
Elk Ridge	7	5		.400	.600	.467
Dolores River	9	11	8		.467	.200
Natural Bridges	14	12	11	9		.267
Fish Canyon	12	14	9	5	6	

Table 4. Character differences among the three races of *Erigeron kachinensis* as determined from herbarium specimens from Brigham Young University and University of Utah.

Characters	Elk Ridge Race	Hanging Garden Race	Dolores River Race
<b>Morphology</b>	rays white	rays white-fading pink	rays pink or white-fading pink
	well-developed caudex	caudex simple, less developed	caudex less developed
	many cauline leaves (8-16)	few cauline leaves (3-7)	cauline leaves (5-8)
	leaves lanceolate-ob lanceolate	leaves oblanceolate-spatulate	leaves spatulate-emarginate; often retuse apically
<b>Ecology</b>	exposed rock crevices	alcoves and seeps	alcoves and seeps
	Ponderosa pine/oakbrush communities	hanging garden communities	hanging garden communities
<b>Geology</b>	Navajo Sandstone	Cedar Mesa Sandstone	Navajo Sandstone
<b>Physiology</b>	non-halophytic	halophytic	halophytic
<b>Elevation</b>	high (7200-8640 ft)	mid (5400-6800 ft)	mid (5150-5400 ft)

## Strict

Consistency index (CI)	0.760
Retention index (RI)	0.647
Homoplasy index (HI)	0.240
f-ratio	0.1594

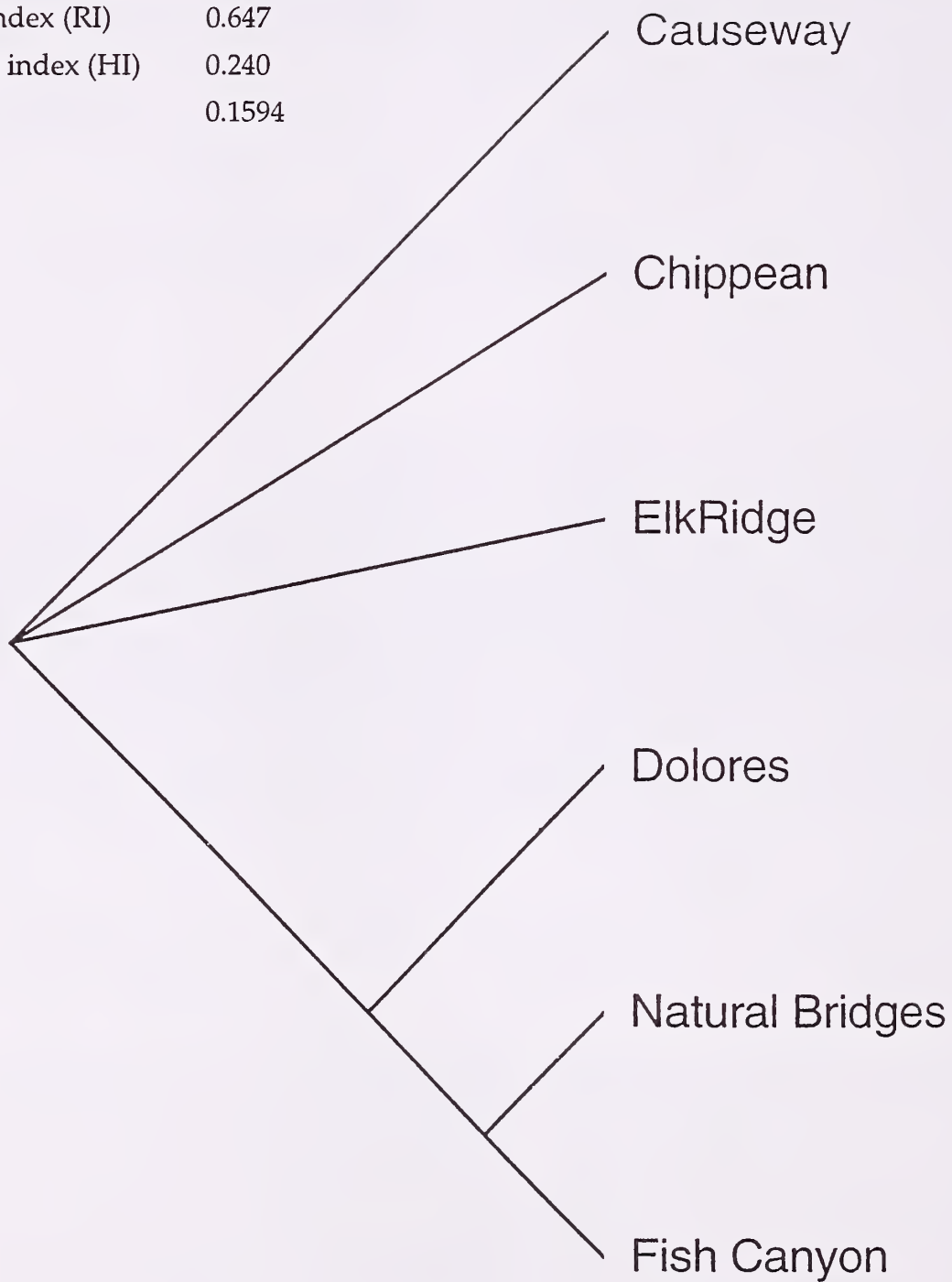


Figure 4. A strict consensus phylogenetic tree generated for all study populations of *E. kachinensis* using PAUP 3.1.

leaves. The Dolores River race can be differentiated from typical materials by its spatulate-emarginate, often retuse leaves (Table 4).

The high-elevation race can also be distinguished from typical materials on the basis of ecology, geology, and physiology (Table 4). It is typically found growing in exposed rock crevices on Navajo Sandstone substrates, which are typically nonsaline. However, the typical materials from Natural Bridges National Monument are found in sheltered alcoves and seeps in hanging garden communities of Cedar Mesa Sandstone substrates with high salt concentrations (Table 4). For all of these characters, the Dolores River race is very similar to the hanging garden race (Table 4).

### Conclusions

Our genetic evaluation suggests that the morphologically distinct race (Dolores River) is more closely related to the type materials than the ecologically distinct, high-elevation race. These data add to the growing body of evidence (King and Wilson 1975, Gottlieb 1977, Cherry et al. 1978, Hillis 1987, Donoghue and Sanderson 1992, Avise 1994) that morphological differentiation isn't necessarily correlated with molecular divergence. Although it might be argued that some allozyme divergence in the ecologically distinct race is adaptive in nature (rather than neutral), it doesn't change the fact that we appear to be dealing with distinct genetic systems.

Consequently, we suggest that the high-elevation race deserves varietal status based upon allozyme and ecological divergence. This variety is recognizable on the basis of morphological characters (such as the caudex and number of cauline leaves) in addition to elevation and ecology. Since the Dolores River race is closely related to the hanging garden race according to allozymes and ecology, we prefer to retain it in the hanging garden variety pending further study. Closely related species of *E. kachinensis* analyzed to date have been too distant with respect to allozymes for phylogenetic comparisons. Future studies should include at least one closely related species to provide a suitable outgroup and should include an assay of DNA variability, since allozymes are not always reflective of total genomic variation (Mitton 1994).

The recognition of two varieties of *Erigeron kachinensis* would require each taxon to be managed separately. The total number of individuals of the hanging garden taxon would be signifi-

cantly reduced, allowing it to possibly remain federally protected. With growing human impact to the canyon country, protection may prove necessary for preservation of total species diversity within *E. kachinensis*.

### Acknowledgments

We would like to thank the Bureau of Land Management and the Utah Native Plant Society for assisting with funding of this project. Special thanks also goes to Stanley L. Welsh, Brigham Young University, for input and loan of herbarium specimens. We would also like to thank Elisabeth Mattes with the Hughes High School Summer Research Program who assisted with enzyme electrophoresis. We also thank the National Park Service, Natural Bridges National Monument, and the U.S. Forest Service.

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# Genetic Variation Among Populations of *Arctomecon* (Papaveraceae)

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**Abstract:** Three species comprise the genus *Arctomecon* (Papaveraceae). All are endemic to the Mojave Desert region of the southwestern United States. *Arctomecon humilis*, endemic to Washington County, UT, was listed as an endangered species in 1979 and is presently known from only nine populations. Habitat for this species continues to decline as human development increases. *Arctomecon californica* is endemic to southern Nevada and the lower Grand Canyon area of Arizona. *Arctomecon merriamii* is confined to southern Nevada and the Death Valley area of California. Both *A. californica* and *A. merriamii* are candidates for listing as threatened or endangered by the U.S. Fish and Wildlife Service. *Arctomecon californica* populations are seriously threatened by development. We have estimated the variation existing among populations of *A. humilis* and the degree of genetic similarity this taxon shares with its congeners. Estimates are based on RAPD (randomly amplified polymorphic DNA) analysis of genomic DNA. DNA was extracted from leaf tissue. Tissue from *Argemone munita* and *Romneya coulterii*, proposed close relatives of *Arctomecon* and *Papaver orientale*, an outgroup, was also included in the analysis. Results show that little genetic variation exists among the populations of *Arctomecon humilis* (populations are over 95% similar on average). Populations of *A. californica* were nearly as similar (~94%). However, *A. merriamii* populations averaged only about 68 percent similarity. Mean interspecific similarity among the three species of *Arctomecon* was between 15 and 23 percent. The results provide information on the genetic composition of individual populations that will prove helpful for management decisions. The data also provided insights concerning phylogeny in the family Papaveraceae.

## Introduction

Three species of *Arctomecon* (*A. humilis* Coville, *A. merriamii* Coville, and *A. californica* Torr. & Frem.) are endemic to the Mojave Desert regions of the southwestern United States (Nelson and Welsh 1993 and Figure 1). *Arctomecon californica* and *A. humilis* are usually found only on gypsic soils. *Arctomecon merriamii* sometimes occurs on gypsic soils as well, but occurs over a much larger area than either of the other species, often on soils derived from limestone or travertine (personal observation). Habitat requirements of *A. humilis* have been described by Nelson and Harper (1991). Alkaloids of *Arctomecon* suggest affinities with *Argemone* and *Romneya*, two North American genera, and *Glaucium* and *Papaver*, which all share tetrahydrobenzylisoquinoline and protoberberine alkaloids, which are not known from other genera in the family (Raynie et al. 1991). Within the genus *Arctomecon*, *A. humilis* contains nearly twice as many kinds of alkaloids as *A. californica* and *A. merriamii*.

*Arctomecon humilis* was listed as an endangered species by the U.S. Fish and Wildlife Service in 1979 (U.S. Fish and Wildlife Service 1979) while *A. californica* and *A. merriamii* are presently cate-

gorized as C2 by the agency. *Arctomecon humilis* and *A. californica* are particularly vulnerable as they occupy sites threatened by rapidly expanding human population centers. Encroachment of residential and commercial developments pose difficult problems for managers charged with preserving these species. As pressures on habitat due to land exchanges and other events continue, it is critical that estimates of genetic variability among populations be understood. The ability of a species to survive various stresses may be limited by the genetic variability within its populations (Barrett and Kohn 1991, Hamrick et al. 1991, Lacy 1987).

For rare and endangered species, conservation of the genomic material must be a primary objective (Schaal et al. 1991). Morphological uniqueness does not always reflect the degree of genetic variation that a particular population may contribute to the total genome of the species.

There are many methods in use to estimate genetic variation in populations (Schaal et al. 1991). RAPD (randomly amplified polymorphic DNA) analysis has proven to be an economical and reliable method for estimating genetic variation within natural populations, especially in the

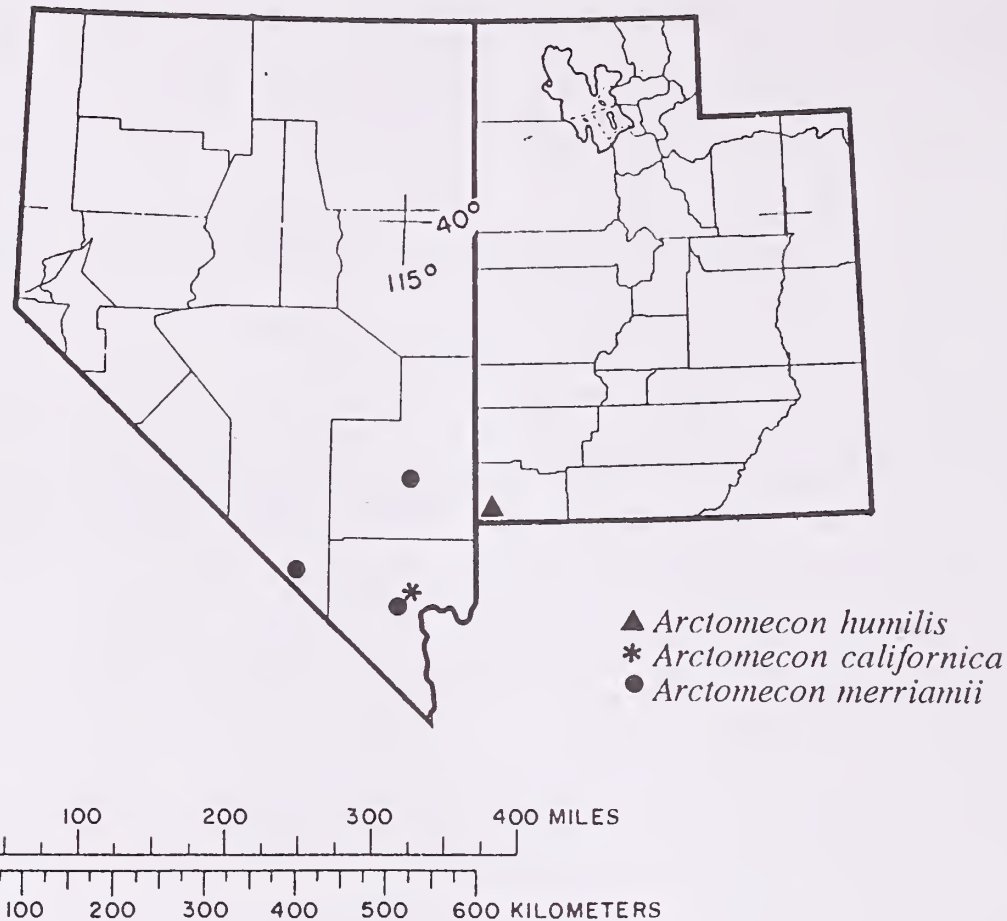


Figure 1. Map showing the general locations of study sites for taxa included in this study.

case of endangered species (Haig et al. 1994, Welsh and McClelland 1990, Williams et al. 1990). In cases where classification is problematic, this method has provided information useful for resolution of problems (Van Buren et al. 1994).

We have employed the RAPD methodology to amplify total genomic DNA. Random primers were used to generate DNA markers for estimation of percent similarity of DNA among populations of *Arctomecon humilis* and with related species. Leaf tissue was collected from all known populations of *A. humilis*. The nine known populations of this poppy are all located near St. George, Washington County, southwestern UT. In order to better understand the degree of variation observed among populations of a single species, we have included tissue from three populations of each of the other species in the genus *Arctomecon*, *A. californica* and *A. merriamii*. To better interpret the variation observed within *Arctomecon*, we have also included tissue from species in three other genera in Papaveraceae: *Argemone munita* Dur. & Hilg. (Welsh et al. 1993), *Romneya coulteri* Harvey (Clark 1993), and *Papaver orientale* L. (Welsh et al. 1993). Such broad sampling of related taxa is helpful for understanding the significance of

various degrees of similarity of DNA markers from species assigned to various taxonomic levels by systematists.

### Materials and Methods

All taxa included in this study were sampled at locations in southwestern Utah and southeastern Nevada (Figure 1). Two disease and parasite-free leaves were collected from 30 individuals at each of the nine populations of *Arctomecon humilis* (see Table 1 for specific locations of populations for all taxa considered). At a previously established demography plot at Red Butte, Washington County, UT, samples were collected to determine whether any DNA differences existed between juvenile and adult age classes (Nelson and Harper 1991). Leaves of 30 juveniles and 30 adults were collected. These samples were treated independently throughout the study. Thirty individuals from each of three populations of *Arctomecon merriamii* and three populations of *A. californica* were sampled in Clark and Lincoln counties, Nevada. These populations represented widely disjunct fragments within each species' range. Leaf tissue of *Argemone munita* was collected from two sites, one in Clark County, Nevada, and the other

Table 1. Locations and abbreviations used for all populations of all taxa included in this study. These abbreviations are used throughout the manuscript.

Taxa	Abbreviation	Location
<i>Arctomecon humilis</i>	AHSC	Santa Clara Butte, Washington Co., UT
	AHBH	Boomer Hill, Washington Co., UT
	AHWD	White Dome, Washington Co., UT
	AHPH	Webb Hill, south side of highway, Washington Co., UT
	AHAW	West of I-15 near Atkinville road, Washington Co., UT
	AHRBj	Demography plot, juv.; Red Bluff, Washington Co., UT
	AHRBa	Demography plot, adults; Red Bluff, Washington Co., UT
	AHBD	Beehive Dome, Washington Co., UT
	AHWV	Warner Valley, Washington Co., UT
	AHSK	Shinob Kibe Hill, Washington Co., UT
<i>A. californica</i>	ACAX	Apex road, north of I-15, Clark Co., NV
	ACFM	Frenchman mountains, Clark Co., NV
	ACLV	North Las Vegas, N Centennial and 5th, Clark Co., NV
<i>A. merriamii</i>	AMLV	N. L. V., NE corner Craig Rd. and Jones, Clark Co., NV
	AMRC	Rainbow Canyon, Lincoln Co., NV
	AMAM	Ash Meadows, Nye Co., NV
<i>Argemone nunita</i>	AGCC	Highway 63, Lincoln Co., NV
	AGGC	Highway 54, Garfield Co., UT
<i>Romneya coulteri</i>	RCJC	Roadside near Visalia, Fresno Co., CA
<i>Papaver orientale</i>	POCV	Cultivated in Spanish Fork, Utah Co., UT

in Garfield County, Utah. *Romneya coulteri* tissue was taken from a single plant growing along a roadside near Visalia, Fresno County, California. *Papaver orientale* leaf tissue was collected from a cultivated individual growing in a garden in Utah County, UT. All sample leaves from each population were combined except for the Red Butte size-class collections, which were treated independently. Leaves were immediately submerged in liquid nitrogen after collection and transferred to an ultra-cold (-70°C) storage unit as soon as possible. Voucher specimens from all populations are deposited in the herbarium at Brigham Young University.

DNA was extracted from population-bulked leaf samples following the procedures of Bult et al. (1992) which were modified from Doyle and Doyle (1987). Approximately 0.3 g of combined leaf tissue from each population was extracted by grinding first in liquid nitrogen and then in 1.2 ml 1X CTAB buffer (1% hexadecyltrimethylammonium bromide, 1.4 M NaCl, 20mM EDTA, 100mM Tris, pH 8.0). The procedures of Bult et al. (1992) were adjusted for the sample volumes described above. Each bulked sample was extracted twice to provide duplicated extraction products for estimating repeatability of the RAPD signals. Five µl of the

original extracts were diluted with 95 µl TE (1 m Tris and 0.5 m EDTA, pH 8.0). After this dilution, most samples yielded between 30 and 85 ng DNA per µl TE. DNA from the diluted samples was quantified using a Beckman DU 640 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). Samples were further diluted in TE to concentrations of approximately 2.5 ng DNA per µl TE.

Amplification was performed using methods described by Williams et al. (1990). Reactions with a final volume of 15 µl each contained approximately 5 ng DNA, 1.5 µl 10X buffer, 100 µM each of four deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP), 3.5 mM MgCl<sub>2</sub>, 0.4 µM primer (Operon Technologies, Inc., Alameda, CA), and 1.2 U Stoffel Fragment (Perkin Elmer-Cetus). Reactions were amplified using a Perkin Elmer-Cetus DNA 48-well thermal cycler with cycling regimes consisting of the following steps: (1) 3-min initiation step at 92°C; (2) 92°C for 1 min, 35°C for 1 min and 45 sec, 72°C for 2 min, repeated for 45 cycles; and (3) 72°C for 7 min. Ramp times between temperatures were limited only by the capabilities of the machine. Amplification of replicated DNA samples was carried out for estimation of repeatability. Control tubes containing

all reagents except for DNA were used to determine artifactual products from the amplification. Amplification products were electrophoresed in 1.4% agarose gels in TAE buffer (40 mM Tris-acetate, pH 7.5 and 1 mM EDTA) and stained for 1 hr in ethidium bromide (0.5 µg/ml). Gels were imaged using a red filter on a Fotodyne high-resolution video camera with a Fujinon RV zoom lens, Mitsubishi video processor, Macintosh IICI color system with high resolution, Fotodyne capture card, and NIH Image and Collage 2.0 software. The gel images were scored from directly digitized photocopies. Molecular weight markers (pUC 19 digest #204, Discount DNA, Inc., Charleston, SC) were included on both outside lanes and at least every sixth lane of the electrophoretic gels for ease of comparison and consistency in scoring.

Forty-nine decanucleotide primers of arbitrary sequence were screened and 27 primers were ultimately

used to produce scorable bands (avg. 11.5 bands per primer) (Table 2). Duplicate amplifications using independent DNA extractions and different thermocyclers were used on 12 of the primers to test for repeatability. These results established sufficient confidence in the RAPD reactions that the remaining primer amplifications were not duplicated.

The presence/absence data were analyzed using the NTSYS-pc program, version 1.8 (Rohlf 1993). Similarity coefficients were derived using Jaccard's coefficient of similarity (Jaccard 1912) and the SIMQUAL option of NTSYS. The similarity matrix was then analyzed using the SAHN option for UPGMA clustering, and finally the TREE option for the construction of a phenetic tree based on percent similarity. We have also analyzed the presence and absence data to obtain indices of uniqueness for each population based on marker polymorphisms.

Table 2. Names, nucleotide sequences, and molecular weights of primers used in RAPD analysis and the total number of scorable markers produced by each primer.

Name	Sequence	MW	Total No. Markers
Operon P-1	5'-GTAGCACTCC-3'	2979	11
Operon P-2	5'-TCGGCACGCA-3'	3004	14
Operon Q-1	5'-GGGACGATGG-3'	3124	15
Operon Q-4	5'-AGTGCGCTGA-3'	3059	12
Operon Q-5	5'-CCGCGTCTTG-3'	2986	7
Operon Q-6	5'-GAGCGCCTTG-3'	3035	11
Operon Q-9	5'-GGCTAACCGA-3'	3028	7
Operon Q-12	5'-AGTAGGGCAC-3'	3068	7
Operon Q-13	5'-GCAGTGGACA-3'	3108	6
Operon Q-14	5'-GGACGCTTCA-3'	3019	14
Operon Q-15	5'-GGGTAACGTG-3'	3099	7
Operon Q-16	5'-AGTGCAGCCA-3'	3028	16
Operon S-1	5'-CTACTGCGCT-3'	2970	9
Operon S-6	5'-GATACCTCGG-3'	3019	10
Operon S-8	5'-TTCAGGGTGG-3'	3090	11
Operon Y-18	5'-GTGGAGTCAG-3'	3099	11
Operon Y-19	5'-TGAGGGTCCC-3'	3035	16
Operon Z-3	5'-CAGCACCGCA-3'	2973	15
Operon Z-4	5'-AGGCTGTGCT-3'	3050	14
Operon Z-6	5'-GTGCCGTTCA-3'	3010	11
Operon Z-7	5'-CCAGGAGGAC-3'	3053	8
Operon Z-9	5'-CACCCAGTC-3'	2924	8
Operon Z-11	5'-CTCAGTCGCA-3'	2979	21
Operon Z-13	5'-GACTAAGCCC-3'	2988	11
Operon Z-14	5'-TCGGAGGTTC-3'	3050	5
Operon Z-18	5'-AGGGTCTGTG-3'	3090	16
Operon Z-20	5'-ACTTTGGCGG-3'	3050	18



## Results

Of the 45 primers screened, 27 produced 311 scorable markers, which were used to determine similarity among samples. For each primer, gels were scored as to presence or absence of bands with molecular weights between 2000 and 400 bp (Figure 2). The phenogram produced using RAPD data demonstrates a high degree of similarity among the populations of *Arctomecon humilis* (Figure 3). Percent similarity among populations of a single taxon, as well as similarity observed among species, is indicated in Table 3. Intraspecific populations of *A. humilis* and *A. californica* display average percent similarities of nearly 95 percent, whereas populations of *A. merriamii* demonstrate only about 68 percent similarity.

The presence/absence data were also used to compare uniqueness of *A. humilis* populations to determine uniqueness indices that are not detectable using similarity only (Table 4). For example, although intraspecific percent similarity was very high for *A. humilis*, when the total number of markers generated from all primers was averaged, the Shinob Kibe population had fewer total bands

Table 3. Percent similarity observed among populations of single taxa and between different species.

	Number of Comparisons	Mean	Standard Error
Interpopulation similarity			
<i>Arctomecon humilis</i>	45	0.956	0.015
<i>A. californica</i>	3	0.939	0.021
<i>A. merriamii</i>	3	0.679	0.168
Interpopulation mean	3	0.858	0.155
Similarity between <i>Arctomecon</i> species			
<i>A. humilis</i> / <i>A. californica</i>	30	0.230	0.017
<i>A. humilis</i> / <i>A. merriamii</i>	30	0.188	0.007
<i>A. californica</i> / <i>A. merriamii</i>	9	0.155	0.008
Between species mean	3	0.191	0.038

than the mean for all populations (mean = 101.4). This population is the most northerly and the smallest of the *A. humilis* populations. The reduced number of markers generated for this population suggests that its genome is somewhat different than that present in the other populations. In the case of *A. merriamii*, one population sampled (Ash Meadows) produced nearly 75 percent of all



Figure 2. Photo of gel produced using RAPD amplification of total genomic DNA with primer OPZ-11.

Table 4. Characteristics of the suite of DNA markers generated for nine populations of *Arctomecon humilis*. Total number of bands generated with 27 primers is reported for each population. Numbers of total markers were adjusted for missing data. The number of markers that are uniquely present or absent (or are present or absent in only two or three populations) is also reported for each population. Population numbers correspond to the following names: (1) Santa Clara Butte, (2) Boomer Hill, (3) White Dome, (4) Webb Hill, (5) Atkinville, (6) Beehive Dome, (7) Red Bluff juveniles, (8) Red Bluff adults, (9) Warner Ridge, and (10) Shinob Kibe.

Parameter	Population Number									
	1	2	3	4	5	6	7	8	9	10
Total markers observed	102	104	100	98	102	97	100	99	97	86
Potentially missed markers	0	0	1	5	1	4	0	4	7	7
Probable total markers (70% probability)	102	104	103	103	103	101	100	103	104	91
Unique markers										
+	1	1	0	0	1	0	0	0	0	0
-	0	0	0	0	0	0	1	0	0	0
Unusual markers										
2 populations only										
+	0	0	0	0	0	0	0	1	1	2
-	1	1	1	1	1	1	0	0	0	0
3 populations only										
+	1	1	1	1	1	1	0	0	0	0
-	0	0	0	0	0	1	1	0	0	0
Unusual marker index										
+	2	2	1	1	2	1	0	1	1	2
-	1	1	1	1	1	2	2	0	0	0

Table 5. Summary of *Arctomecon merriamii* population uniqueness based on RAPD marker presence and absence data. Unique markers were considered to be those that occurred in or were absent from one population only.

Characteristic	Population			Total
	N. Las Vegas	Rainbow Canyon	Ash Meadows	
Total markers observed	95	100	98	129
No. unique markers				
present	2	5	21	28
absent	4	3	24	31
Index of uniqueness (unique + and -)	6	8	45	

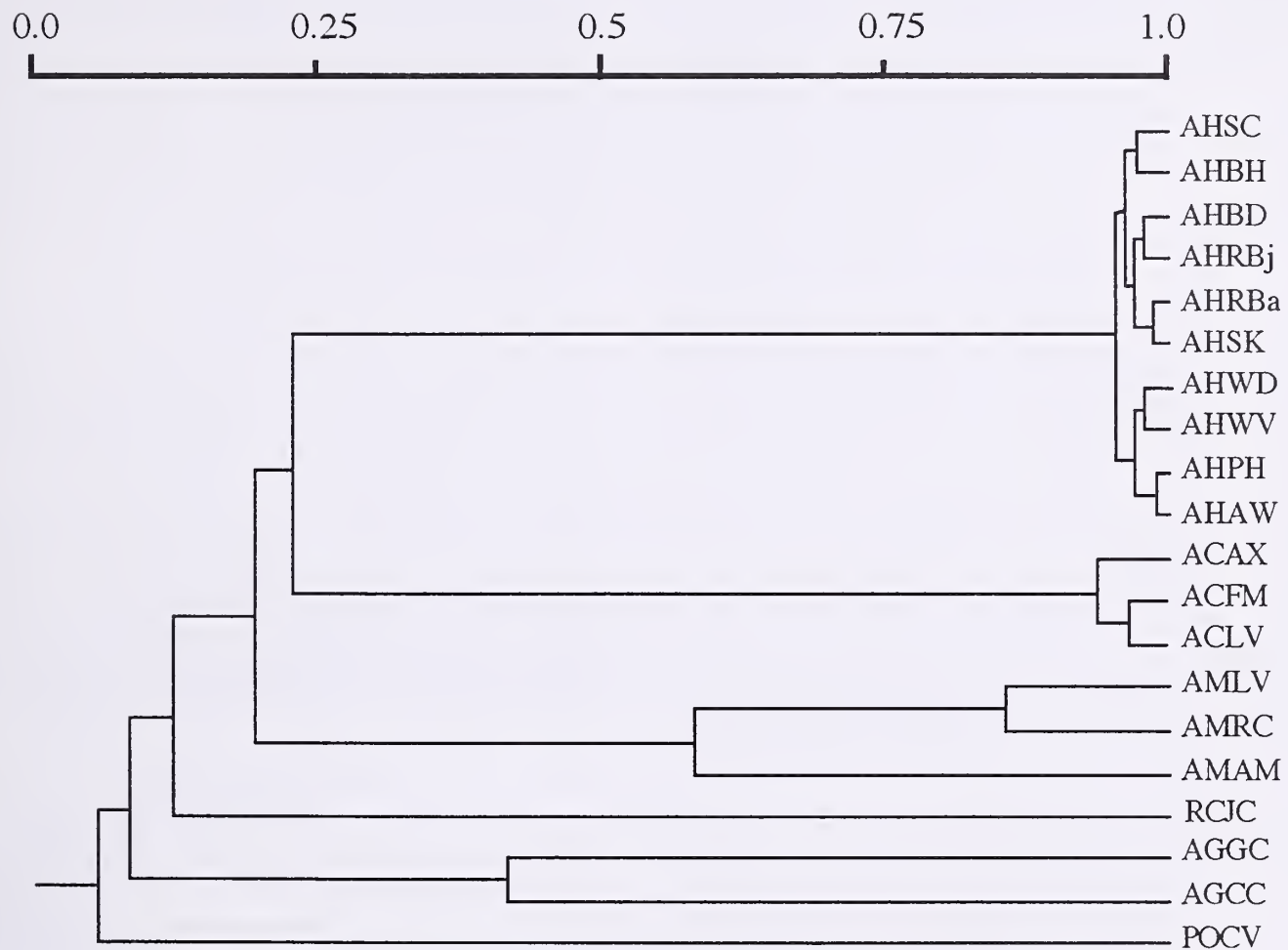


Figure 3. Phenogram produced from RAPD data showing percent similarity among *Arctomecon* populations. See Table 1 for legend abbreviations.

unique markers (those confined to a single population) for this taxon (Table 5). RAPD markers showed a mean percent similarity of about 20 percent among the three species of *Arctomecon*, with *A. humilis* and *A. californica* being most similar (23%) to each other. There was not a large difference between DNA markers for juvenile and adult age-class samples from the Red Butte population of *A. humilis*, but the two age classes do appear in different branches of the phenogram (Figure 3).

### Discussion

The success of a rare species is undoubtedly influenced by genetic variation regardless of external stresses upon the species. If the genetic potential for surviving stresses does not occur in a species' genome, conservation efforts may have little effect on the species' survival. This study provides information concerning the genomic structure of separate populations of the three species of *Arctomecon*. By quantifying the number of unique DNA markers in known populations of

a species, managers can prioritize preservation efforts for the various populations. *Arctomecon humilis* now exists as several disjunct populations derived from what was probably once a large contiguous population that has recently been subdivided as the required gypsiferous beds have eroded away. Such prior conditions would have allowed for a relatively constant flow of genetic information throughout the population. There is evidence that although the present populations are similar, each has some degree of uniqueness (Table 4). This genetic dissimilarity will most likely increase as populations continue to decrease in size and become more disjunct from each other. There is presently some degree of separation between populations occupying the eastern and western sides of the valley. Loss of any one of the populations would reduce the potential variation available to the species. Of the nine populations, the Shinob Kibe population appears to be in the greatest immediate danger of extirpation. It is the most disjunct population and represents the most northerly extension of the species' range. It is also the smallest population and is threatened on every side by development. Table 4 reveals the uniqueness of this population in terms of total bands amplified by the same suite of primers used for all other populations. This reduced number indicates a lesser degree of homology for primer binding sites, or in other words, a greater degree of dissimilarity relative to other conspecific populations. Although *A. humilis* appears to possess little genetic variation among populations, observed differences between juvenile and adult age classes at a single site suggest that genetic variation may be selectively sorted during the developmental process. Alternatively, the results may arise as different genomes are recruited from the seedbank by distinctive climatic conditions associated with individual germination events. In either case, selection on the pool of genetic variation could have important consequences for survival of the species.

Relationships of *Arctomecon* to other genera included in this study are suggested in the phenogram (Figure 3). Our data support the recent phylogenetic relationships reported by Kadereit et al. (1994) for the same genera in the family Papaveraceae. Although RAPD data must be interpreted cautiously when estimating phylogeny, the major divisions observed in Figure 3 are similar to groupings reported by Kadereit et al. (1994) using morphological characters. Our groupings are also and Kadereit (unpublished report) based on

chloroplast DNA restriction fragment analysis.

RAPD markers for individual populations provide managers with valuable information concerning the degree of genetic uniqueness resident in each population. In the absence of such information, managers must resort to plant numbers alone to decide the fate of individual populations that occur on sites for which requests have been made for developments that will either destroy or modify the habitat. Under such circumstances small populations would be preferentially sacrificed unless they were known to harbor unique genetic material.

### Acknowledgments

Funding for this study was provided by the Bureau of Land Management (BLM) and the College of Biology and Agriculture, Brigham Young University, Provo, Utah. The authors thank Valorie Armstrong, BLM, UT, Gayle Mars-Smith, BLM, NV, Janet Cooper, and Julie Tolman for assistance in locating the study sites, collecting material, and manuscript preparation. We thank Kaye Thorne, assistant curator at the herbarium at Brigham Young University, for housing and verifying voucher specimens.

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# Use of Random Amplified Polymorphic DNA (RAPD) Markers for Genetic Analysis of *Lilium parryi*, a Rare Arizona Plant

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**Abstract:** Genetic variation in several different Arizona and California populations of the rare plant species *Lilium parryi* Wats. was analyzed using the random amplified polymorphic DNA (RAPD) marker technique. The plants included two representatives of a newly identified population from east Turkey Creek in the Chiricahua Mountains. These recently discovered plants were strikingly different from all of the other populations studied, including both Arizona and California populations, indicating a significant level of genetic divergence. The observation that this population is genetically unique suggests a distinct taxonomic identity and long-term genetic isolation for this population. In contrast, the majority of the remaining Arizona plants showed very little genetic variability and appeared to represent a single interbreeding group. Considering the overall genetic variability, the small population sizes, and the rareness of the habitats required by these plants, we propose that this species should qualify for additional protection.

## Introduction

*Lilium parryi* Wats., the lemon lily, is native to the San Gabriel and San Bernadino mountains of southern California (Mistretta and Parra-Szjij 1991), southeast Arizona, and Sonora, Mexico (Malusa et al. 1993) at elevations of 1700 m to 3000 m. The plant is a bulbous herbaceous perennial that produces showy, fragrant yellow flowers with red spots in the throat. In California, populations numbering in the thousands are found in high-elevation meadows, and smaller populations are found in canyons with perennial water. Much smaller populations, most numbering less than 100, are also found in the Santa Rita and Huachuca Mountains of southeast Arizona and in the neighboring mountain ranges of Sonora, Mexico. An exception is a population of about 500–1000 plants in Miller Canyon of the Huachuca Mountains (Falk and Warren 1994, Warren et al. 1989, our surveys). In these locations, the lemon lily grows along wet canyon bottoms near a perennial source of water. The species is currently listed as category 2 by the U.S. Fish and Wildlife Service.

A recent study of California and Arizona populations of *Lilium parryi* using allozyme data showed that, with respect to the California populations, the Arizona populations (1) had less variability, (2) usually carried only a subset of the alleles present in the California populations, and (3) usually retained only the more common alleles present in the California populations at a given locus. These patterns of genetic variation were con-

sistent with colonization of Arizona locations by California plants, followed by loss of alleles due to founder and inbreeding effects (Linhart and Premoli 1994). Another possibility is that all Arizona populations of *L. parryi* are relicts of a once more widespread distribution that is now confined to small populations, which have lost genetic variability due to drift.

In order to investigate the origin of the Arizona plants further, we have examined genetic variation in *Lilium parryi* using RAPD loci, which are more sensitive to genetic variability than the allozyme method (Rieseberg and Ellstrand 1993). We have acquired some experience in using these markers to analyze rare plant populations and we describe these procedures and methods of analysis. In addition, a newly discovered and genetically divergent population of *L. parryi* from east Turkey Creek in the Chiricahua Mountains of southeast Arizona is analyzed here for the first time. The present paper summarizes the results of this study, which will be described in detail elsewhere.

## Materials and Methods

Two leaves were collected from each of the 28 plants at the locations indicated in Table 1. Tissues of plants from two locations in southern California were generously provided by Mailie Neel. Leaves were stored in plastic bags on ice and were placed into storage at -70°C within 48 hours.

Table 1. Origin of *Lilium parryi* plants used in this study.\*

Plant	Location
1	Ramsey Canyon 1, Huachuca Mountains
2	Ramsey Canyon 2, Huachuca Mountains
3	Ramsey Canyon 3, Huachuca Mountains
4	Ramsey Canyon 4, Huachuca Mountains
5	Ramsey Canyon 5, Huachuca Mountains
6	Miller Canyon Site B 1, Huachuca Mountains
7	Miller Canyon Site B 2, Huachuca Mountains
8	Miller Canyon Site B 3, Huachuca Mountains
9	Miller Canyon Site B 4, Huachuca Mountains
10	Miller Canyon Site B 5, Huachuca Mountains
11	Miller Canyon Site C 1, Huachuca Mountains
12	Miller Canyon Site C 2, Huachuca Mountains
13	Miller Canyon Site D, Huachuca Mountains
14	Metcalf Creek 1, Boulder Group Camp, CA
15	Lemon Lily Spring 1, CA
16	Lemon Lily Spring 2, CA
17	Lemon Lily Spring 3, CA
18	Lemon Lily Spring 4, CA
19	Lemon Lily Spring 5, CA
20	Florida Canyon 1, Santa Rita Mountains
21	Florida Canyon 2, Santa Rita Mountains
22	Ramsey Canyon Site E, Huachuca Mountains
23	Metcalf Creek 2, Boulder Group Camp, CA
24	Bear Canyon 1, Huachuca Mountains
25	Bear Canyon 2, Huachuca Mountains
26	Bear Canyon 3, Huachuca Mountains
27	Turkey Creek 1, Chiricahua Mountains
28	Turkey Creek 2, Chiricahua Mountains

\*Plant numbers refer to field notes of DM and MF. The Huachuca and Chiricahua mountains are in Cochise County, and the Santa Rita Mountains are in Santa Cruz County, Arizona. The Lemon Lily Springs plants are from the SW1/4 of sec. 25, and the Metcalfe Creek plants from the SE1/4 of sec. 26, township 2N, range 1W.

DNA was isolated using the procedure of Doyle and Doyle (1990), except that the CTAB extraction step was repeated. The step improved reliability of PCR amplification.

Seven decanucleotide primers, purchased from Operon Technologies Inc., were used to amplify fragments from genomic DNA. The primers and their sequences are listed in Table 2. PCR reaction mixtures contained 25 nm DNA, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01% w/v gelatin, 50 mM each dATP, dCTP, dGTP, dTTP, 1 unit Taq polymerase (Stratagene) and 0.5 mM primer, for a total reaction volume of 30 ml. Reactions were carried out in a Cetus/Perkin-Elmer thermocycler with the following reaction conditions: 94° for 3 min., followed by 45 cycles of 94° for 1 min., 36° for 1 min., and 72° for 2 min. ending with 1 cycle of 72° for 5 min. The amplified

products were then size fractionated on a 1.4% agarose gel containing ethidium bromide and visualized under UV light. PCR amplification using a given primer was performed on all plants at the same time, along with controls lacking plant DNA, and the products were then scored on the same gel. In a small fraction of cases, it was necessary to rerun the reactions of certain plants since no products were obtained. In such cases, control plants previously scored as positive or negative were included. Using these methods, the production of RAPD bands was highly reproducible.

Presence or absence of all scorable bands was determined for each plant. The amplification of an RAPD band of the same approximate size in two plants was interpreted to mean that the plants share a particular DNA sequence and are similar in the corresponding regions of the plant genome. For within-population comparisons, the percentage of total scorable bands that was polymorphic in each population was determined. For between-population comparisons, genetic distances were calculated between all pairs of plants, using the formula

$$D_{xy} = 1 - (nXY / (nX + nY - nXY))$$

where nXY is the number of shared bands between plants X and Y, nX is the number of bands present in plant X, and nY is the number of bands present in plant Y (Marsolais et al. 1993, Nei 1972). These pairwise genetic distances were then used to create a dendrogram showing the relationship between individual plants using the Neighbor-Joining algorithm from the Phylip suite of phylogenetic programs (Felsenstein 1993) using mid-point rooting.

Genetic variation in the rare Arizona plant *Lilium parryi* was analyzed using random amplified polymorphic DNA (RAPD) markers, as

Table 2. Primers used for RAPD marker amplification.

Primer ID Code*	Sequence
F3	5'-CCTGATCACC-3'
F13	5'-GGCTGCAGAA-3'
G7	5'-GAACCTGCGG-3'
G12	5'-CAGCTCACCA-3'
G14	5'-GGATGAGACC-3'
H6	5'-ACGCATCGCA-3'
H16	5'-TCTCAGCTGG-3'

\*The primer ID code is that assigned by Operon Technology Corp.

described above. This method depends upon the selective amplification of random DNA sequences in the target DNA. In normal polymerase chain reaction (PCR) amplification, two different primers of specified sequence and approximately 20 bp long are used to amplify a target DNA fragment (Figure 1A). The sequence of these primers is based upon prior knowledge of a gene sequence in another organism. In RAPD marker amplification, a single primer of randomly selected sequence and approximate length 10 bp is used. These primers amplify DNA sequences that carry a sequence complementary to that of the primer on both DNA strands. As indicated in Figure 1B, these priming sites must be located approximately 1 kb apart for successful amplification to occur. Presence or absence of a particular amplified product is scorable as a polymorphism between individuals. The loss of a product is thought to occur because of a point mutation in one of the priming sites, which prevents primer annealing (see Rieseberg and Ellstrand 1993).

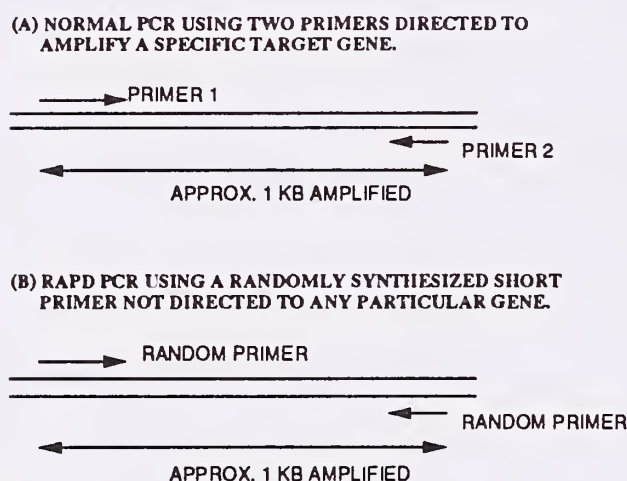


Figure 1. Comparison of normal PCR amplification of genomic DNA with RAPD PCR amplification.

RAPD loci have several distinct advantages for analysis of rare plant DNA: (1) very little plant material is needed, (2) the method is relatively simple and inexpensive, and (3) polymorphisms are found quickly. However, the method also has several problems and limitations. First, not all bands show clear-cut intensity changes scorable as polymorphisms. Second, two bands at the same gel position may not have the same sequence, that is, they may not originate from the same chromosomal location. The likelihood of sequence identity is increased when analyzing closely related individuals. Identity may also be confirmed by cutting the amplified fragment with restriction endonucle-

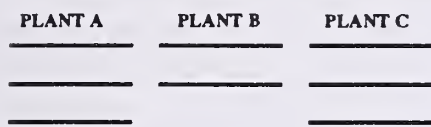
ases or by sequencing. Third, absence of a band in two individuals may not mean that these two carry the same sequence change which prevents amplification. Fourth, the method is sensitive to contaminating DNAs from molds or skin through lab handling. Fifth, RAPD bands are not always reproducible at a later date or in another lab. These last two problems are largely circumvented by careful experimental technique, and by amplifying all of the DNA samples with a given primer at the same time. For example,  $Mg^{++}$  concentration and ratio of genomic DNA to primer concentration must be carefully controlled, and the age of specimen DNA and primer taken into account.

For genetic analysis using RAPD data, we define a locus as a specific chromosomal site that can potentially give rise to an RAPD band. Each locus is assumed to be a two-allele system, a marker allele that is amplified to give an RAPD band and a null allele that is not amplified. Further, the marker allele is assumed to be dominant (Friar, Robichaux and Mount, in preparation). When DNA derived from somatic tissues is used, a band is obtained whether the marker allele is present on one or both chromosomes. Accordingly, three categories of RAPD loci are defined in a population using this system. First, detectably polymorphic loci when the marker allele is present in some individuals, but not others. Second, detectably monomorphic loci when the marker allele is not present in any individual in the population. Third, noninformative loci when the marker allele is present and their variances have been described (Lynch and Milligan 1994). A phylogenetic analysis can also be performed using RAPD loci, but such an analysis assumes sequence identity of the marker and null alleles in the compared populations, an assumption that may be difficult to test.

Two possible types of variation in RAPD loci among populations are shown in Figure 2. In (a), there is very little variation among the three individuals shown, indicating a strong degree of genetic similarity. Such a population may be identical due to the recent introduction of a few individuals (founder effect), inbreeding within the population, some other reproductive or biological property of the species, or selection which maintains genetic similarity. In Figure 2(b) there is a higher degree of RAPD loci polymorphism, indicating more genetic variation in the population. However, some loci are shared in this example, also indicating genetic relatedness. As the number of shared loci decreases, the likelihood that similar sized bands have the same sequence and that



(a) POPULATION WITH LOW LEVEL OF POLYMORPHISM



(b) POPULATION WITH HIGH LEVEL OF POLYMORPHISM

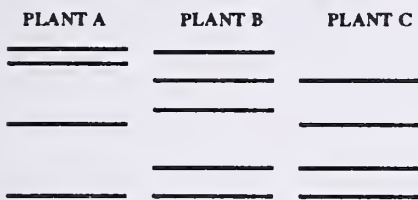


Figure 2. Analysis of RAPD band variation in populations.

absence of a band is the same allele also decreases. In spite of these limitations, RAPDs can provide a quick and reliable analysis of genetic variation, as demonstrated below for *Lilium parryi*.

## Results

### Variation Within *Lilium parryi* Populations

The presence vs. absence of amplified fragment bands was scored in 28 plants using seven RAPD primers. Every primer used showed variability in at least one plant. Of a total number of 84 RAPD loci scored, 77 were detectably polymorphic. Among the Arizona populations, the percentage of loci that were detectably polymorphic varied from less than 1 percent for the Miller Canyon population to 42 percent for the Turkey Creek population (Table 3). Thus, the amount of genetic variation in these populations appears to

Table 3. RAPD marker polymorphism in populations of *Lilium parryi*.\*

Population Location	Estimated Population Size**	Percent Variable Bands
Huachuca Mountains		
Miller Canyon	500-1000 (8)	<1
Ramsey Canyon	10 (6)	5
Bear Canyon	5 (3)	13
Santa Rita Mountains		
Florida Canyon	5 (2)	4
Chiricahua Mountains		
Turkey Creek	20 (2)	42
California Plants		
Lemon Lily Springs	>1000 (4)	6
Metcalf Creek	>1000 (1)	-

\* 77 bands were scored & 84 of these bands were polymorphic.

\*\* Number of plants sampled is shown in parentheses.

be highly variable. The level of detectable polymorphism in RAPD loci was least in the Miller Canyon population, which has the largest number of individual plants among the Arizona populations. The other Arizona populations showed intermediate levels of variation with 4-13 percent of the loci being detectably polymorphic. Not enough California plants were analyzed for an accurate comparison with the Arizona plants. An intermediate level of 6 percent of the RAPD loci in two Lemon Lily Spring plants were detectably polymorphic.

### Variation Between *Lilium parryi* Populations

The fraction of RAPD loci not shared between each pair of plants listed in Table 1 was determined and was then used to calculate the average distance between populations, as previously. These averages were then used to produce a dendrogram which is overlaid on a location map for the populations in Figure 3. The newly discovered Chiricahua Mountain population was dramatically distant from the remaining populations studied. The range of distances between these plants and the others corresponded to 0.42-0.51 of RAPD loci not being shared, a highly unusual amount of variation for populations of the same species. The remaining Arizona plants fell into one group, of which the Bear Canyon plants appeared to be the most unique. Collectively, all of the Arizona plants occupied a common region of the tree, indicating a genetic difference from the California plants.

## Discussion

These studies of genetic variation in Arizona populations of *Lilium parryi* have revealed unusual variation both within and between populations. The most dramatic result was the unique genetic composition of the newly identified population in the Chiricahua Mountains. These plants showed approximately 47 percent nonsharing of RAPD loci with all other Arizona plants and California plants. In addition, there was considerable variation between the two plant representatives from this population (42% of RAPD loci were detectably polymorphic). There was also no evidence of discernible morphological differences among these plants. Although this sample was small, the results clearly indicate that this population is genetically both unique and variable. These results raise the possibility that these plants are representatives of a larger interbreeding population close to the Chiricahua Mountains, perhaps from other sites in

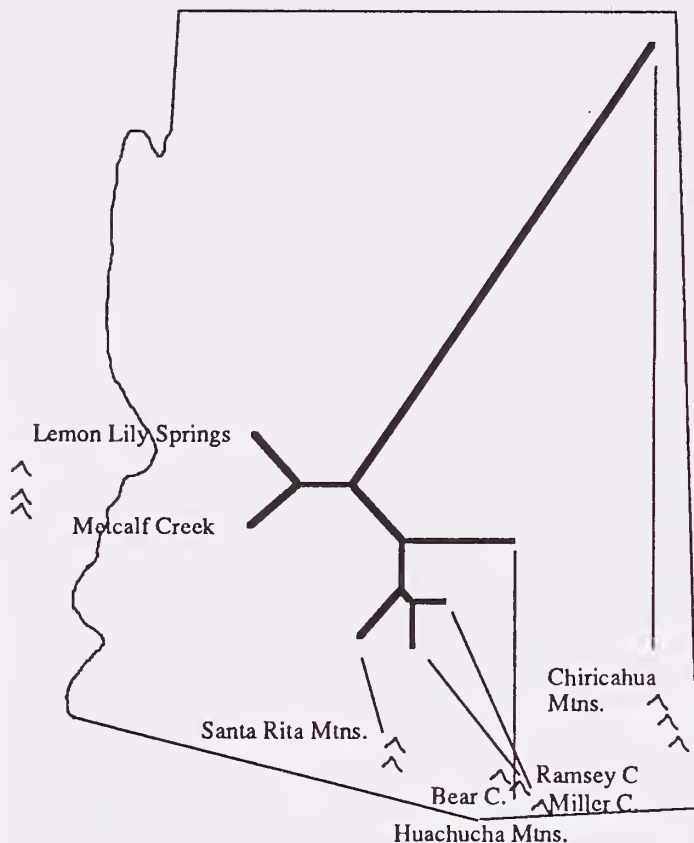


Figure 3. Genetic relationships among Arizona and California populations of *Lilium parryi*. The distance between the populations of the Chiricahua and Huachuca mountains corresponds to approximately half of the RAPD bands not shared, and the other distances are similarly scaled. The map is not to scale.

southeast Arizona, southwest New Mexico, or Sonora, Mexico. However, it is also possible that these plants are recently derived from a larger variable population that has been recently reduced in size, but have not yet been subjected to loss of variability through inbreeding. Unfortunately, a large forest fire in the Chiricahua Mountains in the summer of 1994 resulted in subsequent flooding and extensive erosion of east Turkey Creek, including the site of the *L. parryi* population. In the summer of 1995, these plants were no longer present. It will be interesting to monitor this site to determine whether or not this population can re-establish itself in Turkey Creek Canyon.

The genetic differences between the Chiricahua Mountain plants and the other Arizona plants suggest that there has been no significant gene flow between these populations. In contrast, there is very little RAPD marker differentiation between the Santa Rita and Huachuca Mountain populations. Since RAPD markers are highly sensitive indicators of genetic variation, these results show that *L. parryi* from the Santa Rita Mountains and most *L. parryi* from the Huachuca Mountains are

genetically very similar. This lack of segregation of genetic similarity with geographic separation argues for considerable long-distance gene flow occurring within the Huachuca Mountains, via pollen spread by pollinators or seed movement. This situation would promote the exchange of genetic information between widely spaced plants, and reduce the amount of genetic differentiation between separate populations. Therefore, it may be reasonable to consider the Huachuca Mountain population to be composed of a large interbreeding group of individuals, rather than a group of several smaller populations. Since the Miller Canyon population is the largest and least variable in Arizona, this population could have been recently derived from a small number of individual plants or by inbreeding, without sufficient time having passed to reintroduce a higher level of variability.

The above results are in good agreement with an enzyme polymorphism study of many of these same populations of *L. parryi* (Linhart and Premoli 1994). The raw percentage of RAPD markers that were detectably polymorphic (<1–13%) in the Arizona plants was comparable to the percentages of enzyme loci that were polymorphic (0–15% at the 95% probability level). In their study, Linhart and Premoli also found Miller Canyon plants to be the least variable. Our results support a model of a former widespread distribution of *Lilium parryi* followed by a reduced distribution into isolated populations and long-term regional isolation of certain of these populations from each other. These results do not rule out an introduction of California plants into certain Arizona locations, as proposed by Linhart and Premoli (1994).

### Acknowledgments

This work was supported by contracts from the Coronado National Forest. We are grateful to Mailie Neel for providing plant material from the California populations of *Lilium parryi*, to Mark Fishbein, Peter Warren and Tom Wood for information and advice, and to Y. Linhart and A.C. Premoli for providing their manuscript prior to publication.

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# Molecular and Adaptive Variation: A Perspective for Endangered Plants

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**Abstract:** Molecular data are currently being widely used in decisions concerning endangered and threatened plant species. Although molecular data can be valuable under many scenarios, they may not always be correlated with or able to identify adaptive variation, a central concern in conservation biology. Below we first present data from Scots pine, *Pinus sylvestris*, that show a lack of association of individual allozyme heterozygosity and fitness. As a result of this study and an examination of the published results from other studies, it appears that what positive associations have been observed are probably not, in large part, due to the presence of intrinsic heterozygote advantage. Second, while the level of molecular variation for allozymes and DNA markers is similar to that for a quantitative trait within Scots pine populations, the extent of differentiation between populations is much greater for the quantitative trait than for the molecular markers. From these observations and other considerations, we feel that molecular information should be used very cautiously in attempting to understand adaptive differences within and between populations and should be used in conjunction with other information, such as that relating to the geographical, ecological, life history, historical, and other aspects of the taxa to determine evolutionarily significant units.

## Introduction

Molecular genetic variation—for example protein or DNA variation within or among individuals, populations, or higher taxa—has been widely used to examine various issues in biological conservation and has been particularly useful in a number of arenas. For example, neutral molecular genetic markers are extremely well suited for investigation of questions related to the history and structure of a population, the mating or reproductive system of an organism, or the extent of reproductive isolation from other populations. However, when investigating adaptation within a population or adaptive differences among populations, the same nuclear markers that are so useful in determining non-selective evolutionary factors may be inappropriate, because they may be uncorrelated with adaptive variation. Further, in determining the unit of conservation for a species, one should be cautious in relying completely on molecular genetic variation, because of the potential lack of correlation with adaptive differences. As a result we advocate a comprehensive evaluation of all available information: genetic, distributional, ecological, historical, etc., in determining conservation units, an approach similar to that used in determining evolutionarily significant units in salmon in the northwestern United States (Waples 1995).

The application of molecular techniques to document the large amount of genetic variation in many species was one of the major breakthroughs

in evolutionary genetics (see Lewontin 1991). However, since this discovery, a heated debate has continued concerning the mechanisms responsible for maintaining this genetic variation. Although the overall pattern of variation for allozymes (allelic enzyme variants), both within and between populations, is generally consistent with expectations assuming that variants are equally fit or neutral with respect to each other (e.g., Nei 1987), some studies appear to indicate that different allozyme genotypes may have different fitness consequences (e.g., Koehn et al. 1988). Further, there have been reports that the extent of allozyme heterozygosity in an individual may be correlated with various quantitative traits, including a number of fitness components and life-history traits, although this finding is far from universal (for recent reviews, see Houle 1989, Bush and Smouse 1992, Pogson and Zouros 1994). In addition, some studies suggest that the level of heterozygosity within a population may be correlated with the fitness of the population (Quattro and Vrijenhoek 1989, however, see Sheffer et al. 1995) or that the level of molecular differentiation between populations is predictive of the level of adaptive differentiation between them.

The data presented below on these questions are generally not from rare and endangered plants of the Southwest, but are from a species, Scots pine (*Pinus sylvestris*), for which there are extensive data sets, mainly because of its commercial importance. From these studies, we can understand the

basic mechanisms responsible for the relationship (or lack thereof) between molecular variation and adaptive variation and thereby have a reasonable basis for inferring any possible relationships in endangered plants, species in which such extensive experimentation or study is generally not possible (or at least not yet done).

### Individual Heterozygosity and Fitness

Determining the generality of the correlation between molecular measures of genetic variation and adaptive variation is of great significance in evolutionary genetics, plant breeding, and conservation of endangered or threatened species. For example, in tree breeding (and captive breeding of endangered animals), it has been suggested that

individual heterozygosity, the proportion of sampled loci heterozygous in a given individual, be measured and individuals with the highest heterozygosity be selected as breeders. Although these ideas have not been widely adopted in silviculture, there is a general interest in finding a molecular genetics approach to complement the recognized plant breeding methods generally used (e.g., Bush and Smouse 1992).

There are three major genetic explanations for positive associations between individual heterozygosity and components of fitness, such as viability, fecundity, or mating success, or other quantitative traits, such as diameter or height (e.g., Charlesworth 1991, Houle 1989). First (Figure 1a), heterozygotes at the molecular locus itself may have a

#### (a) Intrinsic heterozygote advantage

Genotype	$A_1A_1$	$A_1A_2$	$A_2A_2$
Fitness	$1 - s_1$	1	$1 - s_2$

#### (b) Apparent heterozygote advantage due to linkage disequilibria

Genotype	$\frac{A_1 + -}{A_1 + -}$	$\frac{A_1 + -}{A_2 - +}$	$\frac{A_2 - +}{A_2 - +}$
Fitness	$1 - s_1$	1	$1 - s_2$

#### (c) Apparent heterozygote advantage due to genotypic association

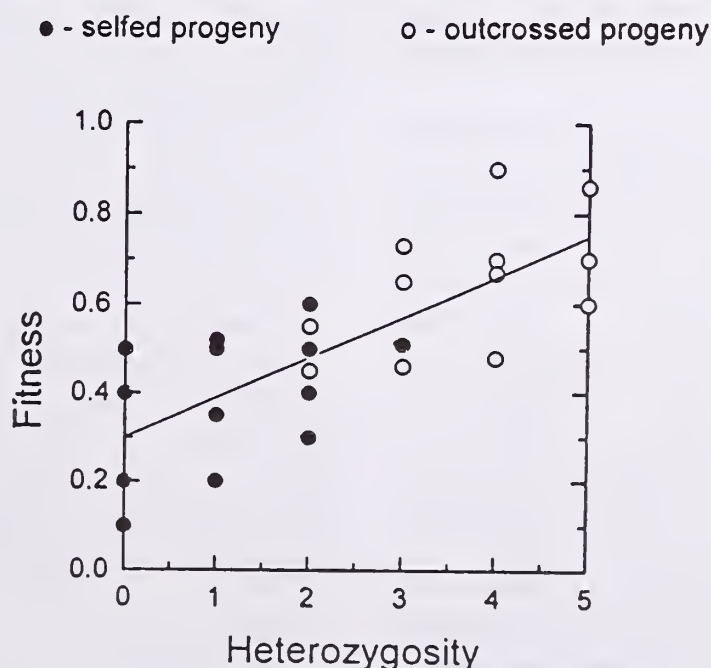


Figure 1. The three different explanations for an association between individual molecular heterozygosity and fitness: (a) intrinsic heterozygote advantage, (b) apparent heterozygote advantage due to linkage disequilibria, and (c) apparent heterozygote advantage due to genotypic association.

higher fitness or quantitative trait value than homozygotes; that is, there is intrinsic heterozygote advantage (or overdominance, as it is often called) at the loci being examined. Although intrinsic heterozygote advantage is a commonly cited mechanism for maintenance of genetic polymorphism (two or more genetic variants at a given locus), its presence has been extraordinarily difficult to document both in its simplest form (e.g., Allison 1964) or as an average over various environments (e.g., Hedrick 1986).

Second (Figure 1b), statistical associations between the alleles at molecular loci and alleles at other, generally unidentified selected loci may result in an apparent heterozygote advantage at the molecular loci. Usually the loci showing such associations are linked in random-mating populations, but may likely be unlinked in highly selfing populations. The extent of such statistical associations is determined by a measure termed linkage (or gametic) disequilibrium (e.g., Hedrick 1985). The unidentified, selected loci are probably dominant, masking the detrimental effects of recessive alleles at several loci so that overall they may result in the appearance of heterozygote advantage, also termed associative overdominance, at the molecular loci (Frydenberg 1963, Houle 1989, Zouros 1993). The statistical associations (linkage disequilibria) between alleles at molecular loci and selected loci, an essential part of this scenario, may be generated by such evolutionary factors as genetic drift (sampling effects in small populations) or by population structure (genetic isolation among various parts of the population) (e.g., Hedrick 1985).

Finally, there may be associations of molecular genotypes and components of fitness or quantitative traits due to the presence of high amounts of inbreeding or different levels of inbreeding in the population, a phenomenon that can be called multilocus genotypic association and quantified using the concept of identity disequilibrium (see Charlesworth 1991 for a detailed explanation). In this case again, neutral molecular alleles may show apparent heterozygote advantage, here due to the genotypic associations caused by inbreeding effects and generally enhanced by linkage. As a simple example (Figure 1c) of what may occur even when there are no linkage disequilibria between the molecular loci and selected loci, if inbred individuals that have both low molecular heterozygosity and low fitness are unknowingly lumped or pooled in a sample with outbred individuals that have relatively higher values of

both heterozygosity and fitness, then a positive association of individual heterozygosity and a fitness component may occur (e.g., Ledig et al. 1983, Ledig 1986, Hedrick 1990). Because the type of parents (related or unrelated) is generally not known, such lumping of individuals may occur, for example, when there is a mixture of selfed and outcrossed progeny either within or between progeny groups.

In the data presented below (and given in greater detail in Savolainen and Hedrick 1995), we have attempted to determine if intrinsic heterozygote advantage is likely to be a major factor resulting in a correlation between allozyme heterozygosity and a number of quantitative traits related to fitness in Scots pine, *Pinus sylvestris*. For the two types of apparent heterozygote advantage to be operative, a statistical association between the allozyme loci and other selected loci needs to have been generated by genetic drift, population structure, or inbreeding. In this study, there are two factors that indicate that if such a correlation is observed, it is very likely to be the result of intrinsic heterozygote advantage.

First, Scots pine in Finland (and other areas) has a very large population size with extensive pollen flow so that any associations caused by linkage disequilibria generated by genetic drift or population structure are unlikely (e.g., Muona and Szmidt 1985, Muona and Harju 1989). Second, even though Scots pine is self-compatible, at the mature seed stage less than 5 percent of the selfs remain (Muona and Harju 1989). While seeds show an excess of homozygosity due to selfing, adult tree genotypes in Scots pine populations are in Hardy-Weinberg proportions (e.g., Muona and Harju 1989) and there is no genetic evidence of inbreds surviving. Other data, such as the very high genetic load and extremely high seed-to-adult mortality in Scots pine in Finnish forests, also make it extremely unlikely that there are any inbreds among adults (e.g., Savolainen and Karkkainen 1992). As a result, it is unlikely that we would observe in Scots pine an association generated by any of the non-selective evolutionary factors mentioned above, such as genetic drift, population structure, or inbreeding, unless the selected loci involved were very closely linked to the allozyme loci.

In addition, several experimental aspects of these data may also allow documentation of intrinsic heterozygote advantage in Scots pine if it is indeed present. First, six different quantitative traits related to fitness were measured, including

measures related to viability and to both female and male reproduction. Second, because of the high allozyme heterozygosity in Scots pine, 12 loci were polymorphic so that there was a large range of individual heterozygosity. Third, genotypes in two of the populations (Viitaselkä and Vilhelminmäki) have been cloned and measurements were taken on multiple ramets of the same clone so that an excellent measure of the intrinsic genotypic value for the quantitative trait is possible. Fourth, the other population, Yllästunturi, was close to tree line above the Arctic Circle, one of the most extreme and stressful natural environments for Scots pine anywhere.

To determine whether there is an association between heterozygosity and these quantitative traits, we used several different approaches. For example, for each combination of locus, population, and trait, we carried out an ANOVA between homozygotes and heterozygotes. Overall, of 156 such comparisons, only 12 or 7.7 percent were significant, very close to the 5 percent expected by chance. And in the 12 significant tests, the heterozygotes had higher values in six and the homozygotes had higher values in the other six. We also looked to see if any loci showed a greater than 5 percent proportion of tests significant and no locus had more than two significant tests over the three populations and the six different traits.

Effects of individual alleles were also studied by regression analyses (see Savolainen and Hedrick 1995 for details). Over all these tests, the Viitaselkä population had the highest number, 15 out of 144, or 10.4 percent, of the tests (ANOVA and regression) significant while Vilhelminmäki had 6 of 72 or 8.3 percent significant and Yllästunturi had 4 of 96 or 4.1 percent significant. Because the quantitative traits in both Viitaselkä and Vilhelminmäki were based on means of five or six trees of the same genotype, one might expect that if there was any intrinsic association, it would most likely be picked up in these populations. On the other hand, because the Yllästunturi population is in a very extreme environment at tree line above the Arctic Circle, one might expect that any associations would be more apparent in such a marginal environment. In any case, the proportion of significant tests in all the populations was not much different from 5 percent, suggesting that these effects had only marginal, if any, influence.

A useful way to illustrate these results is given in Figure 2 (after Houle 1989) in which the level of significance is given for the three populations and 12 different loci for ANOVA. Obviously, there is no pattern for these results, such as a clustering near the 5 percent significance vertical line for different loci or populations. This supports the above conclusion that there are no locus- or

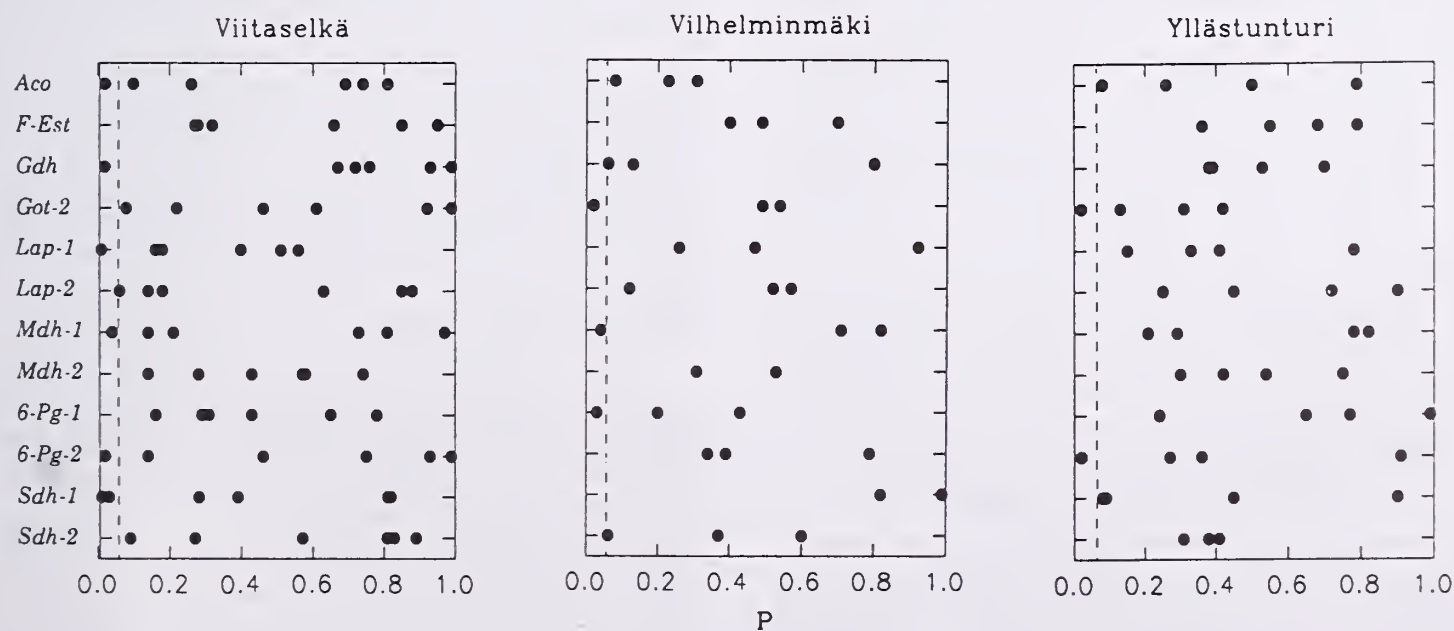


Figure 2. The probability of significance level for a difference between heterozygotes and homozygotes from the ANOVA for the 12 polymorphic loci in the three different populations. The individual points are the probability level for a given quantitative trait. The vertical broken lines indicate significance at the 5 percent level.

population-specific effects on these traits, favoring either heterozygotes or homozygotes.

Because these traits individually appear to be unrelated to the allozyme variants, we made a simple composite fitness measure of the traits that may give a value more closely related to overall fitness (see Savolainen and Hedrick 1995). This composite measure of fitness can be calculated for each tree and then compared to its heterozygosity. None of these tests were significant for the three populations. In other words, this composite measure of fitness does not detect any effects of the allozymes that were missed by examining only one of the quantitative traits related to fitness.

As another approach to examining the association of heterozygosity and the various quantitative traits, the association of the number of heterozygous loci in individual trees and the value of the six different traits was examined in the three populations using multiple regression. Out of 16 combinations of traits and populations, none were significant at the 5 percent level. Over all these combinations, only 5.8 percent of the variation (average  $R^2$  value) was explained by the multiple regression analysis. In fact, much of this variance was explained by a negative association of heterozygosity and the quantitative traits.

As a graphical way to illustrate these results, Figure 3 gives for individuals with different numbers of heterozygous loci, the values of three traits, pollen production, cone production, and height, for the three different populations. Because there is high polymorphism at a number of loci in these populations, the range of individual heterozygosity is high compared to many other studies. Obviously, there is no apparent positive association in Figure 3; for example, low individual heterozygosity does not necessarily imply low values for these traits and high heterozygosity does not necessarily imply high values for these traits. In fact, there appears to be a general lack of pattern between these traits (and the other traits that are not shown here) and individual heterozygosity.

### Molecular and Adaptive Differences Between Populations

Genetic differences between populations may be the result of several evolutionary factors (e.g., Hedrick 1985). For example, isolated or peripheral populations may have less molecular variation than other populations because of genetic drift, either through small population size, repeated bottlenecks, or low founder numbers. However, if

peripheral populations have not been isolated for long, the cumulative effects of genetic drift may not yet have had much impact. Populations may become differentiated if they are somewhat reproductively isolated from each other (not connected by gene flow) so that genetic drift and mutation result in cumulative molecular genetic differences. Genetic variation may be restored to isolated populations by gene flow or mutation and differentiated populations may become similar for molecular variants if gene flow is substantial (approximately one or more migrants per generation).

For adaptive genetic variation to be accumulated in a population, the size of the selective difference needs to be larger than the effect of genetic drift. In a similar manner, the extent of selection must be larger than the amount of gene flow for selective differences to occur even though there is gene flow from outside the area of selection. Of course, selective differences can only occur if genetic variation is present that allows adaptation. If it is not present, it may be introduced by mutation, gene flow, or recombination. If some populations exist in habitats with different environments than other populations, then adaptive genetic differences between the populations may result because of differential selection.

With this context, let us discuss the molecular and adaptive variation that is found in Scots pine in Finland. Scots pine colonized Finland after the last glaciation and has been in areas of northern Finland for less than 10,000 years (Eronen and Huttunen 1993) or only a few hundred generations. The environment of northern Finland is quite severe because of the extreme cold and the very short growing season. As an illustration of this severity, the probability of a Scots pine seed crop here (an area with only 50% germination ability) is less than 5 percent, because the growing season is generally too short for seed maturation (Henttonen et al. 1986).

As we discussed above, Scots pine populations are highly variable for molecular markers. The level of allozyme heterozygosity is high in nearly all populations, north and south, and the populations from north to south, about 1000 km, are hardly differentiated (see Table 1) (Karhu et al. 1995). Notice that the mean heterozygosity value is 0.35 in both southern and northern groups of populations and that the level of differentiation (0.02) is near its minimum of zero, which would occur if the allele frequencies were identical in the two groups.



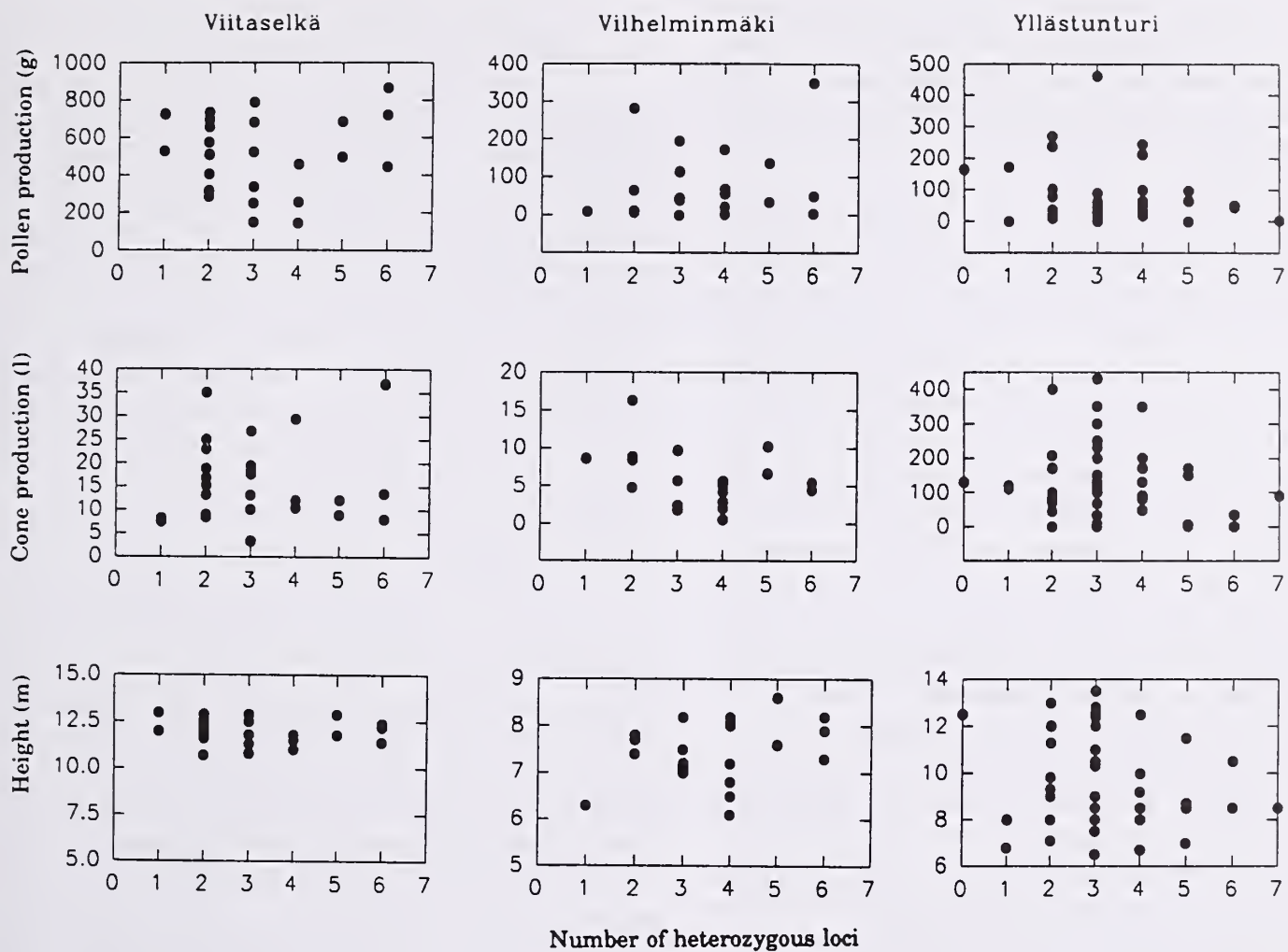


Figure 3. The phenotypic values of three quantitative traits, height, cone production and pollen production, for clonal means (Viitaselkä and Vilhelminmäki) or individuals (Yllästunturi) with different numbers of heterozygous loci.

Table 1. The percentage of variation that occurs within and between southern and northern populations for molecular traits and adaptive traits in Scots pine (after Karhu et al. 1995). The molecular genetic diversity within populations (measured as expected heterozygosity) and genetic differentiation between populations for all traits have ranges from a minimum of 0.0 to a maximum of 1.0. The genetic diversity for the budset date was measured as the phenotypic standard deviation and genetic differentiation as the proportion of the between-population variance component (see Karhu et al. 1995 for details).

Character	Genetic Diversity Within		Genetic Differentiation Between N'n & S'n
	Southern	Northern	
Molecular			
Allozymes	0.35	0.35	0.02
RFLPs	0.44	0.54	0.02
Microsatellites	0.74	0.80	0.02
Adaptive budset date	169.0	171.6	0.36

This same pattern is also observed for the two types of DNA markers, restriction fragment length polymorphisms (RFLPs) and microsatellite loci, given in Table 1. For both types of markers, the level of variation is high within both population groups and quite similar (the slightly higher diversity values in the northern populations are not significantly so). Again, the level of differentiation between northern and southern populations is very small for these two different DNA markers and is consistent with the allozyme similarities.

On the other hand, common garden experiments and transplants have shown that the northern and southern populations are highly differentiated with respect to a number of traits that confer adaptation to the severe northern conditions. From a number of such experiments in Finland and Sweden, a rule of thumb is that trees moved more than 100 km north suffer greatly in performance. For example, experiments in Sweden have shown

that survival is reduced by 7 percent per degree increase in latitude (and also decreased 16% per 100 m increase in elevation) (Persson and Stahl 1990). Similar adaptive local differentiation has been found in a number of other conifers (e.g., Rehfeldt 1979).

Table 1 also gives the extent of variation from common garden experiments for a quantitative trait related to fitness, budset date, which is related to frost tolerance (Karhu et al. 1995, Hurme et al., in preparation) for southern and northern populations. As for the molecular traits, the two groups of populations have similar and high amounts of within-population variation. On the other hand, when the level of differentiation of date of first budset between the populations is examined, there is a very large difference (0.34), much larger than the differentiation for the molecular traits and reflecting the differences seen from the transplant experiments mentioned above.

For all the traits studied, both molecular and quantitative, there is an extensive amount of variation within a population. This is consistent with the evidence suggesting that the effective population size of Scots pine is quite large, both because of the large number of individuals and the extensive pollen gene flow that takes place over many kilometers. As a result, there is little differentiation for the molecular markers, because presumably any selective differences that may occur are small in comparison to the level of gene flow. On the other hand, the selective differences between environments south to north are quite substantial so that genes that confer adaptive differences become highly differentiated in a relatively short time in spite of the high level of gene flow. In other words, in this context the lack of correlation of the pattern of molecular and adaptive traits over space is not unexpected. These results demonstrate that studies of allozyme or DNA markers cannot be a substitute for examining the genetic variation for adaptive traits themselves.

There are extensive efforts to identify the genes involved in traits that may confer differential adaptation for quantitative traits. In the case of Scots pine, if a large part of the variation in budset is determined by only a few genes, it may be possible to find molecular markers associated with the genes causing these adaptive differences. On the other hand, if many genes of small effects are responsible for the adaptive variation, then such markers would be of limited use.

## Discussion

### *Individual Heterozygosity and Fitness*

From the data presented above for Scots pine, it appears that intrinsic heterozygote advantage is probably not a common explanation for an association of heterozygosity and fitness. In endangered species, if populations exist in which such an association occurs, then it is likely due to one of the two mechanisms discussed above that result in apparent heterozygote advantage. However, one should fully evaluate the complete situation, that is, examine the potential for genetic drift, the type and extent of population structure, and the level and variation in inbreeding, as well as both the reliability and power of the molecular and fitness data, before making conclusions.

The extent of apparent heterozygote advantage resulting from linkage disequilibria of molecular markers with selected loci is the result of the effects of finite population size and population structure generating interlocus associations, an impact that could be large in an endangered species. In this case, heterozygous individuals for a molecular marker would have at least one copy of a favorable allele at the associated fitness loci. As a result, such heterozygotes may be better than homozygotes, which would have two detrimental alleles at some fitness loci. The extent of apparent heterozygote advantage resulting from multilocus genotypic association depends on the level of inbreeding and the variation of inbreeding among individuals. For example, variation in the degree of inbreeding among individuals may be high in self-compatible plants in a low enough density so that they receive little pollen from other plants, thereby allowing selfing to occur as well as outcrossing. In this case, individuals with high heterozygosity could have high fitness, because they most likely would be the result of outcrossing. However, the effects from both of these causes of apparent heterozygote advantage may not be large so we suggest that caution be used in any recommendations without detailed knowledge of the evolutionary factors that could contribute to the causes of apparent heterozygote advantage.

A number of previous studies have searched for a relationship between individual heterozygosity and traits related to fitness in trees. We do not know how many studies have been carried out that found no such association and therefore their results were not reported, but any such reporting bias would overestimate the ubiquity of the phe-

nomenon (for a discussion of an analogous bias for studies of natural selection, see Endler 1986). In addition, a majority of the positive reports for trees have been made by a relative few researchers and their colleagues, suggesting that there may be some bias from these researchers in reporting only results that show a positive association between heterozygosity and a trait related to fitness.

In our opinion, the support for a positive association due to intrinsic heterozygote advantage is not strong even in the studies reporting a positive relationship if they are critically evaluated. There are a number of other published studies that have concluded that there is little evidence for intrinsic heterozygote advantage in conifers. Ledig et al. (1983) found a positive relationship between heterozygosity and growth rate in *Pinus rigida* and concluded that this association was due to inbreeding depression from partial selfing. Strauss and Libby (1986) studied clonal replicates of 10-year-old *Pinus radiata* seedlings and concluded that intrinsic heterozygote advantage was a very unlikely explanation for an association of allozyme heterozygosity and quantitative traits. Strauss (1986) also studied a more controlled situation, where he included selfs and crosses between different trees of *Pinus attenuata*. Outcrosses did not show consistent relationships, but among the inbreds, heterozygosity was positively correlated with trunk growth and cone production. A study of family mean heterozygosity versus growth rates provided no evidence for heterozygote superiority for growth in *Pseudotsuga menziesii* (Bongarten et al. 1985). Bush and Smouse (1991) studied selfs and crosses in *Pinus taeda* and found little evidence for heterozygote advantage.

There have also been a large number of studies examining the association of individual heterozygosity and quantitative traits, particularly growth rate, in mollusks (see Zouros and Foltz 1987). However, in many of the studies that do show a positive relationship between heterozygosity and a quantitative trait, there also appears to be a deficiency of heterozygotes in the early age classes (e.g. Gaffney et al. 1990). This suggests that population structure or some other factor influencing genotypic frequencies may be causing an association between the allozyme loci and loci affecting the trait being examined.

In a sophisticated extension to the previous approaches, Pogson and Zouros (1994) detected some positive association between multiple-locus heterozygotes and a measure of size in scallops for allozyme loci, but not for other loci identified by

DNA markers in the same population. However, only one allozyme locus had a significant positive effect on size, and regression analysis showed that the allozyme data explained only 3 percent of the variance for size. As they state, "the differences observed between the effects of allozyme and RFLP heterozygosity on growth rate provide evidence against the associative overdominance hypothesis, but a strong case against this explanation must await corroboration from similar studies in different species."

In a large and comprehensive laboratory study of *Drosophila melanogaster*, Houle (1989) looked for associations of allozyme heterozygosity and size, developmental rate, and fluctuating asymmetry (differences between measures on the left and right sides of the fly). As is the case for Scots pine, *D. melanogaster* is unlikely to exhibit either extensive linkage disequilibrium or genotypic associations because of its large population size and lack of inbreeding. In other words, any observed associations would likely be the result of intrinsic heterozygote advantage. Overall, Houle found no significant correlation of heterozygosity at eight different loci for four different traits in two different experiments. Based on these results and a review of the literature, he concluded that "until there is evidence that allozyme heterosis occurs in large, panmictic, natural populations, the hypothesis of functional overdominance must be regarded with considerable skepticism."

Based on these findings and the above discussion, we feel that the use of individual allozyme heterozygosity (or heterozygosity at other molecular marker loci) to select parents for use in conservation must be approached with extreme caution. Our results, and those of others, indicate that in populations without factors that can cause interlocus correlations, individuals with high allozyme heterozygosity may not be any different for fitness-related traits than individuals with low allozyme heterozygosity. Only when there are very small population sizes, extreme population structuring, or a high or variable level of inbreeding is it likely that a positive association would be present, and in these cases it would not be due to the intrinsic advantage of heterozygotes. In other words, selection in these cases would be for genotypes that have an ephemeral advantage generated by these secondary factors. Such a breeding or management program may be temporarily effective in some cases but it would be difficult to support it over traditional techniques in conservation genetics (e.g., Hedrick and Miller 1992).

*Molecular and Adaptive Differences  
Between Populations*

From the Scots pine data we presented on differences between populations, it is obvious that there may be no molecular differences between populations but important adaptive differences. In such an instance, the picture provided by molecular markers does not reflect differences in traits related to fitness. Further, in other species there may be large differences at the molecular level because of genetic drift, but if the populations are in similar environments, there may be little significant adaptive differences. In fact, several recent studies have shown that population differentiations for molecular and morphological traits are often not concordant (Schwaegerle et al. 1986, Prout and Barker 1993, Podolsky and Holtsford 1995, Spitze 1993, Yang et al. 1995). There is some indication from these studies that the extent of local differentiation is higher for quantitative traits than for molecular markers, but it is not clear how general this observation is.

Quantitative traits may diverge at a different rate than that for molecular variation (see discussion in Lewontin [1984] for a perspective on the basis on such changes). There are a number of examples in which there is rapid adaptive genetic change in response to extreme environmental conditions (e.g., Bradshaw 1972). Adaptive changes in allozyme variation may be quite slow in comparison, because any selective differences present at these loci are probably small. Frequencies of allozyme alleles may change because of non-selective forces, such as genetic drift, but this would not necessarily be associated with any adaptive changes at other loci. Differentiation at molecular variants that have a high mutation rate, such as microsatellite loci, may be quite fast and they could differentiate as fast as adaptive quantitative traits. However, the forces that would cause any differentiation for microsatellite loci would be the combination of mutation, genetic drift, and low gene flow and not selection. Low gene flow would allow even small adaptive differences to accumulate and therefore there may be some concordance between the level of differentiation for these types of traits.

Lessica and Allendorf (1995) have suggested that peripheral populations may often be of great conservation and evolutionary significance. For example, if a population has been isolated for a long time, then this would allow time for genetic

differentiation to build up by genetic drift. Further, if a population exists in an ecologically marginal habitat, then selection could result in adaptive genetic differentiation from the rest of the taxa. In fact, Lessica and Allendorf (1995) suggest that genetic drift and selection may act synergistically to increase the extent of genetic differentiation over that expected if they acted independently (e.g., Cohan and Hoffmann 1986). Overall, they suggest that populations that are peripheral or physically isolated, have life history attributes that reduce gene flow such as limited pollen and seed dispersal, have short generation times, and exist in habitats that differ with respect to soil type, climate, and competitors, may have important genetic differences that should be conserved. In addition, they suggest that such populations may be the source of new adaptations (or even new species). Of course, these suggestions are based on generalities and some peripheral or isolated populations may not have any unusual genetic attributes or may in fact be fixed for detrimental variants.

There have been several different strategies for identifying units for conservation purposes. Perhaps the most developed is the concept of an evolutionarily significant unit (ESU) that has been developed to use for protection of vertebrates under the Endangered Species Act (e.g., Waples 1995; for other perspectives, see also Dizon et al. 1992, Moritz 1994, Moritz et al. 1995, Vogler and DeSalle 1994). To identify an ESU, an integrated approach is advocated by Waples (1995) in which all available information is taken into account. This includes genetic information, such as genetic distance determined between populations from various molecular markers as an indicator of reproductive isolation, information on other characters such as morphology, behavior, physiology, and life history variation, experimental data such as transplants and common garden information, as well as other information such as geographical and distributional parameters, ecological differences, and historical information. Obviously, when all this information is concordant, then the answer is unambiguous. When not all of the information is consistent, then a careful evaluation of the reliability and significance of the different types of information must be undertaken before a conclusion is made. In some such cases, a definitive conclusion may not be possible with the available data.

## Acknowledgments

This research was financially supported by the Finnish Research Council for Agriculture and Forestry (O.S. and P.W.H.) and the National Science Foundation (P.W.H.). Katri Kärkkäinen helped in collecting some of the field data.

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# Current Hybrid Policy and the Importance of Hybrid Plants in Conservation

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**Abstract:** While man-caused disturbances and the introduction of exotics can result in hybridization, which may then lead to genetic assimilation, natural hybridization among native species can have many positive effects and should be preserved as part of a dynamic ecosystem. For example, by conserving native hybrid plants and hybrid zones we may preserve centers of plant speciation, centers of biodiversity for arthropods and fungi, essential habitat for rare arthropods, superior nesting habitat for birds, and sources of economically important plants. We review current U.S. hybrid policies and develop two examples of current efforts to conserve hybrid populations (hybrid cliffrose in Arizona and hybrid palms in Texas). We also propose guidelines for distinguishing between "good" and "bad" hybridization and discuss an experimental approach for using hybridization to rescue rare species.

## Introduction

The controversy over the 'goodness' and 'badness' of hybrids is not new. At the end of a series of benchmark papers in genetics dealing with hybrids, Levin (1979, p. 300) concludes:

There seems to be a lingering notion that hybridization is bad: that gametes are wasted, hybrids are inferior, and the infiltration of alien genes will pollute an otherwise "pure" gene pool that must be on some adaptive peak already. Wagner (1970) refers to hybridization as an evolutionary mode producing something abnormal. However, Epling (1947) suggested that hybridization and gene exchange may actually be advantageous in some groups, and others are in accord.

The "bad" view of hybrids seems to currently prevail. For example, in the United States, the hybrid policy advocated by O'Brien and Mayr (1991) is to "discourage hybridization between species," because hybridization may "disintegrate the genetic organization of the species in contact." Although the examples presented in that paper were examples of hybridizing animals, the same logic has been applied to plants. There are, however, fundamental differences between plants and animals that make animals poor role models for developing management policies for plants. For example, the propensity of plants to clone and reproduce asexually by diverse means, while higher animals generally reproduce only sexually, has led to different speciation mechanisms for plants and animals (Grant 1981). Furthermore, hybridization has long been thought to be important in plant evolution (e.g., Stebbins 1950), but has not

been thought to be important in animal evolution (e.g., Mayr 1963).

We believe that part of the controversy over the good and bad aspects of hybridization arises from pooling two major types of hybrids. We define "native" hybrids as those that arise naturally among native species. These hybrids are an integral part of a dynamic ecosystem and should be conserved. Conversely, we define "exotic" hybrids as those that arise from the introduction of exotic species, which then hybridize with native species and/or the hybrids between native species that arise from man-caused disturbances. Exotic hybrids, like exotic species, pose ecological and evolutionary problems when they are invasive and have the potential to eliminate native species. Thus, from a conservation perspective, it is naive to consider all hybrids as good or bad. As we judge species on their own ecological and evolutionary merits, so must we judge hybrids.

When viewed in this light, the conservation of hybrids is much the same as the conservation of species. We believe that the primary goals in conserving hybrids should be:

1. To conserve the genetic diversity of species, including their natural hybrids.
2. To conserve the evolutionary potential of species in which native hybrids may play an important role.
3. To conserve characteristics that have economic value.
4. To conserve the interactions among parental species.

5. To conserve the diverse trophic-level interactions that include the dependent organisms that are associated with species and their natural hybrids. In the case of plants, this would include the insect herbivores and their parasites, some of which are unique to hybrid plants.

With these points and those that follow in mind, we believe that the hybrid policy of the U.S. should be the following.

A "Better Hybrid Policy" would recognize the important role that hybridization has played in the ecology and evolution of plants, which in turn has affected the ecology and evolution of the rest of the community. Just as we discriminate between native and exotic species in formulating management policies, so should we discriminate between native and exotic hybrids by conserving the former and discouraging the latter.

### Current U.S. Hybrid Policy and Two Examples

Hybrids have no official protection under current U.S. law. The Endangered Species Act does not have any specific statement endorsing the protection of either plant or animal hybrids. However, the U.S. Fish and Wildlife Service has developed a draft policy on hybrids or intercrosses, which has not been given official endorsement as of this date. R. Lohofener, Division of Endangered Species and Habitat Conservation, USFWS, Washington, D.C., explained USFWS hybrid policy as follows:

The USFWS recognizes that there may be a need to keep gene flow between disjunct populations, especially when populations that have been isolated due to human activities develop low levels of heterozygosity. In addition, where hybrid individuals are morphologically indistinct from pure species, hybrids may be protected to facilitate law enforcement.

For example, the Florida panther has an appearance similar to cats introduced from South America. Both are protected to ease law enforcement issues.

According to Angela Brooks, USFWS-Arizona Ecological Services Office, "evaluation of a species and its potential hybrids is made on a case-by-case basis with the major goal being to protect the rare species. Species with minor evidence of introgression may be protected particularly if there is no distinct morphological or geographical separation of the pure and hybrid forms."

We present two examples where private and/or government agencies have recognized the need

to conserve hybrids, have made relevant policy changes, and are attempting to study native hybrid populations.

### Hybrid Cliffrose in Arizona

The USFWS and other federal agencies often fund studies to determine the taxonomic status of a species before any listing proposal is made. In the case of the endangered Arizona cliffrose, *Purshia subintegra* (Kearney) Henrickson, the Bureau of Reclamation funded electrophoretic studies (Phillips et al. 1988) and the USFWS funded morphometric (Reichenbacher 1993) and random amplified polymorphic DNA (RAPD) studies (Mount and Logan 1993) to determine the taxonomic status of the pure rare species and its putative hybrids with the common Stansbury cliffrose, *P. stansburiana* (Torrey) Henrickson (USFWS 1995). Given this broad base of information, the USFWS determined that *P. subintegra* can usually be clearly differentiated from *P. stansburiana*. Nevertheless, variability in both species sometimes makes it difficult to classify individuals.

The Arizona Cliffrose Recovery Plan (USFWS 1995) illustrates the current treatment of hybrids. The recovery plan first presents the taxonomic history of the species, describes the hypothetical evolutionary origin of *P. subintegra*, and suggests that the hybrid swarms are worthy of conservation. It is hypothesized that following the last glacial period, *P. subintegra* was an endemic to small areas of uncommon limestone soils in central Arizona. During the wetter glacial periods of the Pleistocene, the common Stansbury cliffrose, *P. stansburiana*, expanded southward creating a zone of contact between the two species. The two species are believed to have hybridized or introgressed during this period and today hybrid swarms in the Verde Valley and Tonto Basin still exist. Although the USFWS (1995, pp. 12-13) considers the plants in these introgressing hybrid swarms to be outside the definition of *P. subintegra*, they recognize that the hybrid swarms "illustrate the migratory and dynamic nature of evolving plant populations. Plants in the hybrid swarms are genetically and phenotypically variable, represent a piece of the evolutionary history of *Purshia*, and may provide the key to the future of the genus and species. For these reasons, conservation of these hybrid swarms is important. If the Tonto Basin and Verde Valley forms are described as distinct taxa in the future, the Service may consider providing protection under the Endangered



Species Act. For those plants not within *Purshia subintegra*, other conservation strategies should be pursued."

### *Hybrid Palms in Texas*

Lockett (1991) describes the efforts to conserve a naturally occurring population of hybrid palms (*Sabal minor* (Jacq.) Pers. x *S. mexicana* Mart.) that is concentrated on approximately 90 acres in Brazoria County, Texas. The dwarf palm, *S. minor*, co-occurs with the hybrid population and is found east of this site to the Atlantic coast. The tall palm, *S. mexicana*, is found in Mexico and as far north as the central coast of Texas (Lockett and Read 1990). While it may have once co-occurred at the hybrid site, the nearest *S. mexicana* is now 60 miles away as it has been almost totally extirpated from its range in Texas due to lumbering and use as pilings in the construction of wharves.

These hybrid palms, up to 9 m in height, are the only known hybrids of *Sabal* species; they are the only naturally occurring and reproducing palm hybrids in the United States, and they represent the northernmost natural population of "tall" palms west of *S. palmetto* (Walt.) Lodd. ex Schultes & Schultes in Florida and east of *Washingtonia filifera* (Linden ex Andre) H. Wendl. in Arizona. The palm specialist, Balick (1988, p. 30, cited in Lockett 1991) argues that "since hybrid progeny can develop into distinct species over time, it must be recognized that these are distinct taxa worthy of conservation efforts." Lockett (1991) and palm specialists cited therein believe that this hybrid "could eventually stabilize into a new species, perhaps a tall palm for the middle Gulf Coast, a region which has no such native palm." It is also important to note that no molecular evidence or experimental breeding efforts have yet been completed to clearly establish the hybrid status of these palms (Lockett, personal communication), but there can be little doubt that they are morphologically distinct from the putative parental species (Lockett 1991).

At the time of Lockett's 1991 publication, the 43-acre site containing most of these palms was up for sale. Although Lockett's initial efforts for preserving this hybrid palm met with great resistance due to their hybrid status, the Texas Nature Conservancy became convinced of their value and in 1994 purchased 23 acres of this site. This purchase will be turned over to USFWS and they in turn will incorporate the site into the newly formed Columbia Bottomland National Wildlife Refuge. This new status will protect both hybrid plants

and a forest that is important to migrant birds coming across the Gulf (Landon Lockett, Aug. 1995, personal communication). Lockett's contributions were recently recognized by the Native Plant Society of Texas when they gave him a special award for his efforts to conserve these unique hybrids (Ellis 1995).

It is also interesting to note that the Plant Committee of the Texas Organization for Endangered Species (an organization with no official capacity made up mostly of professional biologists) has changed its protection guidelines to include hybrid plants. They state that "whereas hybridization can be viewed as a natural evolutionary process—though it would be expected to be a rare situation—the TOES Plant Committee may recommend a hybrid population for conservation protection. Such situations may be considered for action, on a case by case basis, provided they are recommended to the Plant Status Committee with good background information and scientific documentation" (TOES 1993). Furthermore, TOES has recently given the hybrid palms in Brazoria County a category V protection status. This is defined as "any species that lacks legal protection and at present has either low population numbers or restricted range in Texas and is not declining or being restricted in its range but requires attention to insure that the species does not become endangered or threatened" (TOES 1994).

With these current policies and examples in mind, we now review the negative and positive aspects of hybridization and propose management guidelines for dealing with hybrids and rare species that hybridize.

### Negative Aspects of Hybridization

There are three major concerns about hybridization of rare plants with more common species. (1) The genetic integrity of rare species will be swamped or assimilated into widespread species leading to a loss of genetic diversity. (2) Hybrid individuals may suffer reductions in fitness due to outbreeding depression (i.e., Price and Waser 1979). (3) Detecting hybrids using classical taxonomic methodologies is difficult and/or impossible. Thus, hybrids may become problematic for management when hybrids are not given the same protective status as "pure" species (Rieseberg 1991b).

Some of these concerns have recently been re-examined and found to not always hold true. For example, Rieseberg (1995) dispelled the myth that

hybrids are always less fit than their parents and cited studies showing that F1s can exhibit the entire range of fitness responses. F1 hybrids were more fit than both parental species (Langevin et al. 1990), less fit than either parent (Heiser 1947, 1949), intermediate in fitness (i.e., Potts and Reid 1985, 1990) or equal in fitness (Levin and Schmidt 1985). Therefore, the automatic assumption of fitness reduction with hybridization is not supported by the evidence.

Similarly, the management of hybrid populations may not present problems uniquely different to the management of "pure" rare species. Often hybrid zones abut populations of rare species, as is the case with hybrids of *Purshia subintegra* and *P. stansburiana* in the Verde Valley of Arizona. Although ecological requirements of the hybrids and the endangered parent, *P. subintegra*, may differ slightly, the major threats to the species, habitat destruction and livestock grazing, apply to both parent and hybrids. Consequently, management objectives for the two do not differ. Furthermore, as molecular genetics techniques become more widespread and easier to use, problems in discriminating between hybrids and their parental species will be simplified (e.g., Keim et al. 1989).

The one concern about hybridization with rare plants that has not been resolved in our minds is that hybridization may lead to the loss of uniquely adapted, genetically distinct plant populations. As Rieseberg (1991b) noted, "I can think of no justification for exchanging several distinct rare plant species, each with its own unique growth form and habitat requirements, for a single widespread compilospecies." This argument for the aesthetic "value" of rare species—the right to live for its own sake—has however little merit or appeal for those who are guiding political policy. A more cogent argument is that ecotypes and morphotypes of rare species can be lost through hybridization and in the process traits of economic importance, such as chemical composition, could be lost as well. Other potentially negative consequences of hybridization include increased mutation rates, fixation of recessive alleles, and reduced developmental stability (Grant 1975).

Is there any way to predict how serious a problem hybridization can be? Reproductive isolating mechanisms serve to minimize the amount of hybridization, even in species known to hybridize. Recent chromosomal studies with *Helianthus annuus* L. and *H. petiolaris* Nutt. have shown that chromosomal structural differences may reduce or eliminate introgression such that species differ-

ences can be maintained even in the face of extensive introgression (Rieseberg 1995). In addition, in many plant species, intraspecific pollen has a higher probability of success than interspecific pollen. For example, when mixed loads of pollen were applied to stigmas of *Iris fulva* Ker Gawler and *I. hexagona* Walt., in both species intraspecific pollen was much more likely to fertilize ovules (Carney et al. 1994).

The threat of assimilation may be particularly severe when rare species hybridize with common species. Carney et al. (1994) and Ellstrand (personal communication) suggest that when a rare species is in proximity to a congeneric species and is in the minority, there is a high probability of hybridization. Ellstrand suggested eliminating the common species to avoid genetic assimilation in cases where this scenario occurs.

The quandary is that hybrids may also serve as centers of plant speciation, as was suggested by Stebbins (1959). In addition, hybrids may comprise a major proportion of the genetic diversity of a rare species. Such is the case with *Cercocarpus traskiae* Eastwood, which is restricted to five adult trees, two hybrids with *C. betuloides* var. *blancheae* C. Schneider and 28 pure seedlings (Rieseberg 1991b, Rieseberg and Gerber 1995). Because critically endangered species have limited genetic diversity by definition, conserving hybrids should conserve a portion of the genes of the rare species, if they are not lost through assimilation.

### Positive Aspects of Hybridization

Hybridization in plants has long been thought to serve as an important avenue of speciation (Stebbins 1950, 1971, Lewontin and Birch 1966, Grant 1971). Estimates from these and other studies suggest that 30–80 percent of all plant species arose from hybridization events (e.g., Stace 1987). Recent studies by Gallez and Gottlieb (1982), Arnold et al. (1991), Rieseberg (1991a), and Wendel et al. (1991) suggest that hybrid swarms and hybrid zones can serve as founding populations for new species. This is in part due to the presence of novel morphological characters in hybrids (Rieseberg and Ellstrand 1993), which may serve as the fodder for evolution. Although the role of hybridization in speciation events in the earlier studies was clearly linked to polyploidy, later studies showed that diploid speciation may also be important.

If hybrids zones are sites of future speciation events, then it seems important to preserve the evolutionary potential of such lineages. Failure to

protect centers of speciation is basically to accept that all meaningful evolution has taken place and/or that sites of speciation are so unpredictable that we cannot hope to protect them. If worldwide estimates of environmental change and extinction are as grave as many believe, then it seems especially prudent to preserve genomes and sites that have the greatest potential to survive in a changing environment. In the case of hybrid zones, it seems clear that they do have evolutionary potential and they are in many cases easily identifiable.

#### *Sources of Economically Important Plants*

Naturally occurring hybrids should also be conserved for their potential economic value. For example, the native intersectional hybrid *Populus x acuminata* Rydberg is planted widely as an ornamental throughout the Great Plains of North America where its hardiness and attractive foliage make it a superior street tree (Eckenwalder 1984).

Species of hybrid origin have also been used by man to produce economically important hybrids. The artificial hybrid, *Populus x canadensis* Moench (also known as *P. x euramericana*), is used extensively in forestry and is the progeny of *P. deltoides* Bartr. ex Marsh. and *P. nigra* L. (cited in Smith and Sytsma 1990). Considering that *P. nigra* is thought to be the product of an ancient hybridization event (Smith and Sytsma 1990), and has subsequently been used by man to produce hybrids of commercial importance, the conservation of hybrid lineages is important from both evolutionary and economic perspectives (Whitham et al. 1991). Beyond the few examples presented here, the general significance of hybrids in agriculture is illustrated by Simmonds's (1984) review of the evolution and geographical origins of crop plants of the world.

#### *Centers of Biodiversity and Unique Community Interactions*

Because plants represent the basis of the food chain it seems likely that nearly any factor that affects plants will also have ripple effects on the rest of the community. Organisms such as insect herbivores and their parasites, fungal mutualists (endophytes and mycorrhizae), and pathogens might be likely candidates for ecologically responding to the patterns of hybridization in plants. Several studies have now examined the responses of insect herbivores to hybridizing plants and while some have found little or no responses (e.g., Boecklen and Spellenberg 1990), others have shown strong responses.

An example of a strong community response to hybrid plants is illustrated by several studies of naturally hybridizing *Eucalyptus* in Australia. In Tasmania, Whitham et al. (1994) found the hybrid zone between *E. amygdalina* Labill. and *E. risdonii* Hook. f. to be a center of insect and fungal species richness and abundance. Of 40 taxa examined, 73 percent were significantly more abundant in the hybrid zone than in pure zones, 25 percent showed no significant differences, and 2 percent were most abundant on a pure host species. The average hybrid tree supported 53 percent more insect and fungal species, and relative abundances averaged four times greater on hybrids than on either eucalypt species growing in pure stands. Thus, while diverse taxa exhibit all possible responses to hybrids, the overall community response is that hybrids accumulate the insects and fungi of both parental species. This accumulation of species also results in otherwise rare species assemblages; 50 percent of the species coexisted only in the hybrid zone making hybrids sites of unique species interactions. Similar results were obtained with *E. obliqua* L'Herit. x *E. baxteri* (Benth.) Maiden et Blakely in Victoria, and with *E. caliginosa* Blakely et McKie x *E. stellulata* Sieb. ex DC. in New South Wales (Morrow et al. 1994). The genetic basis of these field studies has been confirmed in common garden plantings with known crosses in which biodiversity was greater on hybrids than on the parental species (Dungey et al. 1994).

Because insect conservationists argue that it is especially important to save sites of rich diversity (e.g., Morris et al. 1990), plant hybrid zones, especially those with high biodiversity, should receive special consideration.

#### *Hybrid Zones as Centers of Insect Speciation*

If plants speciate via hybridization, then one might also expect their dependent organisms such as insect herbivores to speciate along with their host plants. Floate and Whitham (1993) argued that intermediates resulting from hybridizing plants could act as stepping stones for insects to shift from one plant species to another. In the absence of intermediates, host shifts would have to occur in jumps, whereas with hybrid intermediates, insects could gradually adapt to the foreign defenses of another species. Since host shifting has been proposed as a mechanism that promotes insect speciation (e.g., Bush 1969), in examining the importance of conserving hybrid plants, we must also consider how they might affect arthro-

pod evolution.

If hybridization in plants affects the ecology and evolution of arthropods, we should expect specific associations of arthropods with hybrid plants and their parental species. Floate and Whitham (1995) found that the distributions of some arthropods are sometimes so specific that their presence may rival traditional plant morphological traits in discriminating between specific classes of hybrids (i.e., F1 vs. backcross hybrids) and parental plant species. For example, when cottonwood trees in a hybrid swarm (*Populus fremontii* S. Wats. x *P. angustifolia* James) were classified based solely on morphological traits, and then classified solely on their arthropod assemblages, the level of agreement between these two methods was 98 percent.

An example of how hybridization in cottonwoods has apparently affected insect evolution is the life cycle evolution of the aphid, *Pemphigus betae* Doane (Moran and Whitham 1988). In the hybrid zone where susceptible trees are common, this aphid annually alternates between cottonwoods and herbaceous plants. In the adjacent narrowleaf zone where trees are more resistant and aphid survival is reduced, *P. betae* has abandoned cottonwoods and remains almost solely on its herbaceous hosts. Field and lab experiments show that the differences between the simple and complex life cycle is in part genetically based (Moran and Whitham 1988, Moran 1991). The presence of such different aphid life cycles in close proximity, i.e., a simple life cycle in the narrowleaf zone and a complex life cycle in the hybrid zone, suggests that hybridization in plants can affect insect evolution and perhaps even speciation.

#### *Hybrid Habitat for the Preservation of Rare Insects*

While numerous countries have enacted laws to protect rare and endangered species, few have legislation to protect the habitats of those species. As a case in point, Australia currently protects rare and endangered insects, but not their habitat (Greenslade and New 1990). Here we argue that if hybrid zones support rare species then they should be conserved as essential habitat for those species. Although little research has been conducted in this area, three examples illustrate the issue. First, in a hybrid zone between *Eucalyptus amygdalina* and *E. risdonii* in Australia, 5 of 40 insect taxa were nearly restricted to the hybrid zone. Considering that *E. risdonii* is threatened and endangered (Pryor 1981), the hybrid zone is small (ribbons less than 100 m wide), and that both are near a big city that is expanding, it is important to

contemplate what would happen to these insects if either the hybrid zone were lost, or *E. risdonii* were to go extinct.

Second, in the United States, the 13 km hybrid zone between the riparian species, *Populus fremontii* x *P. angustifolia*, supports 85 to 100 percent of the populations of both the gall aphid, *Pemphigus betae*, (Whitham 1989) and the leaf-feeding beetle, *Chrysomela confluenta* Rogers (Floate et al. 1993). Considering that just 13 km out of a total 500 km of potential riparian habitat support such a large proportion of these insects' populations, it is interesting to consider what would happen if the hybrid zone were lost. The importance of the hybrids to both species is especially apparent in low-density years when nearly 100 percent of the populations of both species are confined to the hybrid zone (Whitham 1989, Wimp et al. unpublished data). If hybrids provide superior habitat for these organisms during hard times, then they act as a refugium and their conservation value becomes especially important as essential habitat for such species (Whitham et al. 1991). Furthermore, these hybrid zones are susceptible to two major kinds of human development. First, cottonwood hybrid zones often form at the mouths of canyons (Eckenwalder 1984) where dams are often built (Whitham, personal observation). Second, the entire Salt Lake Valley in Utah is threatened by housing developments as Ogden, Salt Lake City, and Provo expand to fill the valley floor and nearby riparian flood plains.

Third, Ericson et al. (1993) noted that in Scandinavia, the smut *Anthracoidea fischeri* (Karst.) Kukk. is nearly restricted to hybrid sedges and suggests that it could not persist in the absence of hybrids. While we are not advocating the conservation of disease organisms, it is important to emphasize that if hybrid zones are essential for the support of such diverse taxa, they are also likely to support many beneficial species. For example, many parasites that are used as biocontrol agents for economically important pests forage where their prey are most abundant. If hybrid zones support high concentrations of herbivores (i.e., prey), they are also likely to support these beneficial insects (Whitham et al. 1991). Thus, protecting hybrid zones may protect potential future sources of biocontrol agents.

#### *Hybrids as Superior Avian Nesting Habitat*

Riparian forests are recognized as contributing disproportionately to biodiversity, especially in the arid Southwest and Great Plains where they

represent the only available forests (e.g., references in Finch and Ruggiero 1993). Within these forests, cottonwoods are often dominant and are reported to contain the highest bird densities (Carothers et al. 1974, Johnson et al. 1977, Strong and Bock 1990). Recent studies by Martinsen and Whitham (1994) suggest that F1 type hybrid cottonwoods and the hybrid zones in general have the highest value as nesting habitat for birds. For example, within the hybrid zone, they found twice as many bird nests in F1 hybrid trees as they did in either parental type. Additionally, comparisons among zones showed a similar pattern; bird nest densities were significantly greater in the hybrid zone than in adjacent pure zones.

These studies demonstrate that plant hybridization can be important in plant speciation and that many economically important plants have hybrid origins. Hybrid plants also impact the rest of the community and may serve as centers of insect speciation and biodiversity. Furthermore, hybrid zones may provide essential habitat for rare arthropods and superior habitat for nesting birds. Thus, there are many valid reasons for conserving hybrids.

### Hybrid Guidelines and Their Use in Species Recovery

Since few management decisions are clear cut, we must first try to identify what should be considered good and bad hybridization in the management of hybrids and endangered species that hybridize with other species. We consider it bad when natives hybridize with exotic species, because hybridization has the potential to introduce truly foreign genomes with unknown consequences. Note that we consider man-induced hybridization between native species in the same undesirable exotic category. In contrast to these exotic hybridization events, we consider good hybridization as those that occur naturally among native species.

The primary distinction between bad and good hybridization is that the latter is likely the result of long-term interactions that predate European settlement of North America in which natural selection has contributed to the process. For example, due to the cloning nature of many plants, hybrid zones have the potential to be very old. Also, because hybridization has often been tied to periods of environmental disturbance such as glaciation, some current hybrid zones may date back to the Pleistocene or farther (Rattenbury 1962, Grant 1971, Kat 1985). With such hybridizing

species, gene flow may have been evolutionarily favored, partial isolating mechanisms may be in place, and the parental species, as well as the hybrids, may be locally adapted.

In contrast, hybridization among exotics and natives may introduce genomes from another continent that may or may not be adapted to the new continent, and the relationship is so new that few isolating mechanisms may be in place. For example, this may be the case with *Populus nigra* in Europe. As a result of extensive plantings of numerous cultivars of *P. x canadensis* (a hybrid between the European *P. nigra* and the North American *P. deltoides*), which then hybridize with the native *P. nigra*, the latter is being displaced from its habitat (cited in Smith and Sytsma 1990). To prevent such problems with crop trees, sterility should represent a prerequisite for introduction.

Just as it is crucial to know the biology of weed species to make appropriate management decisions, it seems clear that to differentiate between good and bad hybridization, we must know the biology and origins of the hybrids in question. Since many view hybridization as a rare event, the attention it has received has been mostly negative (e.g., O'Brien and Mayr 1991), and the positive aspects have been less advertised. As hybridization is known to occur in all major taxonomic groups and all floras of the world (e.g., Grant 1971), hybridization can no longer be considered a rare event. For example, even by 1891, A. Kerner listed over 1000 interspecific wild hybrids in the European flora that came from groups as diverse as mosses, ferns, horsetails, conifers, flowering trees, and herbaceous angiosperms (cited in Grant 1971, p. 154). Since then, the list has continued to grow. By recognizing that hybridization can be common and even desirable, we can then give it the research attention that it deserves so that appropriate management decisions can be made on a case-by-case basis.

### Gray Areas of Hybrid Conservation

Although we have argued for the conservation of native hybrids, we recognized that there are "gray" areas between what we consider native and exotic hybrids. As a case in point, the evolution in this century of *Tragopogon mirus* Ownbey and *T. miscellus* Ownbey (goat's beard, Asteraceae) in Washington and Idaho (U.S.A.) presents an interesting dilemma. Originally sterile F1 hybrids between species of diploid *Tragopogon* introduced from Europe, they became fertile following chromosome doubling events (Ownbey 1950). These

new species have since increased their distributions and may be regionally more common than their parent species (Novak et al. 1991). Having arisen from exotics after their introduction, are these new species native or not, and how should they be conserved? Although their exotic origins confound the conservation issues, these examples nevertheless illustrate how hybrids can result in speciation and demonstrate that the origins of species can be very recent. The above example is not an isolated one; Reiseberg (1991b) cites three examples of endemic, rare sunflower species (*Helianthus paradoxus* Heiser, *H. exilis* Gray, and *H. bolanderi* Gray) arising from hybridization between exotic and native species.

#### *The Potential Use of Hybridization in Species Recovery*

Despite potential problems that hybridization may pose, hybridization may be a useful tool for conserving rare taxa (Stebbins 1942), especially "in captive-breeding programs as a last-ditch effort to preserve the germplasm of a rare and endangered taxon" (Reiseberg 1991b). For taxa that have such a low population that few options are available for recovery, we recommend a two-stage recovery approach. First, at one site use asexual propagation of remaining plants to build up the population. The danger in this is that all resulting plants are genetically the same and similarly susceptible to disease and other problems associated with monospecific stands. At this site, we further recommend that crosses of the most dissimilar phenotypes be performed to prevent inbreeding depression and to maintain maximum variation.

Once stage one is accomplished, at another site isolated from the first to prevent gene flow, we recommend that hybridization be performed as a potential recovery tool. When the population of a rare species is so low (i.e., only a few individuals) and perhaps suffering from inbreeding depression, hybridization may be a viable avenue for preserving a portion of the genome. Otherwise all that remains are artificial means of propagation or slower processes leading to alterations in the breeding system. The effects of hybridization are likely to vary among species. Only through experimentation can we gain predictive power about the use of hybridization as a recovery tool.

A critical aspect of the recovery program is the quantitative monitoring of the performance of plants at both sites. Based upon genetic and phenotypic identity, growth, survival, reproduction, and other performance parameters of two sources

of plants, we then have crucial information upon which to make informed recommendations for restoration and reintroduction. This gives options in a rescue effort for which few options remain. We recognize that this is a commitment to long-term research, but with critically endangered species, intensive propagation is also a long-term commitment. Since we're concerned about the extinction of a species, we very much need the predictive power of the experimental approach to develop a viable conservation strategy.

### Acknowledgments

We thank Landon Lockett for providing information on hybrid palms. Financial support was provided by NSF grant DEB9408009, USDA grant 95-37302-1801, and DOE grant 94ER61849.

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# Is *Hymenoxys helenioides* (Rydb.) Ckll. a Species or a Hybrid? Morphological and Molecular Evidence

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**Abstract:** *Hymenoxys helenioides* (Rydb.) Cockerell is a category 2 candidate for federal listing as a threatened or endangered species. It is a poorly known taxon, occurring in small, widely scattered populations of 20 individuals or fewer in the central Rocky Mountains of Colorado, Utah, Arizona, and New Mexico. These small populations always co-occur with large populations of two related members of the Heliantheae, *H. richardsonii* (Hook.) Cockerell and *H. hoopesii* (Gray) Bierner (also placed in the genera *Dugaldia* and *Helenium*). These two species only infrequently occur together, usually growing in different habitats and elevations. Chromosome number, morphological intermediacy, pollen sterility, and an additive pattern in nuclear molecular markers show that *H. helenioides* is a largely sterile hybrid of independent origin at each locality. It should be referred to as *H. xhelenioides*. Because taxonomic recognition of this entity as a species is unjustified, federal protection is unwarranted.

## Introduction

Skinner et al. (1995) have listed over 200 possibly threatened or endangered taxonomic entities in California as obscure in either taxonomy or distribution and requiring further study to determine their true status regarding endangerment and the need for listing. *Hymenoxys helenioides* (Rydb.) Cockerell, a category 2 candidate species for federal listing, is an example of just such an obscure, rarely collected, and poorly known taxonomic entity from the central Rocky Mountains (Figure 1). The few scattered, small populations of this taxon cover a 400-mile-wide range, from the Sangre de Cristo Mountains in south-central Colorado (the type locality) on the east to the Wasatch Plateau in central Utah on the west and the Chuska Mountains of northeastern Arizona and northwestern New Mexico on the south (Figure 2). This study was undertaken to help provide some explanation for the highly dispersed, small (always fewer than 20 individuals) populations.

The holotype of *Hymenoxys helenioides* (Cockerell 1904), collected on Sangre de Cristo Creek in the Sangre de Cristo Mountains of Costilla County, Colorado, on July 2, 1900 (Rydberg and Vreeland 5495; holotype at NY, isotype at RM), was named *Picradenia helenioides* by Rydberg (1901). Rydberg described its habitat as "mountain valleys at an altitude of 2700 m" (Rydberg 1901). *Hymenoxys helenioides* was only collected once more in Colorado, over a hundred miles to the west, along upper Cebolla Creek between Lake City and Cathedral in 1940 (Penland 1548 COCO) in the San

Juan Mountains of Hinsdale County (Barrell 1969).

A collection made in 1939 (Peebles 14401 ARIZ) from the Lukachukai (Chuska) Mountains, Apache County, Arizona, was identified as *Hymenoxys helenioides* by Parker (1960); however, it was referred to as *H. lemmonii* (Greene) Cockerell in the *Arizona Flora* (Kearney and Peebles 1951).

In Utah *Hymenoxys helenioides* was first collected in 1940 on the Aquarius Plateau (Maguire s.n. UTC), Garfield County, and then again 6 years later on the Wasatch Plateau (Parker et al. 6369), in Carbon County. Unlike the Arizona and Colorado locations, *H. helenioides* has been collected at the Utah localities by several others up to the present.

At the initiation of this study in 1987, the Colorado (and Arizona) localities had not been rediscovered despite intensive searches (Johnston 1979). To gain familiarity with *Hymenoxys helenioides* and its habitat, they were evaluated at a known locality in Utah. On the Wasatch Plateau, Sanpete County, *H. helenioides* grows with shrubby cinquefoil (*Potentilla fruticosa* L.) on rocky hillocks among sedge meadows in the spruce/fir/aspens zone at 8500 feet. *Hymenoxys helenioides* is found in very low numbers in association with two other species of the Heliantheae that are common at the site, *Hymenoxys richardsonii* (Hook.) Rydb. var. *floribunda* (Gray) Parker and *Hymenoxys hoopesii* (Gray) Bierner. In the field, the general morphology of *H. helenioides* appears to be intermediate to *H. richardsonii* and *H. hoopesii*. These attributes (low numbers of individuals and morphological intermediacy) are often considered characteristics of a putative hybrid. Furthermore, the putative



Figure 1. *Hymenoxys helenioides* (Rydb.) Cockerell.

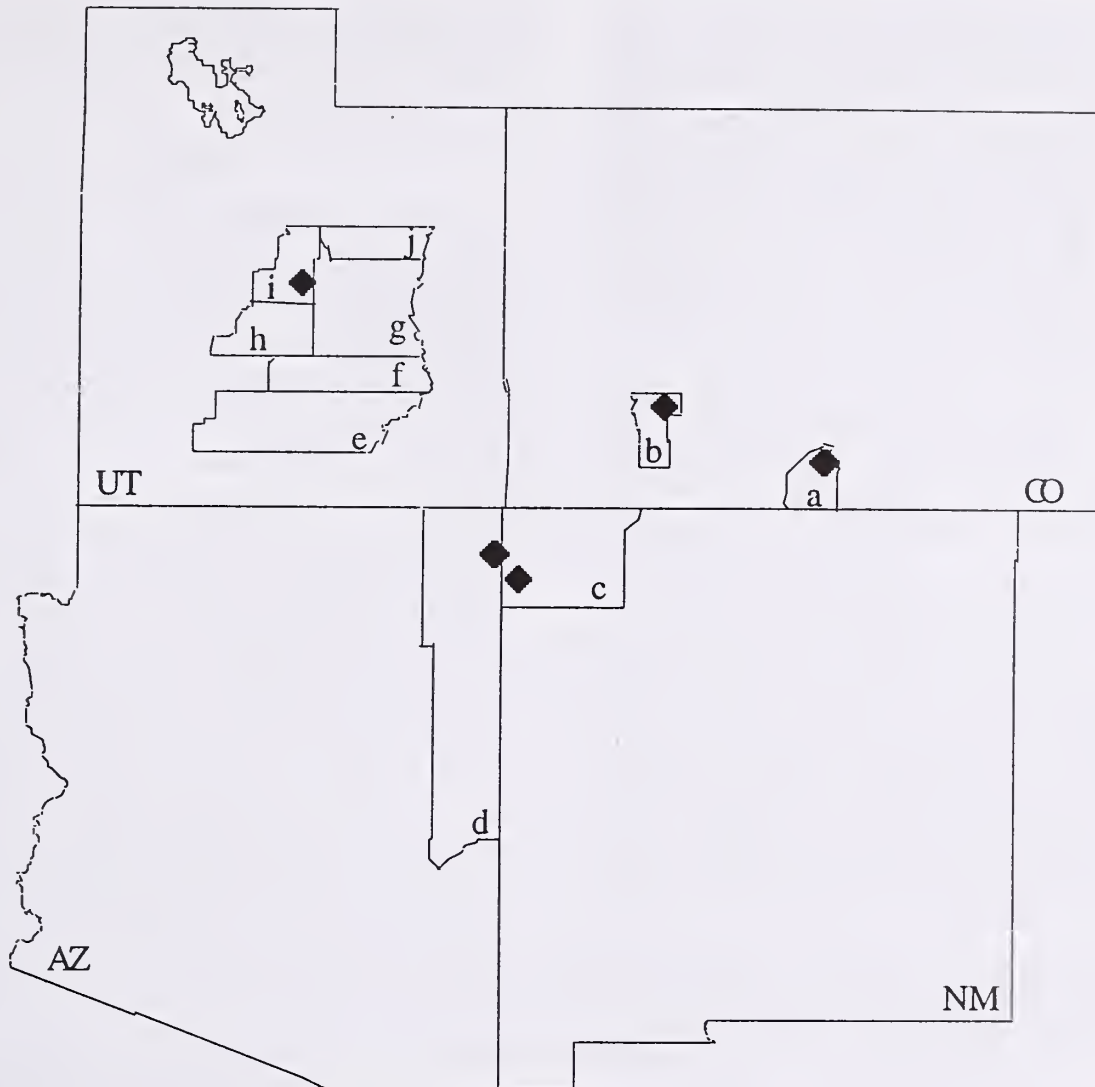


Figure 2. States, counties, and collection sites mentioned in the text. Counties: a = Costilla, b = Hinsdale, c = San Juan, d = Apache, e = Garfield, f = Wayne, g = Emery, h = Sevier, i = Sanpete, j = Carbon; ♦ = collection sites.

parents, *H. richardsonii* var. *floribunda* and *H. hoopesii*, only infrequently grow together. The former is typically a mid-elevation species of pinyon-juniper woodlands on arid sites, whereas the latter is a higher elevation species that grows on mesic sites in mountain meadows of the spruce/fir/aspens zone. The infrequent sympatry of the two putative parents could explain the sporadic, but widespread distribution of *H. helenioides*, if it is a hybrid.

To relocate *Hymenoxys helenioides* in Colorado and Arizona, the unusual co-occurrence of *H. richardsonii* var. *floribunda* and *H. hoopesii* was used as a marker for potential habitat. Using this search criteria, *H. helenioides* was relocated along Sangre de Cristo Creek, the type locality, at two sites approximately 10 miles apart, referred to below as La Veta Pass and Russell (Figure 2). Like the Utah

site, there is a similar pattern of a few *H. helenioides* individuals (eight in 1987 and four in 1988 at La Veta Pass and six in 1987 and eight in 1988 at Russell) among many *H. richardsonii* var. *floribunda* and *H. hoopesii*. The habitat is a montane sedge/grass meadow on a flood plain terrace below spruce/fir/aspens forest between 8400–9000 feet. The San Juan Mountains locality in Hinsdale County was rediscovered along Mill Creek, an upper tributary of Cebolla Creek (Figure 2), with 14 plants of *H. helenioides* in both 1987 and 1988 sympatric with large populations of *H. richardsonii* and *H. hoopesii*. The habitat is similar to that along Sangre de Cristo Creek, a montane meadow on a flood plain terrace below spruce/fir forest at 10,200 feet. *Hymenoxys helenioides* was thus rediscovered in Colorado for the first time in nearly 50 years.

The Arizona site of *Hymenoxys helenioides* was similarly relocated after nearly 50 years; again, there is the same pattern of scattered *H. helenioides* among large numbers of the two putative parents. The habitat here is grassy openings or understory park lands in ponderosa pine/Gambel oak/aspen forests at 8500 feet. *Hymenoxys helenioides* was then traced across the Chuska Mountains to Washington Pass in San Juan County, the first record of *H. helenioides* from New Mexico (Figure 2).

The pattern of co-occurrence of these three species together at every site strengthens the putative hybrid hypothesis for the origin of *Hymenoxys helenioides* in such widely separated sites. To explore the hypothesis that *Hymenoxys helenioides* represents the hybrid progeny of *H. richardsonii* and *H. hoopesii*, we used three lines of inquiry: morphometric analyses, pollen stainability, and molecular evidence for additive patterns of nuclear markers. Although the morphology of *H. helenioides* appears to be intermediate, it is important to demonstrate that statistically. In addition, if *H. helenioides* is a hybrid, fertility levels may be depressed, providing further support of hybridi-

zation. Finally, we sought molecular evidence for hybridization by looking at the putative hybrid for additive patterns of nuclear markers present in the putative parents.

## Methods

### *Morphometric Analysis*

Three Colorado populations (La Veta, Russell, and Mill Creek) were sampled for morphological variation (Table 1). An equal number of individuals of all three taxa were sampled at each locality, but the number of individuals sampled was a function of the number of the rarest taxon (*H. helenioides*) present. The following features were recorded for the sampled individuals in the field: plant height, number of stems, number of capitula, involucre width, number of phyllaries, number of ray flowers, length of ray corolla, width of ray corolla, number of lobes of cauline leaves, length of cauline leaves, maximum width of cauline leaves, number of lobes of basal leaves, length of basal leaves, maximum width of basal leaves, color of ray corolla, color of disc corolla, and amount of pubescence on the basal leaves. All

Table 1. Collections and localities of *Hymenoxys helenioides*, *H. hoopesii*, and *H. richardsonii* used in this study. Vouchers deposited at RSA unless otherwise indicated.

Locality	Taxon	Collector and Voucher Number
La Veta Pass, Sangre de Cristo Creek Costilla Co., CO	<i>helenioides</i>	Anderson 88-94 <sup>ab</sup>
	<i>richardsonii</i>	Anderson 88-105 <sup>ab</sup>
	<i>hoopesii</i>	Anderson 88-106 <sup>ab</sup>
Russell, Sangre de Cristo Creek Costilla Co., CO	<i>helenioides</i>	Anderson 87-116 <sup>cd</sup> ; 88-109 <sup>b</sup>
	<i>richardsonii</i>	Anderson 88-107 <sup>b</sup>
	<i>hoopesii</i>	Anderson 88-108 <sup>b</sup>
Mill Creek, (Cebolla Creek) Hinsdale Co., CO	<i>helenioides</i>	Anderson 88-95 <sup>ab</sup>
	<i>richardsonii</i>	Anderson 88-97 <sup>ab</sup>
	<i>hoopesii</i>	Anderson 88-96 <sup>ab</sup>
Wasatch Plateau Sanpete Co., UT	<i>helenioides</i>	Anderson 87-93 <sup>a</sup> and Thompson
	<i>richardsonii</i>	Anderson 87-92 <sup>ac</sup> and Thompson
	<i>hoopesii</i>	Anderson 87-94 <sup>ac</sup> and Thompson
Lukachukai Mts. (Chuska Mts.) Apache Co., AZ	<i>helenioides</i>	Anderson 87-126 <sup>a</sup> and Heil
	<i>richardsonii</i>	Anderson 87-132 <sup>a</sup> and Heil
	<i>hoopesii</i>	Anderson 87-125 <sup>a</sup> and Heil
	back-cross?	Anderson 87-128 <sup>a</sup> and Heil
Chuska Mts., Washington Pass San Juan Co., NM	<i>helenioides</i>	Anderson 87-147 <sup>ac</sup> and Heil

a = DNA vouchers; b = morphological vouchers; c = pollination stainability vouchers; d = CS.

measurements were made in millimeters and with the exception of plant height, were based on an average of three measurements made on each individual. The three populations were pooled for statistical analysis ( $n = 54$ ).

Summary statistics were calculated. Principal component analyses were performed on both the entire data set (using the correlation matrix) and on continuously varying characters alone (using the covariance matrix). Statistical analyses were performed using Statview statistical software on a Macintosh 840AV.

#### Pollen Stainability

Pollen stainability tests were conducted with cotton blue-lactophenol dye on those collections with sufficient pollen. Five hundred pollen grains were counted per specimen from *Hymenoxys helenioides* at Russell, Colorado, and Arizona (two replicates), and *H. hoopesii* and *H. richardsonii* from Utah (Table 1).

#### Restriction Site Analysis of the nrDNA ITS Region

Total DNAs were isolated from single individuals of *Hymenoxys helenioides*, *H. hoopesii* and *H. richardsonii* from each of the sampled populations (Table 1) using the modified CTAB protocol of Doyle and Doyle (1987). To further purify the DNAs, each DNA pellet was re-extracted a second time using the same CTAB protocol. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (ITS1, 5.8S subunit, and ITS2) was amplified using the protocol of Baldwin (1992), using a 1:1 ratio of the primers "ITS5" (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and "ITS4" (5'-TCC TCC GCT TAT TGA TAT GC-3'). PCR amplification followed Baldwin (1992) using an MJ Research (Watertown MA) thermal cycler. Purified PCR products were obtained using differential filtration in Millipore Ultra-Free-MC microfuge tubes (Millipore UFC-3 THK00). The amplified ITS regions were initially digested with the following restriction endonuclease enzymes: *AclI*, *AluI*, *BsaJI*, *BstUI*, *DdeI*, *HaeIII*, *HhaI*, *HinPI*, *MboI*, *MspI*, *RsaI*, *Sau96I* (4-bp recognition sites); *BamHI*, *EcoRI*, *KpnI*, *PstI*, and *HindIII*. The digested PCR products were separated on 1.4% agarose gels by electrophoresis, visualized by staining with ethidium bromide, and photographed.

## Results

#### Morphometric Analysis

Summary statistics for the 17 morphological characters are given in Table 2. *Hymenoxys helen-*

*ioides* possesses intermediate mean values (relative to *H. hoopesii* and *H. richardsonii*) for all continuously varying characters, except cauline leaf length. Similarly, all meristic measures from *H. helenioides* are intermediate relative to *H. hoopesii* and *H. richardsonii*. However, the intermediate values do not necessarily reflect statistically significant differences between the two more extreme species, although this is frequently the case. Basal leaf pubescence, a qualitative, ranked character, was likewise intermediate in *H. helenioides*. The unusual combination of corolla coloration in *H. helenioides* contains the yellow ray corollas like *H. richardsonii* and the orange disk corollas like *H. hoopesii*.

This general pattern of morphological intermediacy of *Hymenoxys helenioides*, relative to *H. hoopesii* and *H. richardsonii*, is graphically displayed in a principal component analysis (PCA) of the morphological data (Figure 3). The PCA using only continuously variable characters (not shown) demonstrates that most of the variation in the data (79.8%) is due to changes in overall size. The first principal component has high, positive factor loadings. This scaling effect is a contrast between the more diminutive *H. richardsonii*, the intermediate *H. helenioides*, and the more robust *H. hoopesii*.

#### Pollen Stainability

The results of cotton blue staining show high pollen sterility in *Hymenoxys helenioides*. Pollen fertility is only 6.9 percent in *H. helenioides* compared to 95.4 percent fertility in *H. hoopesii* and 57.6 percent fertility in *H. richardsonii* (Figure 4).

#### Restriction Site Analysis of the nrDNA ITS Region

Variable length in restriction fragments was observed in digests of *BstUI*, *HinPI*, and *HhaI*. In addition, because of the presence of an approximately 20 base indel in *Hymenoxys hoopesii*, fragment length differences were observable in several other digestions (e.g. *DdeI*), even though there were differences in the presence of restriction sites. Table 3 shows the size of restriction fragments at the five sampled localities. In all cases *H. helenioides* possessed a pattern of fragments that represented the additive combination of *H. richardsonii* and *H. hoopesii*. Figure 5 shows this additive pattern with *HinPI* from sites in Utah, Colorado, and Arizona. The enzyme *BstUI* showed different restriction site patterns at different localities. *Hymenoxys hoopesii* possesses a different restriction site pattern at the Hinsdale County site compared

Table 2. Mean values and standard errors (in parentheses) of 17 morphological characters from sympatric populations of *Hymenoxys helenioides*, *H. hoopesii*, and *H. richardsonii*, sampled at three localities in Colorado. Because of low sample sizes the three populations are pooled.

	<i>H. richardsonii</i>	<i>H. helenioides</i>	<i>H. hoopesii</i>
Plant height (mm)	202.6 (5.81)	388.3 (17.52)	691.1 (31.23)
Involucre width (mm)	7.3 (0.24)	13.4 (0.57)	24.8 (0.90)
Ray corolla length (mm)	7.4 (0.31)	15.2 (0.71)	28.8 (0.96)
Ray corolla width (mm)	4.2 (0.15)	5.9 (0.31)	6.7 (0.37)
Cauline leaf length (mm)	52.9 (2.42)	105.4 (5.82)	96.3 (4.04)
Cauline leaf width (mm)	1.4 (0.12)	4.1 (0.25)	23.1 (1.63)
Basal leaf length (mm)	62.7 (2.59)	130.8 (6.53)	182.1 (11.00)
Basal leaf width (mm)	1.2 (0.09)	4.1 (0.21)	14.4 (0.76)
Number of stems	11.3 (2.27)	6.5 (1.43)	5.7 (1.04)
Number of capitula per stem	9.9 (0.93)	8.3 (1.85)	6.2 (0.63)
Number of phyllaries	15.6 (0.16)	25.8 (0.51)	44.2 (2.03)
Number of ray flowers	7.8 (0.15)	12.4 (0.23)	22.8 (0.93)
Cauline leaf lobe number	3.7 (0.20)	3.3 (0.31)	1.0 (0.00)
Basal leaf lobe number	3.7 (0.23)	2.8 (0.25)	1.0 (0.00)
Basal pubescence <sup>a</sup>	2.8 (0.09)	2.2 (0.09)	1.4 (0.12)
Ray petal color <sup>b</sup>	2.0 (0.00)	2.0 (0.00)	1.0 (0.00)
Disc color <sup>c</sup>	2.0 (0.00)	1.0 (0.00)	1.0 (0.00)

<sup>a</sup> 1 = glabrous, 3 = pubescent; <sup>b</sup> 1 = orange, 2 = yellow; <sup>c</sup> 1 = orange, 2 = yellow.

Table 3. Length of nrDNA ITS region restriction fragments using two endonucleases. The fragment lengths are reported for *Hymenoxys richardsonii*, *H. helenioides*, and *H. hoopesii* from four localities. Only *H. helenioides* was sampled at the San Juan County population. The Costilla County collection locality represents La Veta Pass.

Enzyme	Collection Locality	Fragment Length (bp)		
		<i>H. richardsonii</i>	<i>H. helenioides</i>	<i>H. hoopesii</i>
<i>Bst</i> UI	Sanpete Co., UT	478, 317	478, 459, 317	459, 317
	Apache Co., AZ	478, 317	478, 459, 317	459, 317
	Hinsdale Co., CO	478, 317	767, 478, 317	767
	Costilla Co., CO	478, 317	478, 459, 317	459, 317
	San Juan Co., NM	–	478, 459, 317	–
<i>Hin</i> PI	Sanpete Co., UT	421, 289, 263	421, 289, 263, 146	289, 263, 146
	Apache Co., AZ	421, 289	421, 289, 263, 146	289, 263, 146
	Hinsdale Co., CO	421, 289	421, 289	421, 289
	Costilla Co., CO	421, 289	421, 289, 263, 146	289, 263, 146
	San Juan Co., NM	–	421, 289, 263, 146	–

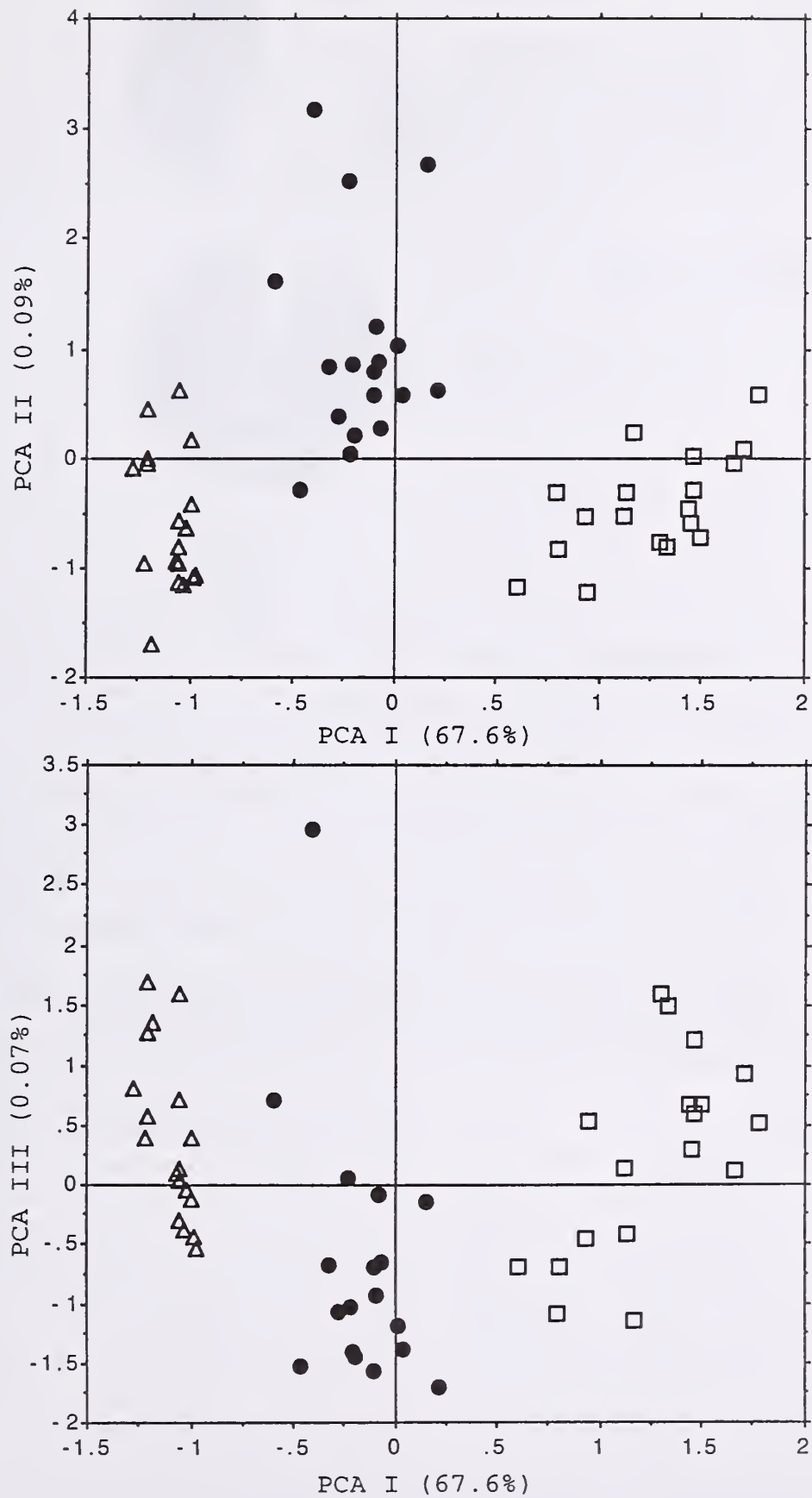


Figure 3. Principal component analysis ordination of 17 morphological characters. The upper figure shows axes 1 and 2, the lower shows axes 1 and 3. ● = *H. helenioides*; □ = *H. hoopesii*; △ = *H. richardsonii*.

*Hymenoxys richardsonii*



*Hymenoxys helenioides*



*Hymenoxys hoopesii*

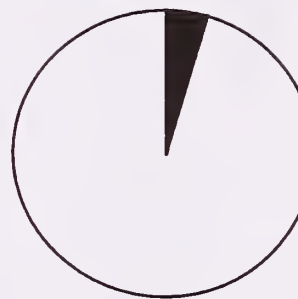


Figure 4. Pollen stainability; frequency of fertile (white) and sterile (black) pollen grains.

to the Costilla County locality, but *H. helenioides* still shows an additive pattern. Figure 6 shows this pattern for *BstUI* at two sampled sites in Colorado.

### Discussion

The suggestion that there exists another hybrid taxon in Asteraceae is consistent with the 2242 cases of hybridization in the Asteraceae reported by Knobloch (1972). These included 43 cases of intergeneric hybridization. At the beginning of this study, *Hymenoxys hoopesii* had long been treated as either *Dugaldia hoopesii* (Gray) Rydb. or *Helenium hoopesii* Gray, which would have made *H. helenioides* a putative intergeneric hybrid. It is possible that previous researchers were less prone to discern intergeneric hybridization in *H. helenioides*. *Hymenoxys helenioides* had also been named *Dugaldia helenioides* (Rydb.) A. Nelson (Nelson 1909), an indication of uncertainty about its true taxonomic position. Bierner (1994) recently transferred all species of *Dugaldia* to *Hymenoxys* (see also Beaman et al. 1962), making the new combination

*Hymenoxys hoopesii* (Gray) Bierner.

The co-occurrence of *Hymenoxys helenioides* with two species that are infrequently found together, *H. richardsonii* and *H. hoopesii*, coupled with the highly dispersed nature of populations, does not by itself indicate that *H. helenioides* is a hybrid. First, for *Hymenoxys helenioides* to be a hybrid, the putative parents must be capable of cross breeding to produce such an offspring, for instance by having the same base chromosome number. A review of the literature (Keil et al. 1988) shows chromosome counts of the same base number of  $n = 15$  for both *H. richardsonii* (Strother 1966) and *H. hoopesii* (Raven and Kyhos 1961). If interbreeding were to occur between species of *Hymenoxys*, it is certainly more likely between two species with similar chromosome numbers. A chromosome count of  $2n = 30$  for *H. helenioides*, based on Parker et al. 6369 from Utah (Speese and Baldwin 1952), is consistent with the hypothesis that *H. helenioides* is a diploid cross between *H. richardsonii* and *H. hoopesii*.



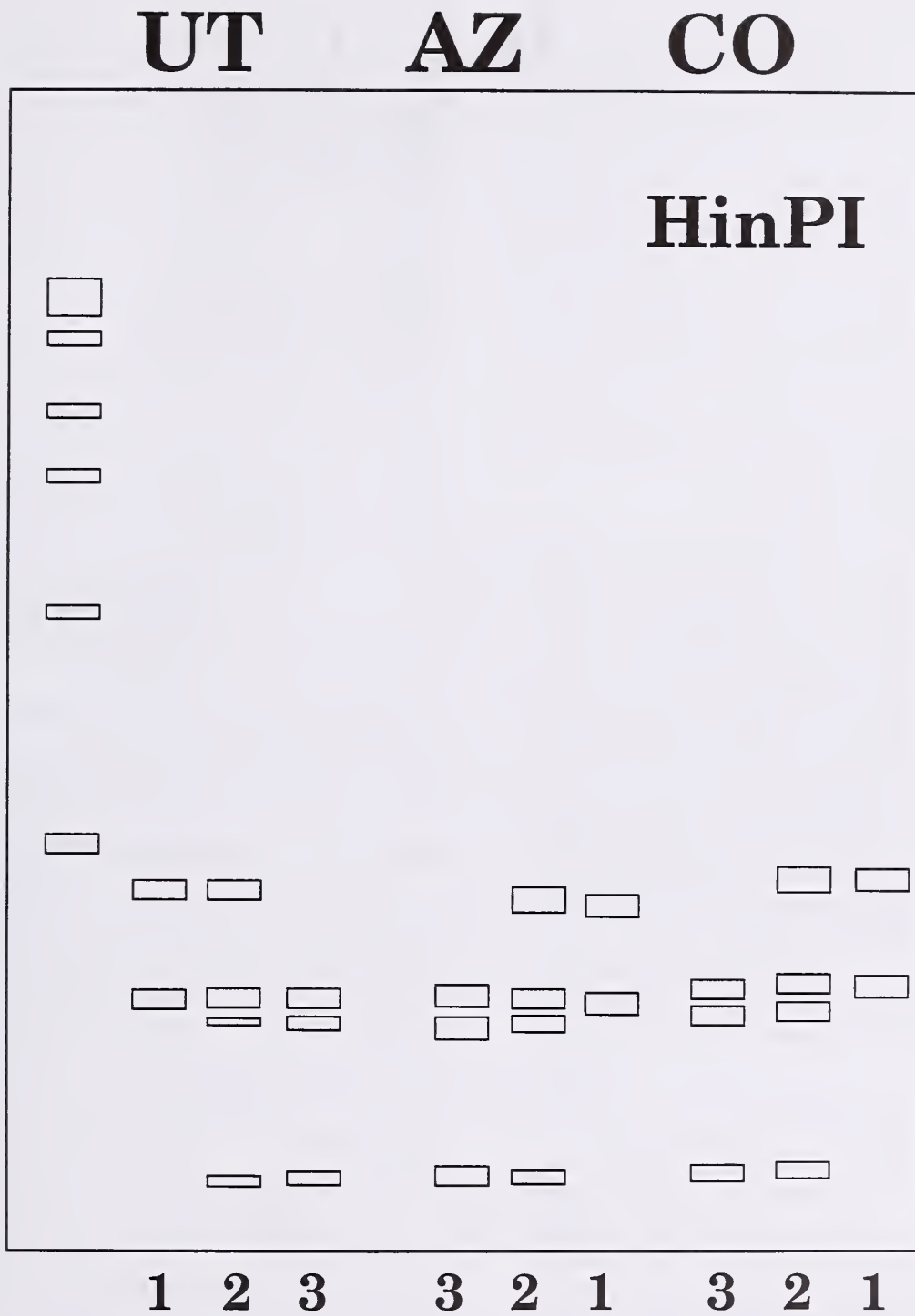


Figure 5. Line drawing representing restriction fragment length polymorphism of nuclear ribosomal DNA (ITS region) from representative sites with *Hymenoxys richardsonii* (1), *H. helenioides* (2), and *H. hoopesii* (3). Localities are from Arizona (AZ), La Veta Pass, Colorado (CO), and Utah (UT). Digests of the endonuclease *HinPI* illustrate the additive pattern of bands in *H. helenioides* at each site.



The results of the three lines of inquiry reported above demonstrated that *Hymenoxys helenioides* possesses hybrid characteristics. The PCA graphs, as well as the univariate statistics, show the morphological intermediacy of *H. helenioides*. Moreover, this taxon is highly pollen sterile, as shown by the pollen stainability. Finally, nuclear molecular markers of both putative parents are additively found in *H. helenioides*. The distribution data, co-occurrence with putative parents, morphological intermediacy, pollen sterility, and additive molecular markers together provide compelling evidence that *H. helenioides* is a hybrid taxon, resulting from crosses between *H. richardsonii* and *H. hoopesii*. The fact that different additive patterns of molecular markers are found in different populations also provides evidence that the hybridization events at the different sites are independent events, arguing that *H. helenioides* is independently derived at each of the sites and arguing against the suggestion that this is a self-perpetuating diploid hybrid species of a single origin.

We suggest that this entity be considered a nothospecies:

*Hymenoxys xhelenioides* (Rydb.) Cockerell pro. sp. et status nov.

*Picradenia helenioides* Rydb. Bull. Torrey Bot. Club 28: 21. 1901.

*Hymenoxys helenioides* Cockerell, Bull. Torrey Bot. Club 31: 481. 1904.

*Dugaldia helenioides* A. Nelson in J. M. Coult. & A. Nelson, New Man. Bot. Centr. Rocky Mts. 562. 1909. (Rydberg & Vreeland 5495, On Sangre de Christo [sic] Creek, Colorado; holotype at NY.)

The investigation of this obscure candidate taxon, *Hymenoxys helenioides*, found it to be unqualified for listing as endangered because, as a hybrid, it is not a valid taxonomic entity. Therefore, federal protection is unwarranted.

Although *H. xhelenioides* is a highly sterile hybrid, it is not necessarily an evolutionary "dead-end." Because some fertile pollen is present, back-crossing is possible, which provides an avenue for gene transfer between *H. richardsonii* and *H. hoopesii*. Moreover, with continued back-crossing it is possible that *H. xhelenioides* might eventually significantly increase fertility (Grant 1981).

### Acknowledgments

The U.S. Fish and Wildlife Service supported the field work. Mr. Robert Thompson from the Manti-La Sal National Forest Supervisor Office in

Price, Utah showed the first author *Hymenoxys helenioides* in the field in Utah. Mr. Ken Heil from San Juan College, Farmington, New Mexico provided navigation and field assistance in the Chuska Mountains. The Forbes Trinchera Ranch provided access to the Sangre de Cristo Creek area in Costilla County, Colorado. Dr. Dieter Wilken of the Santa Barbara Botanical Garden conducted the initial statistical tests of the morphological data and gave suggestions on its analysis. Dr. Donald Pinkava of Arizona State University provided laboratory materials and space for the pollen stainability research and reviewed the manuscript. Rancho Santa Ana Botanic Garden provided the same for the molecular research. The herbaria at ARIZ, ASU, ATC, BYU, COLO, CS, RSA, SJNM, and UTC were consulted. The drawing of *Hymenoxys helenioides* was done by Ms. Kaye Thorne.

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# Genetics and Conservation Biology: Assessing Historical Trends in the Demography of Populations

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**Abstract:** In many ways the long-term demography of rare plant species is particularly important in determining the likelihood of their persistence. Unfortunately, demographic parameters such as population size and mating system generally fluctuate from generation to generation. As a result, direct demographic observations over the short term may not provide a representative view of either the long-term situation or historical trends. Genetic signatures observable in DNA sequences, however, depend on the demographic properties of populations and can be used to infer long-term demography. Comparisons between these long-term estimates and current demographic observations can be useful for elucidating historical trends and can therefore provide information essential to the evaluation and management of rare plant species.

## Introduction

While genetic information has played an important role in the development of conservation biology by focusing attention on the evolutionary properties and population genetics of small populations, the demographic properties of small populations are often regarded as of paramount importance in determining long-term viability (Lande 1988). The importance of demographic properties stems in part from the fact that the alternative—a connection between genetic variation, usually at marker loci, and long-term fitness—is rather indirect. For example, several types of genetic variation must be distinguished, each one of which may respond to a small population size in different ways (Lande and Barrowclough 1987, Polans and Allard 1989). Neutral variation at marker loci may require many thousands of generations to recover following an extended reduction of population size, whereas recovery of variation for quantitative traits may require only hundreds of generations. Empirical data also suggest no direct connection. For example, one rare plant, *Plantago cordata*, exhibited a great deal of genetic variation for life history and morphological characters even while its population size declined steadily (Meagher et al. 1978). A quantitative genetic study of two *Senecio* populations that differed in size discovered greater heritability for numerous life history traits in the smaller population rather than in the larger one, as might have been expected (Widén and Andersson 1993). In an experimental setting, neither the population growth rate nor size was related to initial marker locus variability in the mosquitofish (Leberg 1993). It is also not necessarily the case that increased ho-

mozygosity results in decreased fitness, although many examples of the phenomenon exist. For example, no correlation between heterozygosity and fitness was found in forked fungus beetles (Whitlock 1993), brook trout (Hutchings and Ferguson 1992), or a tropical palm (Eguiarte et al. 1992). Thus, the direct importance of genetic variation to conservation biology has not been universally supported.

In contrast, the direct events leading to reduction in population size clearly involve demographic components. As a result, the demographic properties of small populations are of prime interest (Bradshaw and Doody 1978, Goodman 1987b, Lande 1988, Pavlik and Barbour 1988, Thomas 1990), and detailed information on the demography of a species may give the most relevant information for conservation biology. For example, two important demographic parameters, the mean and variance of population growth rate, largely determine the time until extinction for populations (Goodman 1987a), and have been used as the basis for analyzing the viability of mammalian populations (Belovsky 1987). Much more detailed demographic information encompassed in matrix projections of stage classes has been used in analyzing the viability of several plant species (Menges 1990, 1992).

Because demographic properties of populations change with time, the most useful information derives from long-term studies that quantify the long-term trends relevant to population persistence. Unfortunately, such studies often require more time than is currently available, and always require major investments of labor and money. As a result, the most useful information—direct mea-

surements of demographic properties over long spans of time—may not be forthcoming by using traditional demographic approaches. Nevertheless, an understanding of the long-term demographic patterns is likely to be most useful as an indicator of future prospects for a rare species of either plant or animal. It is, therefore, important to consider alternative means of obtaining long-term demographic information on species of conservation interest. A coupling of new genetic techniques that enable estimation of long-term demographic properties of populations with direct observation of demographic properties over the shorter periods accessible today is most likely to provide the necessary information (Milligan et al. 1994).

### Genetic Markers and Demographic Properties of Populations

The demographic history of a population is reflected in its genetic composition; indeed, this is the basis for the development of retrospective population genetics (Ewens 1990). It should be possible, therefore, to extract demographic information of use in conservation biology from studies of the genetic composition of a population or set of populations. For this purpose, the most useful genetic markers are those that provide a means of

classifying individuals into nominal genotypic categories that themselves may be grouped according to degree of relationship. These markers include DNA sequences and restriction site maps.

The importance of markers such as DNA sequences is that the relationships among genotypic categories contain a record of the history of those categories (Figure 1). The hierarchical pattern of similarity among genotypic classes—for example, in number of shared nucleotides—reflects the genealogical similarity of those classes; those haplotypes that share a larger number of nucleotides have a more recent common ancestor than those that share a smaller number. While this, of course, is the basis for an explosion of interest in such markers for phylogenetic reconstruction in the field of systematics, it is equally applicable to samples of gene sequences taken from within a population or set of populations. One can determine which gene sequences are most closely related to each other and when the most recent common ancestor of different sequences occurred. This genealogy of genes is distinct from the genealogy of the individuals carrying the genes.

First formalized in the population genetics literature by Kingman (1982), the study of gene genealogies has led to conceptual clarification of

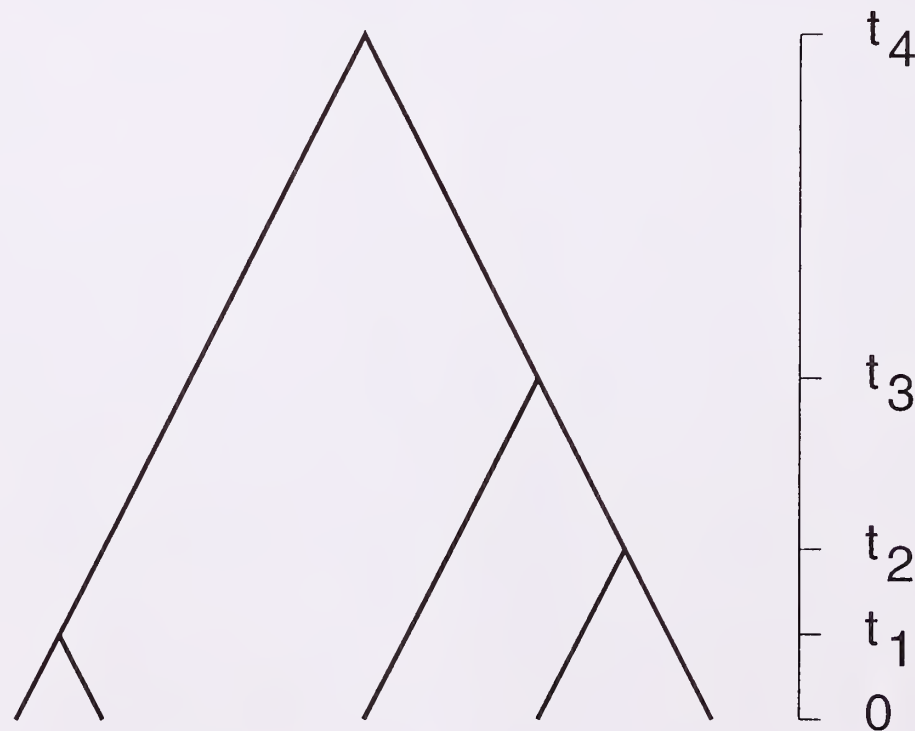


Figure 1. Genealogical relationships among a sample of five DNA sequences. The structure of this genealogy, represented by the pattern of common ancestry and the times to common ancestry given on the right, is determined by such demographic properties as effective population size, mating system, and migration rates.

many classical results (Ewens 1990, Hudson 1990), and will lead to new progress in the field of conservation genetics. Fundamentally, the clarification comes about because of the separation of factors affecting the genetic composition of a sample. Specifically, the genealogical approach makes it clear that genetic processes like mutation that lead to the origin of new genotypes are distinct and independent of demographic factors, including population size, mating, and migration, that influence the structure of the genealogy relating the sampled genes to each other. It is usually these latter factors that are of prime interest in conservation biology.

As an example, consider how population size influences the structure of a genealogy of genes in terms of the times to common ancestry. In a large population a pair of genes is likely to share a common ancestor in the distant past, whereas in a small population a pair of genes is likely to share one more recently (Figure 2). This is simply a consequence of the number of gene copies available each generation from which to derive copies in the subsequent one, and is only a restatement of the

effects of genetic drift. However, by focusing on time to common ancestry one can recognize that the number of mutational events that intervene between that time and the present has no bearing on the relationship between population size and time to common ancestry. It is important to note, however, that the mutation events allow one to reconstruct the topological relationships among genes, and in turn the time to common ancestry, from which population size may be estimated. Therein lies the utility of genetic markers for which genealogical relationships among genotypic categories can be determined. For such markers these two factors—the influence of genetic factors such as mutation and the influence of demographic factors such as population size—are independent of each other.

The major advantage of genetic markers that provide genealogical information is that because the role of genetic and demographic processes are so clearly separable, such markers may be used to estimate parameters governing the long-term demographic processes of interest to a conservation biologist.

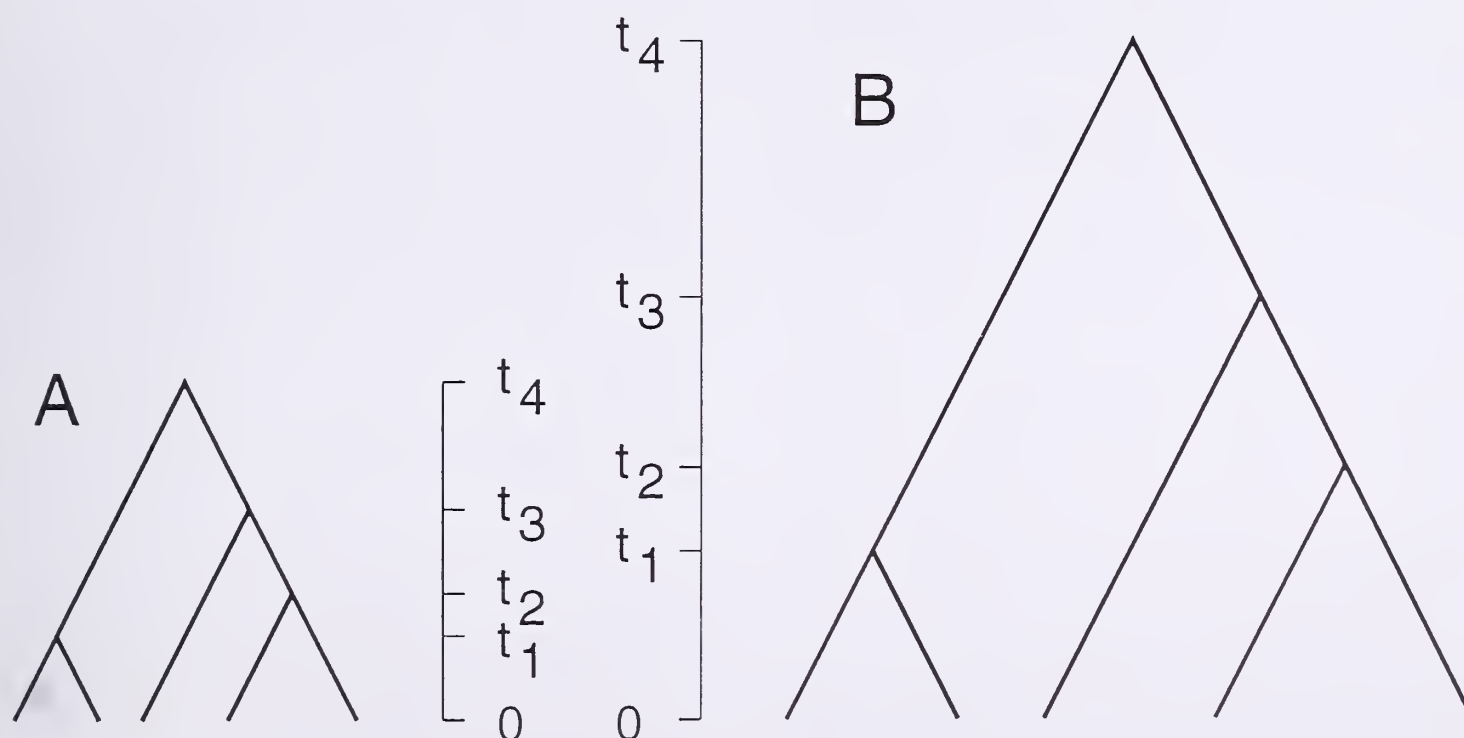


Figure 2. Possible genealogical relationships among a set of five DNA sequences sampled from populations of different sizes, one (A) of which is one half as large as the other (B). Note that the time unit is the same for the two genealogies.

## Comparing Long-Term Genetic and Short-Term Demographic Estimates

Genealogical estimates of demographic parameters such as population size or mating system are inherently based on specific models of the population of interest. Generally, the models that are currently available assume at least relatively constant demographic conditions over long periods of time. As a result, when used to estimate demographic parameters, these same models provide long-term averages. That is, they quantify the properties of an "ideal" population exhibiting constant demography that is equivalent to the "real" population from which DNA sequences were sampled. Because of the temporal lag in the historical information contained within the genealogy of DNA sequences, the estimates truly depict long-term conditions. In contrast, direct observations of current demographic properties at best provide an indication of the short-term average and the stochasticity prevailing at the present.

This mismatch in temporal scale is, however, a basis for determining long-term trends in demographic conditions. A comparison between the long-term average obtained from genealogical analyses and the short-term average obtained from direct demographic observations provides an assessment of how the current situation deviates from the long-term one. Hence, an indication of long-term trends is available.

The following sections outline methods involving genealogical analysis of DNA sequences sampled from natural plant populations for the purpose of quantifying several important demographic parameters: effective population size, mating system, and migration rate. Because all are relevant for conservation biology in terms of assessing population viability, it is hoped that this paper will serve not only to illustrate the importance of coupling genetic and demographic studies, but also to underscore the unique ability of population genetics to provide the critical long-term information needed in conservation biology.

### Effective Population Size

Effective population size, a concept first introduced by Wright (1931), has long been recognized as a general indicator of the rate at which genetic drift alters the genetic composition of a population. Defined as the size of an ideal population that behaves genetically in the same way as the real population of interest, a number of effective sizes can be recognized depending on which property is

equivalent; these include the variance effective size, the inbreeding effective size, and the eigenvalue effective size (Kimura and Crow 1963, Ewens 1979, Crow and Denniston 1988). Despite this diversity of effective sizes, we restrict our consideration to the inbreeding effective size. Because this quantity reflects the rate at which identity by descent of genes within an individual increases within a population, it may be most useful in the context of conservation biology where inbreeding and inbreeding depression are concerns. Although they do not distinguish between the various types, Mace and Lande (1991) include effective size as a criterion for establishing official categories of endangerment.

Three genealogical approaches to the estimation of inbreeding effective population size have been developed (Strobeck 1983, Felsenstein 1992, Kuhner et al. 1995). Although the methods differ in detail, all rely on the observation that the probability of a pair of genes in one generation being derived from a single gene in the previous generation depends only on the current population size (in the absence of selection, migration, and so forth). This observation implies a distribution of times to common ancestry for pairs of genes, or by extension a distribution for times on an entire genealogy of many genes. Because such a genealogy (including times to common ancestry) can be inferred from DNA sequence data for a set of genetic markers using the wide array of techniques for phylogenetic reconstruction, and because the distribution of times to common ancestry depends on the effective population size, one can choose the value of effective population size that provides the best match between the predicted times to common ancestry, and hence degree of divergence between sequences, and the observations.

In many instances, long-term measures of effective population size would be extremely useful. For example, if a population has always occurred in small numbers, it might be adapted to its rare condition, whereas if a population has only recently become small, the effects of inbreeding may be catastrophic. Also, the long-term degree of variability in population size may be an important predictor of extinction probability (Goodman 1987b). Comparison of long- and short-term measures of effective size may provide an indication of historical trends.

### Mating System Estimation

Genetic transmission from one generation to the next, mediated by the pattern of matings



linking generations, is a major focal point in plant conservation biology because of its dual role in determining the genetic composition of a population and in indicating the dependence of reproductive success on pollen vectors, characteristics that may determine long-term persistence. In plants, the pattern of mating varies considerably and includes regular systems of inbreeding, often with extensive self-fertilization, breeding among relatives within small neighborhoods, and negative assortative mating maintained by incompatibility systems (Clegg 1980); plant populations rarely exhibit random mating (Fryxell 1957, Willson 1984). Characterization of mating systems is, therefore, of prime concern in the study of plant conservation biology.

Quantification of plant mating systems often involves a mixed mating model in which matings consist of either selfing or outcrossing to a common pollen pool (Brown 1989, Clegg 1980, Ritland 1984). The degree of selfing estimated by this model positions a population along a continuum between complete selfing and random mating and provides a useful summary of many mating systems.

Estimation of the degree of selfing has traditionally been based on surveys of allozyme segregation in sets of progeny arrays collected from a population (Figure 3) (Brown and Allard 1970, Brown et al. 1975, Brown et al. 1986, Brown et al. 1989, Holtsford and Ellstrand 1990, Warwick 1989). This approach offers the advantages of being experimentally tractable and efficient at quantifying mating systems for a single generation. However, because of the numerous demographic and ecological factors known to affect the mating process (Brown 1989, Brown et al. 1989, Lloyd 1980, Willson 1984), single-generation estimates may not be useful for studies concerned with the long-term evolution of mating systems. For example, single-generation estimates of selfing rate made over multiple years demonstrate temporal variation (Vasek and Harding 1976, Hamrick 1982, Holtsford and Ellstrand 1990, Dole and Ritland 1993). As a result, this approach is limited to providing a short-term measure of the mating system.

A long-term estimate of the mating system can be made by taking advantage of the fact that the outcrossing rate within a population influences the times to common ancestry of alleles within versus among individuals. In the case of a population with a high degree of selfing, the times to common ancestry of two alleles sampled from within an

individual will, on average, be shorter than that between two alleles sampled among individuals. In contrast, under random mating the times to common ancestry are the same for alleles sampled within or among individuals. Figure 4 illustrates these points by following the fate of pairs of alleles through one generation of either selfing or random mating.

Because the time to common ancestry may be estimated from DNA sequence information, the contrast between sequences sampled in these two ways provides estimates of long-term mating system parameters within the context of the mixed-mating model (Milligan 1996). Figure 5 contrasts the genealogical estimator of mating system with the only other long-term estimator of mating system, one based on the degree of homozygosity ( $F$ ) within a population. Over a wide array of population sizes and over the full range of selfing rates, the genealogical estimator is essentially unbiased, whereas the alternative based on homozygosity is severely and variably biased.

The two general approaches to estimating mating systems outlined above attempt to answer separate questions: the first focuses on short-term measures, whereas the second focuses on long-term measures. A comparison between them, however, can provide an indication of whether or not the current mating system reflects the long-term average. Major discrepancies may signal situations placing populations at risk of extinction, or at least of potential decline. For example, if a long-term estimator indicates that a population has historically outcrossed, but a short-term estimator based on recent generations indicates a high level of selfing, the total reproductive output of the population may be declining due to pollen limitation. Therefore, both types of estimators should be useful to conservation biologists in estimating the risk to rare taxa due to mating system dynamics.

### Genetic Structure and Gene Flow

Population structure is a basic element of the biology of most, if not all, species because dispersal limitations result in some degree of both genetic and demographic isolation among discrete populations (Levin and Kerster 1974). This isolation has genetic (Ellstrand 1992, Ellstrand and Elam 1993) and demographic consequences that may be of concern for conservation biologists. For example, migration among populations may increase the viability of rare species by reducing the threat of demographic stochasticity within a

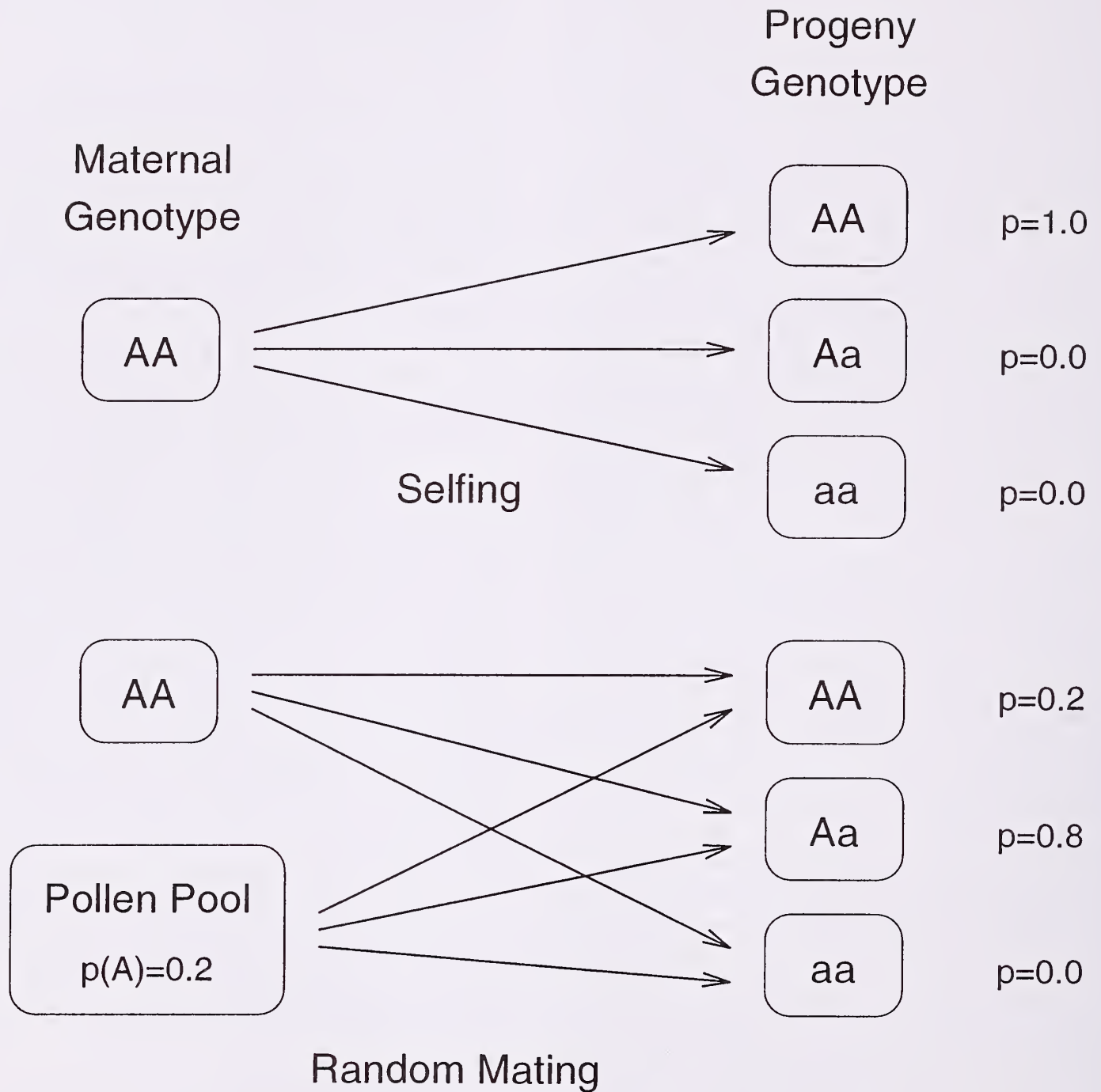


Figure 3. Transmission of genes from parents to progeny under the two components of mixed mating, selfing and random mating. The progeny genotype distributions derived from a homozygous female parent and a pollen pool with fixed allele frequencies are illustrated.

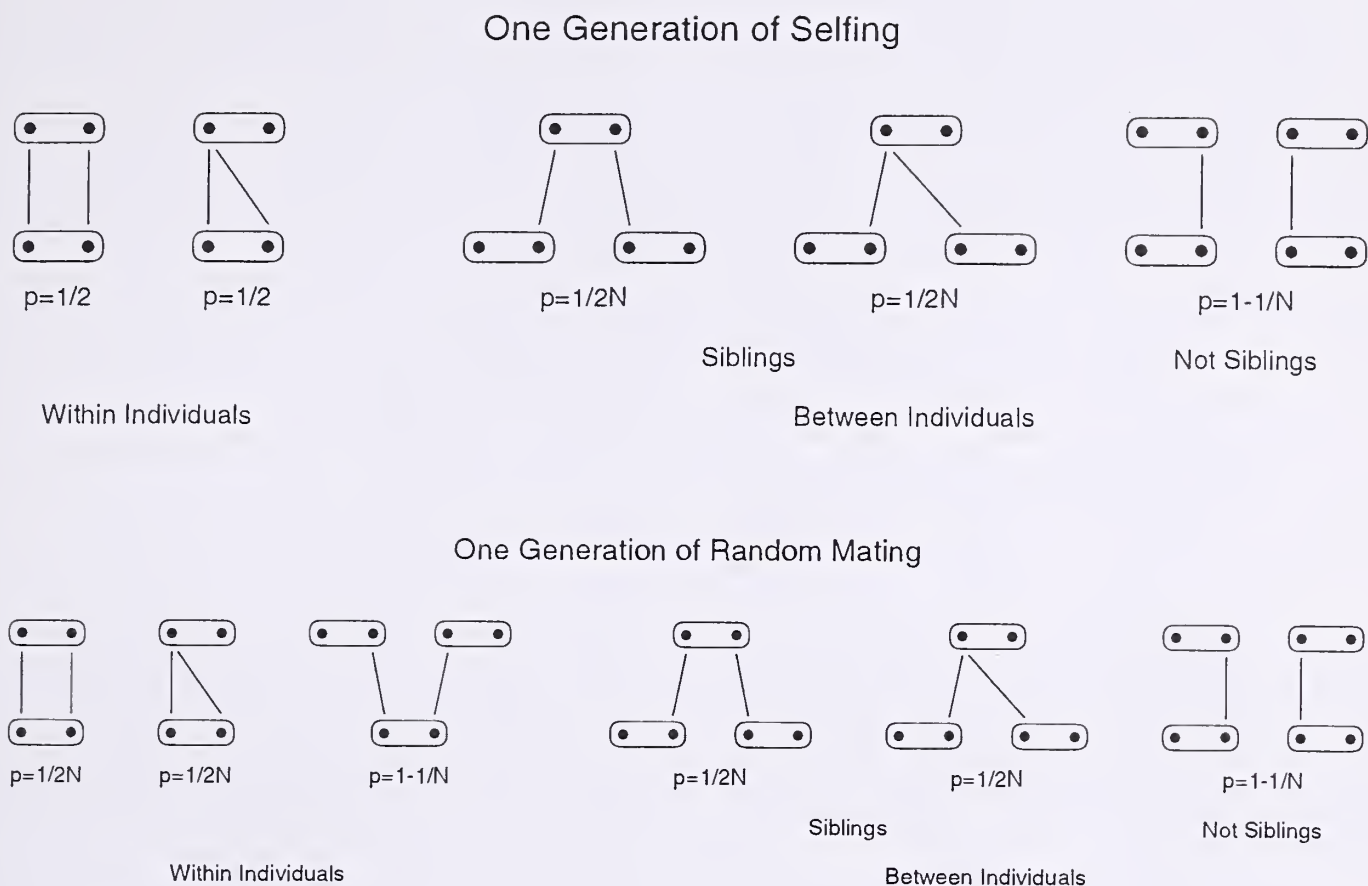


Figure 4. Possible genealogical relationships between pairs of alleles sampled within and between individuals. The upper panel depicts the effects of a single generation of selfing, while the lower panel depicts the effects of a single generation of random mating. Beneath each diagram is the frequency ( $p$ ) of the corresponding relationship within a population only undergoing either selfing or random mating.

population and by increasing the chance for colonization of suitable habitat (Menges 1990). The degree of isolation among populations may also be important in deciding upon the appropriate geographic scale at which to pursue conservation efforts. Thus, distinguishing among alternative population structures and distinguishing actual from apparent gene flow (Strand et al. 1996) may play an important role in conservation biology.

As for estimating other demographic properties of populations, genealogical analyses are appropriate for assessing the degree of ongoing migration among populations. This is because migration of individuals acts to transfer alleles from one subpopulation to another. When viewed in terms of the genealogical relationships of the alleles, together with their locations, a pattern such as illustrated by Figure 6 might result; the degree to which alleles are transferred between subpopulations is directly related to the rate of migration.

Alternatively, one might consider only the genealogical relationships of alleles, independent of their locations, and map onto that genealogy the location of each allele and its ancestors (Figure 7). Given standard means of constructing such genealogies from DNA sequence data (Swofford and Olsen 1990) and of inferring the number of transitions between localities for a collection of alleles, Slatkin and Maddison (1989) constructed a means of estimating migration rate.

### Complex Life Histories

The preceding sections should make clear that many important demographic properties of populations can readily be estimated from genetic data on DNA sequences sampled from natural populations. As a result, much is to be gained in the field of conservation biology by integrating genetic and demographic studies of rare plants. It should also be evident, however, that estimations of demo-

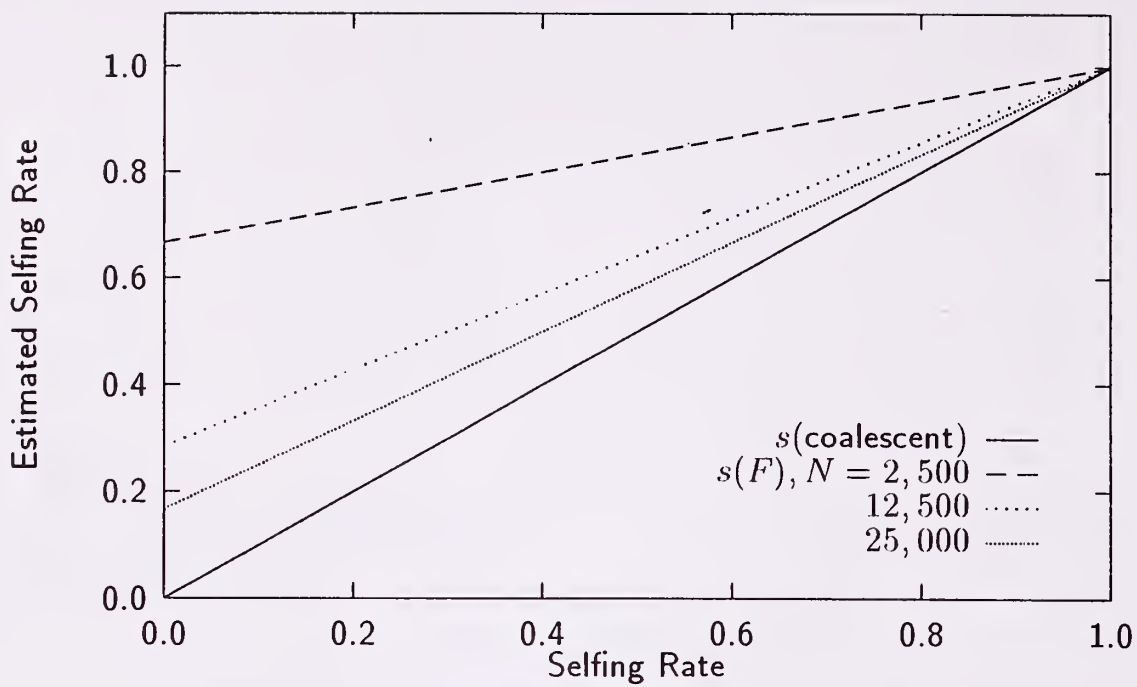


Figure 5. Genealogical (coalescent) and traditional ( $F$ ) equilibrium estimates of the mating system as a function of the parametric values of selfing rate  $s$  and population size  $N$ . Equality between the parametric and estimated values is represented by the solid diagonal line coinciding with the genealogical estimator,  $s(\text{coalescent})$ . Note that the line depicting the geological estimator of selfing rate represents superimposition of values for the same range of population sizes as for the traditional estimator,  $s(F)$ . In all cases,  $\mu = 10^{-4}$ .

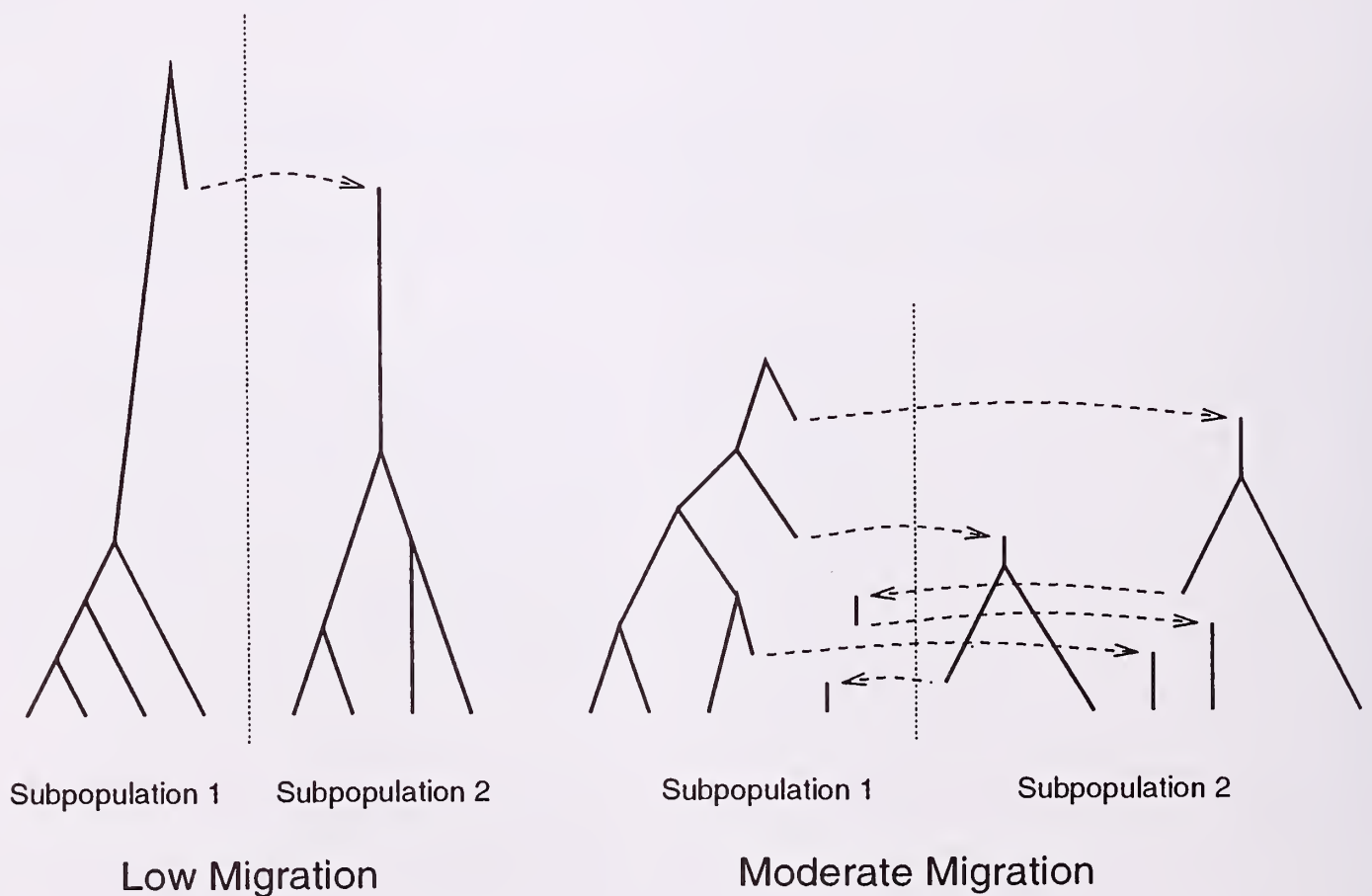


Figure 6. Genealogical relationships of alleles sampled from two populations connected by migration. Migration of individuals transfers the alleles from one subpopulation to another.

graphic properties from genetic data require the use of a model describing how the observable genetic properties relate to the underlying demographic properties. So far, the models considered have all corresponded to extremely simplistic life histories; in particular, they ignore such features common to plants as the seed bank, perennial growth habits, and size or age structure. While this may be a limitation of most currently available models, it does not reflect a fundamental flaw with the general approach of linking genetics and demography. Rather, it points to an area in which future research would be very profitable (Milligan et al. 1994).

In the case of estimating effective population size, however, arbitrarily complex life histories may be incorporated into the models (Orive 1993), thus making them applicable to any plant species. The framework for doing so is the standard matrix-projection model used to describe transitions from one age or demographic stage to another (Caswell 1989). For example, Figure 8 illustrates the transitions between life-history stages of *Pedicularis furbishiae* (Menges 1990). Although such figures are commonly interpreted as giving the probability with which an *individual* of one stage in a particular generation will move into another stage in the subsequent generation, it can equally be interpreted as the probability with which an *allele* moves from one stage to another. As a result, life cycle graphs such as Figure 8 can be used to trace patterns and times of common ancestry between pairs of alleles sampled from natural populations. Because the times of common ancestry are related to the degree of DNA sequence divergence, the genetic and demographic properties of populations exhibiting even very complex life histories can be linked, as is required if we are to estimate the demographic from the genetic properties.

Not only do plants generally exhibit complex life histories, but they also generally exhibit nonrandom mating. It is important, therefore, to determine how the mating system interacts with a complex life history to influence such demographic properties as effective population size. Using the already quite general models of Orive (1993) as a foundation, we have included a description of mating involving a mixture of selfing and random outcrossing, the classical mixed mating model (Brown 1979, Clegg 1980).

Although a wide array of results are possible given the potential complexity of the model, Figure 9 illustrates three general patterns evident in

this analysis of the *Pedicularis* life cycle of Figure 8. First, actual census population size has little effect on the ratio of effective to census size. That is, populations with complex life histories act as an ideal population of somewhat smaller size than indicated by the number of reproductive adults, but how much smaller is independent of the actual population size. Second, the type of mating system has a dramatic effect on how much smaller the effective size is relative to the census size. There is a very large difference between random mating ( $s = 0$ ) and a small degree of selfing (e.g.,  $s = 0.1$ ), but over a wide range of mixed mating systems the actual selfing rate has little effect. This is important when considering the prospect of estimating effective size, because it indicates that, while it is necessary to determine whether any selfing occurs, precise quantification of the actual selfing rate is not critical. Finally, especially from the perspective of conservation biology, the general magnitude of effective size relative to census size is important. For a wide range of selfing rates and population sizes, effective size is less than 10 percent of the number of reproductive adults; it is a much smaller fraction of the total population size because only about 12 percent reproduce. Populations of *Pedicularis* exhibit the demographic properties of much smaller ideal populations. As a result, further reduction in size may have drastic effects should that reduction eliminate any of the individuals responsible for maintaining the populations at even these small levels. Such small effective sizes for plants with complex life histories and mixed mating is in distinct contrast to the larger effective sizes of many animal populations, even those with extremely nonrandom mating (Nunney 1993, Nunney and Campbell 1993). This suggests the need for greater concern when managing rare plant populations.

### Future Prospects

Conservation biology faces a broad array of problems, the most important of which is how to allocate scarce resources to effect the task of preserving biological diversity. An array of techniques, including assays of genetic markers, have been brought to bear on the problems. The role of genetic information has often been limited, however, to the quantification of genetic diversity and how it is partitioned within and among populations. While this is clearly important for the tasks of conserving genetic diversity per se (Falk 1990), it is inadequate for the broader task of providing information on demographic factors affecting

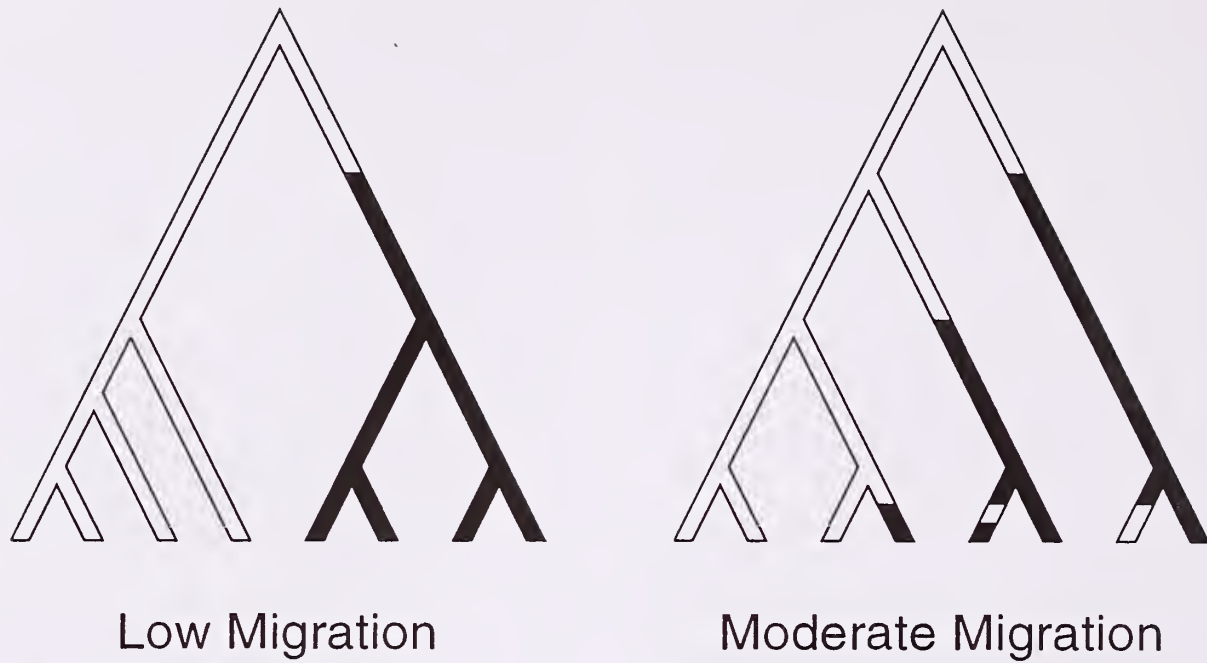


Figure 7. Migration depicted as a characteristic overlaid on the genealogical relationship of alleles. The migration pattern depicted in Figure 6 is shown with the shaded portions of the genealogy representing an allele present in subpopulation 2.

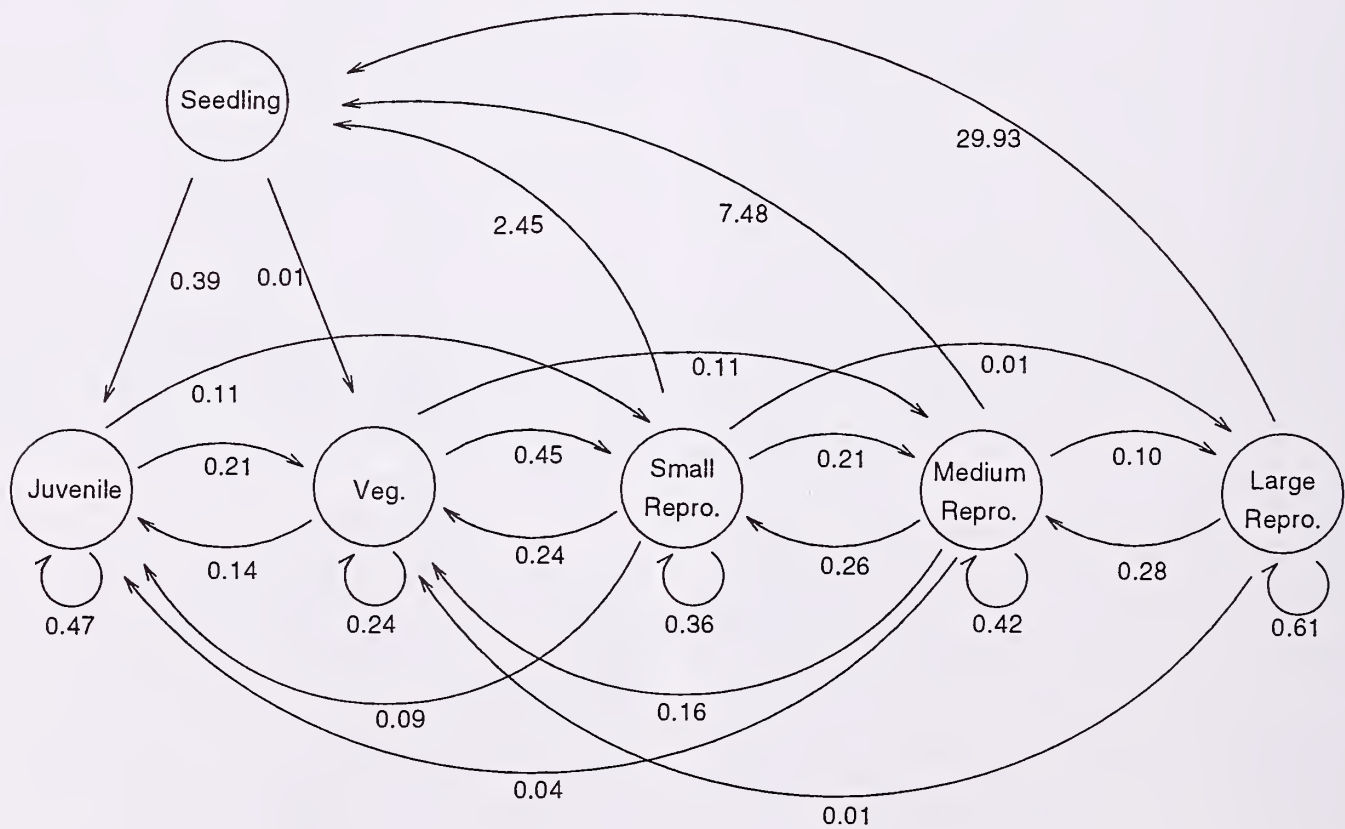


Figure 8. Transitions between life-history stages for *Pedicularis furbishiae* (Menges 1990). Numbers represent the rates of each transition.

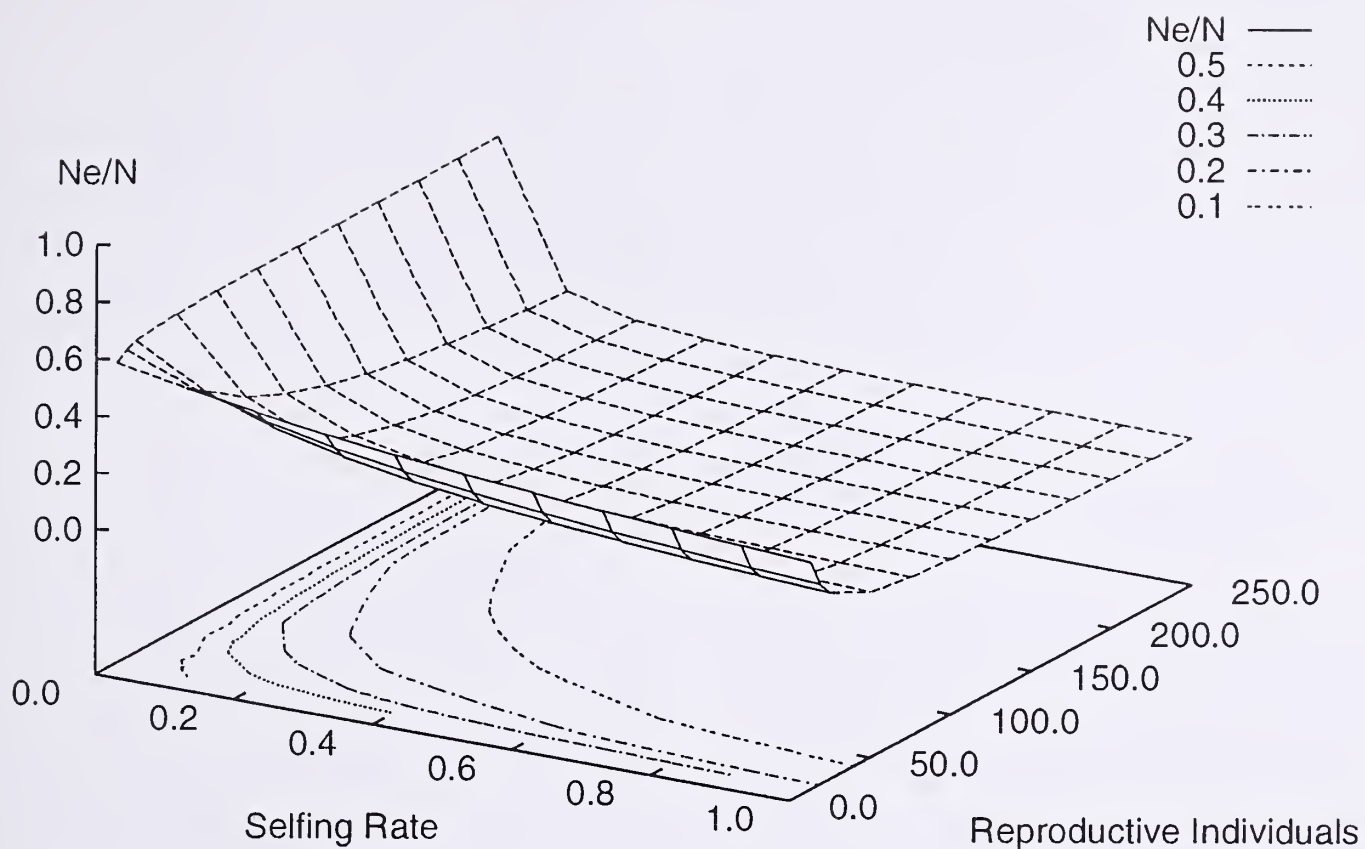


Figure 9. Dependence on adult population size and selfing rate of inbreeding effective population size  $N_e$  relative to total population size  $N$ . Note that adult population size refers to the number of adults from the stable age distribution in the three reproductive life-history stages, whereas total population size includes individuals of all life-history stages.

population persistence. Arguably, the latter is of pressing importance in conservation biology.

In this review we have attempted to illustrate how information obtained from genetic markers can go beyond marker diversity and provide estimates of many demographic parameters of interest to the conservation biologist. The utility of markers in this role is a result of the fact that demographic properties of populations influence their genetic properties; as a result, one may infer the demographic properties from marker data. The success of this endeavor depends largely on the manner in which genetic properties are assayed. Traditional markers, e.g., allozymes, only provide information on the frequency of individual genotypic classes, not on the genealogical relationship among classes. As a result, in certain applications, such as discriminating whether a set of populations are demographically isolated or subject to some form of extinction and recolonization, they are of limited utility. In other applications, such as estimating the short-term mating system or the

effective population size given current conditions, such markers are quite useful.

In many cases, however, one is interested in the long-term demographic properties of a population or set of populations, perhaps as a predictor of long-term prospects. In this instance, short-term observation of marker frequencies cannot provide the necessary information. Genealogies of markers, however, contain additional information that is useful for inferring long-term demographic properties. Because haplotype-based markers such as DNA sequences contain a record of their own history, their genealogies can be reconstructed and used in the inference of demographic properties. For this reason, they appear to be the most promising class of markers in this application of population genetics to conservation biology.

More generally, it is our conviction that comparisons between long-term demographic properties, estimated from genetic data as discussed here, and short-term demographic properties, estimated from direct observation, will provide the informa-

tion necessary to infer trends in population status. In many cases those trends are of prime importance in assessing the degree to which conservation efforts should be mobilized, yet they are also extremely difficult if not impossible to ascertain in the absence of long-term information such as recorded in DNA sequences. Conservation biology has much to gain, therefore, by integrating population genetics and demography with the goal of quantifying not only properties of populations but also historical trends in those properties.

### Acknowledgments

This research was supported in part by National Science Foundation grant 8906129 and by the Big Bend Natural History Association.

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# Genetics and Conservation Biology: Assessing Historical Gene Flow in *Aquilegia* Populations of the Southwest

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**Abstract:** One important demographic process influencing the management of rare plant species is the degree to which geographically distinct populations are interconnected by gene flow. If populations are highly integrated by seed dispersal, a newly extinct one may become recolonized naturally; if populations are essentially isolated from each other, active intervention may be required to reestablish populations. The most tractable means of obtaining information on the rate of migration is based on genetic observations. The genetic approach is also the only means to estimate long-term rates of migration. Unfortunately, the same genetic pattern can arise by the presence of ongoing gene flow or in its complete absence. Using information on chloroplast DNA sequence variation in *Aquilegia*, we have been able to distinguish between these two possibilities and demonstrate that virtually no seed-mediated gene flow interconnects distinct populations.

## Introduction

The degree to which natural populations interact is an important issue in conservation biology. One way in which interaction is important is in allowing the recolonization of locally extinct populations within the range of a species (Menges 1990, Whitlock 1992). Among-population interactions also influence the probability of parasite and pathogen spread (Boeger et al. 1993, Goodwin et al. 1994), the probability of founding new populations, and the degree and partitioning of genetic variation (Hamrick 1987).

Unfortunately, direct measurement of migration among natural populations is often difficult. Propagules must be marked either physically or with unique genetic markers and followed among populations (Ellstrand and Marshall 1985); alternatively, immigrants must be counted as they enter each population under study. While marking and recapturing some species is possible, for plants this task is daunting if not impossible. Not only is it logistically difficult to directly measure migration, such measurements provide data for only a few years at best. Therefore, they may miss relatively rare but important events.

Indirect estimation of migration offers an attractive alternative to its direct measurement. Indirect measures infer the amount of gene flow among populations from the distribution of single gene marker diversity. Although other approaches are available (Slatkin 1985), the distribution of genetic variation within and among populations is commonly quantified by estimating the parameter  $F_{ST}$ , the ratio of among to within population

genetic variance (Wright 1951, 1978), and gene flow is estimated from the relationship between  $F_{ST}$  and the number of migrants among populations per generation (usually denoted as  $Nm$ ) (Wright 1951). Many empirical studies have used this approach to estimate migration in natural populations (Soltis and Soltis 1987, Hamrick et al. 1989, Strand and Wyatt 1991).

Because Wright's (1951) relationship between  $F_{ST}$  and  $Nm$  assumes a population system at equilibrium, naive use of this relationship can result in misleading estimates of migration. For example, if a set of populations is the result of a recent set of founding or vicariance events, the populations will appear to be exchanging migrants even in the absence of any gene flow merely because they share a common evolutionary history prior to the establishment as discrete populations. Therefore, before indirect estimates of migration are interpreted as measures of ongoing gene flow, it is clearly important to verify that the population system has come to equilibrium. Misinterpretation of the migration measured may be particularly costly in conservation biology if recolonization is expected because of high apparent gene flow when in fact that observation only reflects recent subdivision of a more recent widespread population.

In this study we combine two approaches to choose between alternative hypotheses regarding the origin of current patterns of genetic diversity in members of the genus *Aquilegia*: ongoing gene flow versus recent historical subdivision and no ongoing gene flow. Distinguishing between these hypotheses, that is, separating apparent from ac-

tual gene flow, is essential to obtaining meaningful estimates of actual migration rate, a demographic parameter important in conservation biology.

## Materials and Methods

### *Study Organism*

The genus *Aquilegia*, a north temperate taxon of about 70 species (Munz 1946, Whittmore, personal communication), is characterized by dramatic diversity in both floral morphology and habitat preference. At one extreme of this range of diversity are the long-spurred columbines of the desert Southwest. Due to the complex nature of the morphological variation present among *Aquilegia* populations endemic to this region, the members of the genus have received varied taxonomic treatments, resulting in from two to four distinct species (Munz 1946, Lott 1985). Currently, this complex is divided into two distinct species, *Aquilegia longissima* A. Gray ex S. Watson and *A. chrysantha* A. Gray which is divided into three varieties, *A. chrysantha* var. *chaplinei* (Payson) Lott, *A. chrysantha* var. *hinckleyana* (Munz) Lott, and *A. chrysantha* var. *rydbergii* Munz (Lott 1985). Here, we examine populations of *A. longissima*, *A. chrysantha* var. *chaplinei*, and *A. chrysantha* var. *rydbergii*. Throughout this study, we consider populations of these three taxa to have equal potential for interbreeding, a well-known characteristic of this genus (Taylor 1967); therefore, we treat all populations equally without regard to taxonomic status.

All *Aquilegia* taxa in the southwestern United States and northern Mexico are found in rather small, isolated populations in mesic, high-altitude canyons separated by intervening desert. This island population structure provides an excellent opportunity to examine within and among population variation in neutral markers because such a discrete arrangement closely matches many of the assumptions made by classical treatments of population structure (Wright 1951, 1978). In addition, this population structure is present in many rare species (Rabinowitz 1981) and therefore provides a good model system for examining the fate of such groups.

### *Choice of Marker System*

Most studies of population structure have relied on isozymes encoded by nuclear genes (Hamrick 1987). With the advent of DNA-based methodologies, however, other marker systems are being employed. These include randomly

amplified polymorphic DNA (RAPD) markers (Chalmers et al. 1992, Chu et al. 1995), microsatellite DNA polymorphisms (Morgante and Olivieri 1993), and mitochondrial DNA-based markers (Norman et al. 1994, Bowen et al. 1992, Edwards 1993, Zink and Dittmann 1993).

Historically, the chloroplast genome in plants has not often been exploited to examine population-level processes. This phenomenon relates to the perception that the chloroplast genome evolves slowly (Soltis et al. 1992). However, evolutionary rate is not constant across the chloroplast genome. For example, non-coding regions are known to evolve faster than those coding for proteins (Wolfe and Sharp 1988). This observation suggests that population level processes could be estimated using markers based on these regions. Indeed, those studies that have looked for variation in the chloroplast genome within and among populations have found it (Banks and Birky 1985, Milligan 1991, Soltis et al. 1989, Soltis et al. 1992).

The chloroplast genome appears to be maternally inherited in *Aquilegia* (Corriveau and Coleman 1988). Therefore, estimates of migration based on chloroplast markers reflect movement by seed. This component of migration is especially relevant to conservation issues because only seed movement allows the regeneration and colonization of populations.

### *DNA Isolation, Amplification, and Electrophoretic Detection of Variation*

Detailed protocols for DNA isolations, polymerase chain reaction conditions, and electrophoretic systems are provided in Strand et al. (1996). In short, genomic DNA was isolated from 203 individuals collected from 17 populations throughout the range of the three *Aquilegia* taxa examined (Figure 1 and Table 1). A 500 nucleotide non-coding region of the chloroplast genome located between the transfer RNA genes, *trnL* and *trnF*, was amplified from the genomic DNA isolates by means of the polymerase chain reaction. Amplified fragments were compared to one another on two electrophoretic gel systems. First, all individuals were run on 0.9% agarose gels to confirm that no large insertions or deletions were present. Next, denaturing gradient gel electrophoresis (DGGE) was used to detect small insertions and deletions and single base point mutations (Myers et al. 1988, Myers et al. 1989). Genotypes were assigned both by running standard variants on each gel and by rearranging

placement of individuals on subsequent runs to reconfirm scoring. In addition, heteroduplex analysis (White et al. 1992, Lessa and Applebaum 1993) was performed by mixing representative templates from each population with the aforementioned standard variants within a polymerase chain reaction performed under the same conditions used initially (Delwart et al. 1993).

#### Data Analysis

Both approaches to distinguishing the historical subdivision hypothesis from the ongoing gene flow hypothesis utilize variations on an estimate of the number of genetic migrants per generation ( $Nm$ ) derived from estimates of population genetic structure. For our estimate of population structure, Weir and Cockerham's (1984)  $\theta$ , a measure equivalent to Wright's (1951, 1978)  $F_{ST}$ , was calculated from the gene frequency data for all populations. This statistic measures the amount of total genetic variation due to among-population variation. The program HAPLOID.FOR (Weir 1990) was used to calculate  $\theta$ . The estimates of the number of migrants per generation are based on Wright's (1951) relationship between  $F_{ST}$  (here  $\theta$ ) and  $Nm$ :

$$Nm = \frac{1}{2} \left( \frac{1}{\theta} - 1 \right)$$

Note the replacement of Wright's (1951)  $1/4$  with  $1/2$  in this formula. This substitution results from the fact that the chloroplast is uniparentally inherited, and therefore behaves as a haploid marker.

*Isolation by Distance.* Slatkin (1993) has developed a method to test for equilibrium conditions in a population system. This method looks for a pattern of isolation by distance among populations. If the populations in a system have come to equilibrium and are undergoing gene flow, nearby populations will resemble each other genetically more than populations separated by larger distances. Under equilibrium conditions, an exponential decline is expected in the estimates of gene flow between population pairs as geographic distance between them increases exponentially (Slatkin 1993). This characteristic allows a simple, direct test of the relationship between the logarithm of pairwise estimates of gene flow and the logarithm of pairwise geographic distance. In the context of a stepping-stone model of gene flow, a large negative correlation indicates that the population system is in equilibrium and that ongoing gene flow is resulting in a pattern of isolation by distance. The lack of correlation suggests that indications of ongoing gene flow result from

Table 1. Taxonomic designation, population name, abbreviation, and location of the 17 *Aquilegia* populations in the southwestern United States and Mexico examined in this study.

Taxon	Population	Abbreviation	Latitude	Longitude
<i>A. chrysantha</i> var. <i>chaplinaei</i>	Caballero Canyon	CAB	32°52'33"	105°50'2"
	Cloudcroft	CLD	32°57'26"	105°44'31"
	McKittrick Canyon	MKC	31°58'13"	104°41'15"
	Sitting Bull Falls	SIT	32°14'50"	104°41'46"
<i>A. chrysantha</i> var. <i>rydbergii</i>	Bear Creek	BRC	32°57'20"	108°36'6"
	Little Creek	LTC	33°12'14"	108°12'51"
	Palo Amarillo Springs	PAS	29°30'36"	104°8'55"
<i>A. longissima</i>	Cañon Charrateria	CHA	27°7'30"	102°33'30"
	Cañon de la Hacienda	HAC	27°3'30"	102°25'30"
	Cattail Falls	CAT	29°16'24"	103°20'3"
	Cerro del Centinella	CEN	29°6'40"	102°36'5"
	Maple Canyon	MAP	29°17'52"	103°17'5"
	Mt. Livermore	MTL	30°38'7"	104°10'22"
	Nuevo Leon	NUL	25°23'0"	100°18'0"
	Pine Canyon	PIC	29°15'17"	103°12'58"
	Pine Peak	PIP	30°39'18"	104°6'48"
Sierra Mojada	SIM	27°15'40"	103°40'50"	

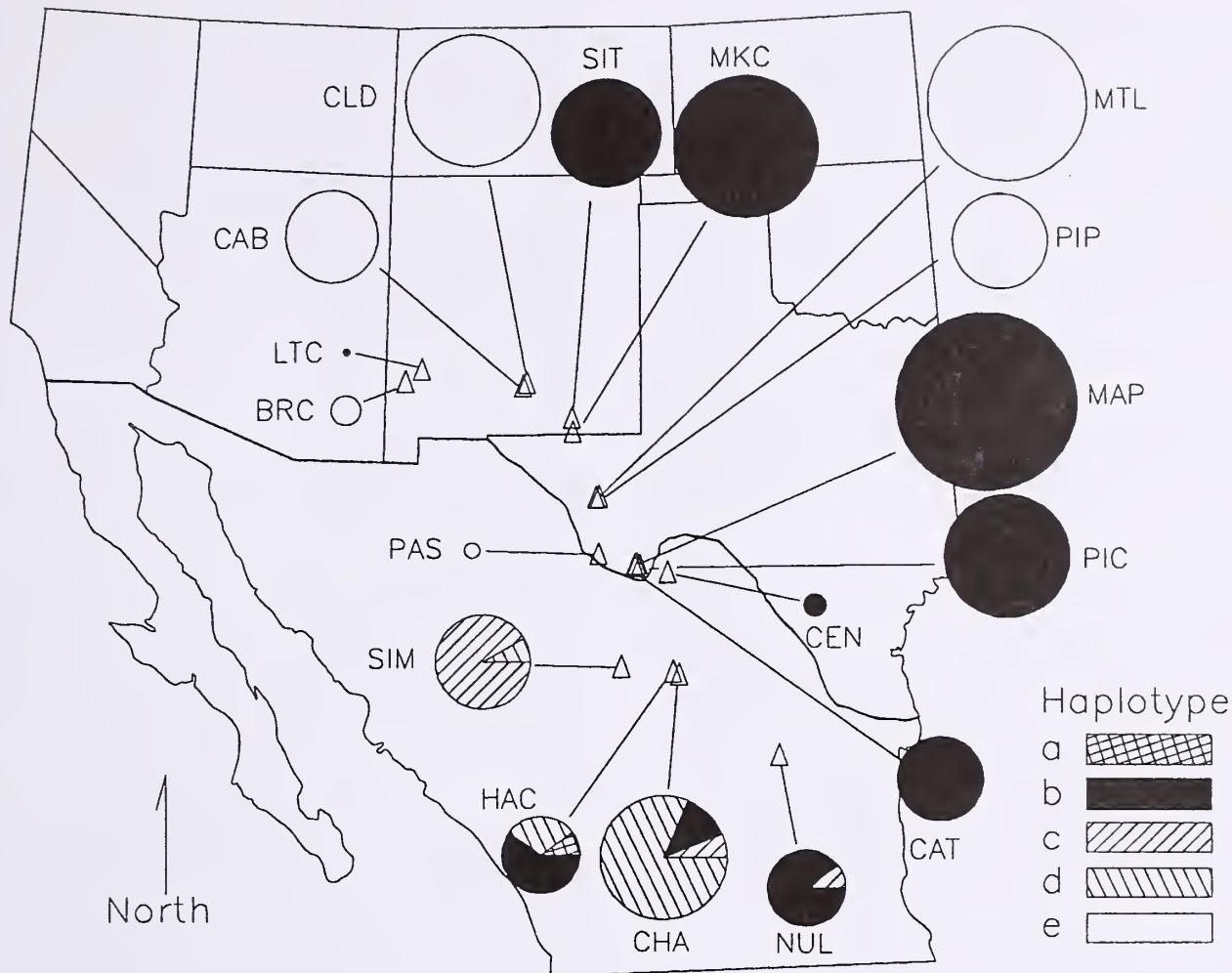


Figure 1. Location of 17 *Aquilegia* populations in the southwestern United States and Mexico. Frequencies of haplotypes in each population are indicated by relative area on each population's respective pie diagram. The diameter of each pie is proportional to the number of individuals sampled in the respective population.

historical associations among populations rather than current interpopulation processes.

Pairwise estimates of gene flow among *Aquilegia* populations, denoted as  $\hat{M}$ , were calculated from pairwise estimates of  $\theta$  using the relationship given above. Estimates of  $\theta$  equal to zero or one were then removed from the dataset to prevent estimation of undefined values of  $\hat{M}$ . This procedure reduced the number of pairwise comparisons used in subsequent analyses from 136 to 64. Pairwise geographical distances were calculated from the latitude and longitude data presented in Table 1. These data were plotted against pairwise  $\hat{M}$  estimates using log-log scales. Finally, linear regression was used to fit a line to these data. Because of the non-independence of the pairwise comparisons, a randomization test was used to determine if a slope significantly less than zero exists between geographic distance and  $\hat{M}$ . To perform this test, we randomly assigned pairwise

geographic distances from the initial dataset to the  $\hat{M}$  estimates and recalculated the regression line 4100 times. This effectively amounts to Mantel's (1967) randomization test. The slope and  $R^2$  were recorded for each randomization. The slope and  $R^2$  obtained from the observed data were then compared to the distributions of both variables generated by randomization.

*Decay of Apparent Gene Flow.* In the absence of actual migration subsequent to population isolation, estimates of gene flow (apparent gene flow) within a population system will decline over time in a manner given by the relationship:

$$\frac{1}{Nm} = \frac{t}{N_e}$$

where  $t$  refers to the time in generations since isolation of populations and  $N_e$  refers to the average effective population size among populations (M. Slatkin, personal communication).

We used estimates of  $\theta$  and Wright's (1951) relationship to provide an overall estimate of  $Nm$ . As an estimate of  $N_e$ , we averaged the number of reproductive individuals in six *Aquilegia* populations and used the relationship  $N_e = N_{\text{reproductive}} = 1/2$  given by Nunney (1993). This estimate of  $N_e$  was then multiplied by  $1/2$  to give the effective size for the chloroplast genome. The estimate of generations since isolation are derived from a range of generation lengths of *Aquilegia* from 10 to 30 years and an estimate of minimum time since isolation of 8000 years (Van Devender 1977, 1987, Van Devender and Spaulding 1979). Using these values gives a range of 266–800 for the number of generations since isolation.

These estimates were used to compare the *Aquilegia* population system to the decay of apparent gene flow curve. If the *Aquilegia* system falls on or below the curve, historical subdivision is sufficient to explain current patterns of genetic diversity. If the estimate of apparent gene flow falls above the curve, ongoing gene flow must be, at least in part, responsible for the current patterns.

## Results

### *Distribution of Variation*

Although length variation was observed among PCR products on agarose gels, denaturing gradient gels resolved five distinct haplotypes. Heteroduplex analysis demonstrated that in each

population, each mobility class did indeed share the same sequence with those same mobility classes in other populations. The frequencies of the haplotypes in each population are presented in Figure 1. Of the 13 populations in which more than five individuals were sampled, four exhibited polymorphism for the chloroplast DNA fragment. Genetic diversities in these four populations range from 0.15 to 0.54. Two haplotypes, b and e, are approximately equally common throughout the sampled range with overall frequencies of 0.51 and 0.34 respectively. Of the three remaining haplotypes, only two, c and d, are present in appreciable frequencies (0.06 and 0.08, respectively) with the final haplotype, a, occurring in a single individual. The distribution of haplotypes among populations can be summarized as follows. Haplotype b is present in 59 percent and haplotype e in 35 percent of the sampled locations, whereas both c and d are restricted to 18 percent of the localities.

### *Isolation by Distance*

Figure 2 illustrates the relationship between pairwise estimates of gene flow and geographical distance (note the log-log scale). The least-squares line through the data is able to explain only 3 percent of the total variation present. The randomization tests revealed that 9 percent of the resampled slopes are more negative than the slope obtained from the observed data. In addition, 18 percent of

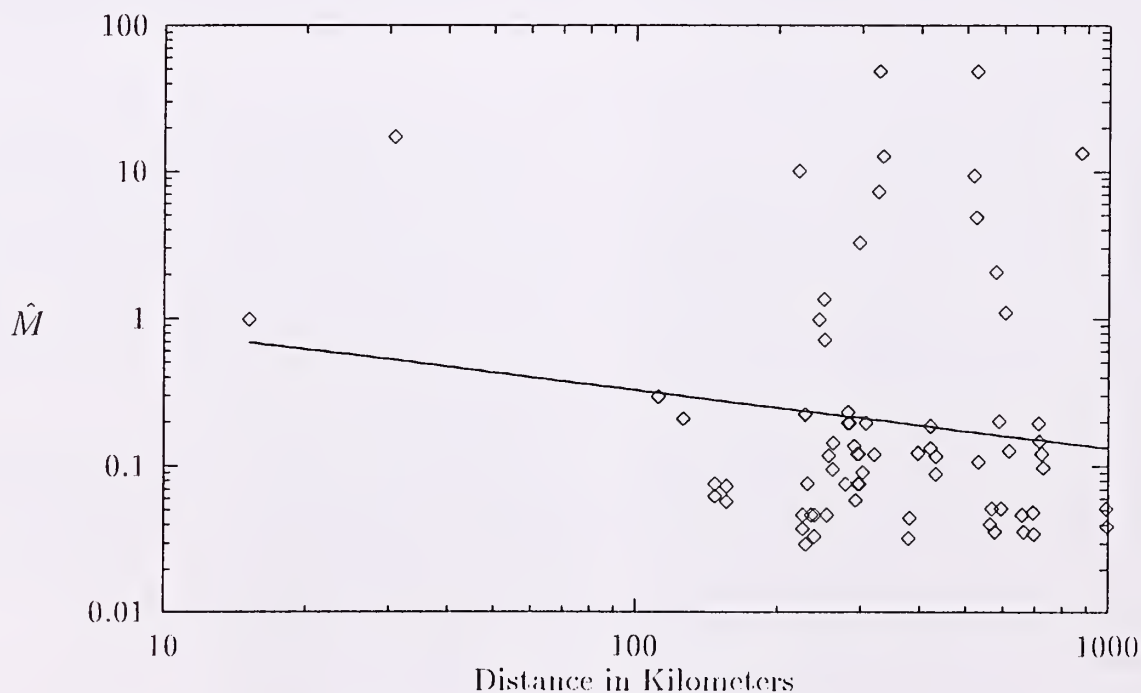


Figure 2. Estimates of  $\hat{M}$  and distances between *Aquilegia* populations in the southwestern United States and adjacent Mexico. Calculation of  $\hat{M}$  is described in the text. The line in this figure represents a linear regression of the logarithm of  $\hat{M}$  on the logarithm of distance and illustrates the relationship  $\ln(\hat{M}) = 1.35 - 0.452 \ln(\text{distance})$ . This regression has an  $r^2 = 0.03$ .

the resampled  $R^2$ s are larger than that obtained from the observed data. Therefore, there is no evidence for isolation by distance.

#### *Decay of Apparent Gene Flow*

The overall estimate of  $\theta$  had a value of  $0.86 \pm 0.06$  (estimate  $\pm$  standard deviation obtained by jack-knifing across populations) over all populations in the study. This high value is congruent with the fact that most populations were fixed for alternative haplotypes. This overall estimate yielded an estimate of  $Nm$  equal to 0.08 (95% CI: 0.01–0.17). The number of reproductive individuals among populations is  $391 \pm 352$  (mean  $\pm$  SD). Therefore average effective population size for chloroplast DNA should be approximately 100. Using this effective size and the estimate of generations since isolation above gives a range of the ratio of  $t/N_e$  from 2.66 to 8.00. These estimates are plotted along with the decay of apparent gene

flow curve in Figure 3. This figure shows that the 95 percent confidence area intersects the curve, which would explain the observed patterns of diversity through historical similarity alone.

#### Discussion

Based on the identical mobility of PCR fragments from all individuals on agarose gels, large changes in the length of this region of the chloroplast genome cannot be the source of the variation observed on the denaturing gels. Therefore, these differences in mobility must be the result of sequence variation. Indeed, comparisons of the sequences of the five haplotypes in this study reveal that differences among haplotypes are due to single base insertion/deletion events (presumably caused by "slippage" during DNA replication), a single base transition, and a single four base insertion. This confirmation by DNA sequencing ensures that the variation resolved by denatur-

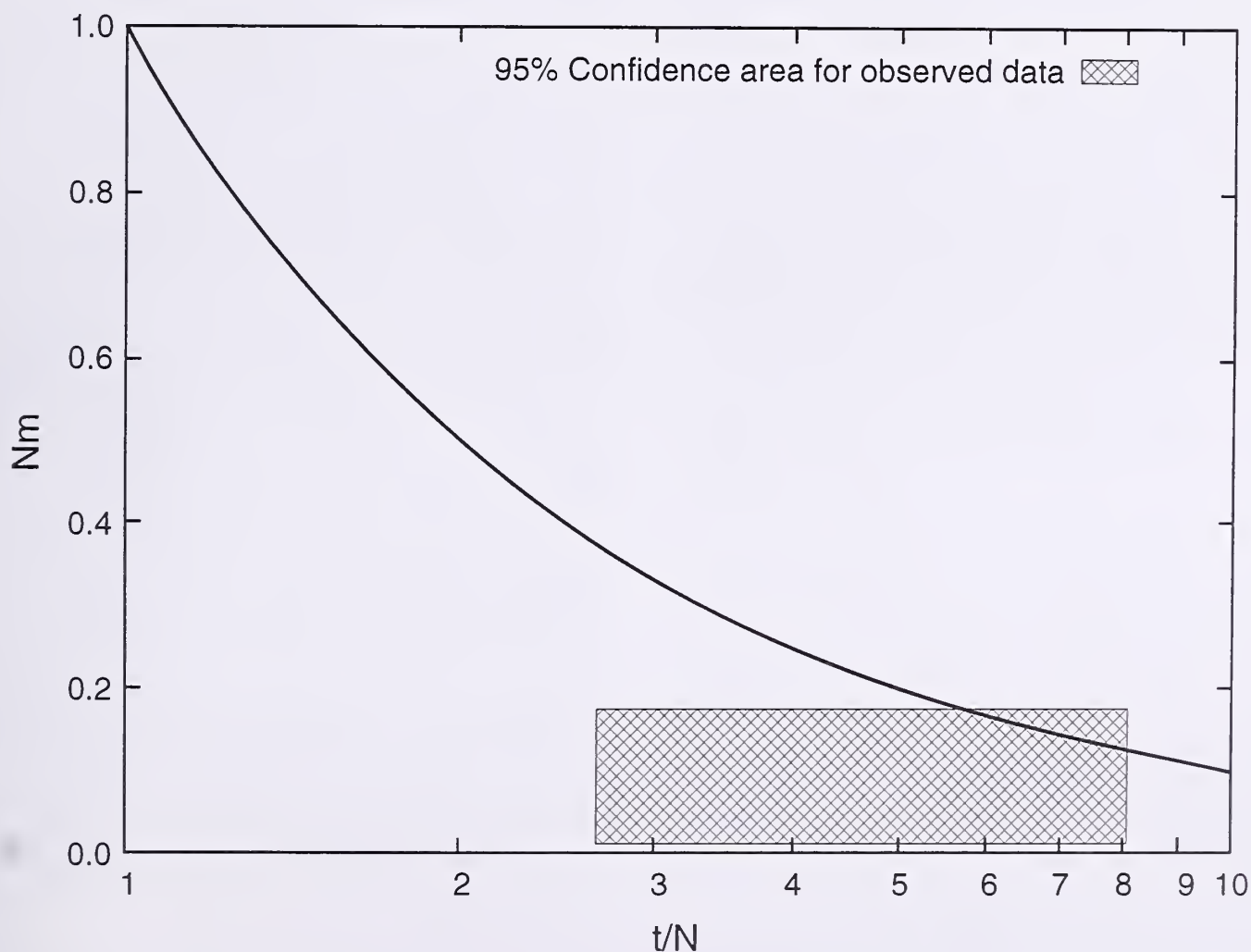


Figure 3. Plot of the decay of apparent gene flow among populations in the absence of actual gene flow since isolation. The shaded box is the 95 percent confidence area for the observed estimates of gene flow  $Nm$  and the ratio of generations since isolation of populations and the average effective population size in the system.

ing gradient gel electrophoresis, a relatively new technique, does reflect variation at the DNA level.

The lack of detectable isolation by distance provides strong evidence that this population system is not in equilibrium with respect to gene flow. This observation could result from either a lack of gene flow or from very large amounts of gene flow. However, the observation that the overall estimate of gene flow is not larger than the levels predicted for a population system undergoing no actual gene flow indicates that there is no ongoing migration via seed among populations. Therefore we strongly favor the hypothesis of historical subdivision accounting for the observed patterns of genetic diversity in the southwestern yellow-flowered *Aquilegia* complex.

The lack of long-distance migration via seed in *Aquilegia* should not be surprising considering the size (1.5–2 mm) of seeds as well as their lack of any specialized adaptations for increased long-distance dispersal, such as fruits that are fleshy or that function in "hitchhiking" on animals. The distances achieved by "gravity" dispersal of *Aquilegia* seeds may reduce structuring within populations, but it appears unlikely to enable dispersal across the several kilometers of desert conditions that separate even the closest populations in this study.

The results presented here serve three purposes. First, we have demonstrated how genetic marker data, when combined with relatively simple to obtain independent information, can be used to infer a demographic parameter important in conservation biology. Second, we have shown that the selection of an appropriate marker system, in this case one based on the chloroplast genome, can greatly influence the value of the markers in terms of conservation biology. Finally, these results have implications for the conservation biology of the southwestern *Aquilegias*. For example, recolonization of newly extinct populations will not occur naturally; some intervention will be required. Also, the lack of connectedness among populations must be responsible in part for the high degree of localized differentiation observed by taxonomists in the southwestern members of the genus.

### Acknowledgments

This research was supported in part by the National Science Foundation and by the Big Bend Natural History Association.

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# Germination Requirements and Genetic Diversity in *Croton alabamensis* var. *texensis*

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**Abstract:** *Croton alabamensis* E.A. Smith ex Chapman, currently listed as a category 2 species, is being considered for addition to the list of endangered and threatened plants under the Endangered Species Act. While previously thought to be restricted to two counties in Alabama, in 1990 a disjunct population was found at the U.S. Army's Fort Hood in south-central Texas. Two additional populations have since been located in Travis County, Texas. The presence of the species at Fort Hood, the probability of eventual listing under the ESA, and the potential for disturbance of populations from military activity prompted us to investigate the requirements for optimal germination and seedling establishment. The information obtained is being used in the development of a management plan that will balance the military mission and compliance with ESA regulations. We also evaluated genetic diversity among the Texas populations using both isozymes and RAPD markers. Little genetic variation was detected among the Texas populations.

## Introduction

### *Species Distribution*

*Croton alabamensis* E.A. Smith ex Chapman (Euphorbiaceae) was described by Farmer and Thomas (1969) as "one of the rarest shrubs in the United States." The species is of particular interest as it combines narrow endemism with a disjunct distribution. Until recently, the species was thought to be restricted to isolated populations in Tuscaloosa and Bibb counties, Alabama (Aplet et al. 1994). A population was discovered in 1990 growing at the U.S. Army's Fort Hood, Texas, by John Cornelius, a wildlife biologist with the Fort Hood Resource Management Department. Two additional populations were later discovered by Chuck Sexton in the Post Oak Ridge area of Travis County, south of Fort Hood. Ginzburg (1992) considered the Texas populations to be morphologically distinct enough to merit varietal status and described them as *C. alabamensis* E.A. Smith ex Chapman var. *texensis* Ginzburg.

*Croton alabamensis* var. *alabamensis* has only been reported from 10 sites occupying less than 100 acres (40 ha) over approximately 30 square miles (77.7 km<sup>2</sup>) along the Cahaba River valley in Bibb County (seven populations) and the Warrior River valley (three populations) in Tuscaloosa County, Alabama (Farmer and Thomas 1969). The species was collected once in 1899 in Tullahoma, Coffee County, Tennessee; however, there have been no subsequent reports of populations from that area.

*Croton alabamensis* var. *texensis* has been reported only from Travis, Coryell, and Bell counties, Texas, with most populations found along the eastern edge of the Edwards Plateau in the Owl Creek Mountains (Ginzburg 1992). The known distribution is limited to Training Areas 2 and 3A in Coryell and Bell counties at Fort Hood and to a few square kilometers in northwestern Travis County (Steed 1993). Plants typically occur on canyon bottoms and slopes in mesic ravines characterized by moderately alkaline, stony clay or clay-loam overlying Cretaceous limestone (McCaleb 1985, Werchan et al. 1974). In contrast to the Alabama populations, which were reported to grow on shallow-soiled slopes (McDaniel 1981), Aplet et al. (1994) found a significant association between the presence of adult *Croton* plants and areas with deep soil. Aplet et al. (1994) reported no correlation between canopy gaps and presence or absence of *Croton* with populations observed in full sun, partial shade, and densely shaded areas; however, Steed (1993) reported a correlation between canopy cover and reproductive habit. In shady areas along canyon bottoms, *Croton* forms dense groves of apparently long-lived adult individuals that produce relatively low numbers of flowers and fruits and very few seedlings. Populations occurring in canopy gaps, which receive full sun most of the day and partial shade the remainder of the day, form less dense groves of shorter-lived individuals and produce considerably more flowers and fruits than those found in

shady areas. Isolated individuals found growing in full sun, with no shade whatsoever during any part of the day, tend to be stunted and less prolific reproductively.

The Texas and Alabama populations of *C. alabamensis* are separated by more than 1000 km (Figure 1). The reason(s) for the disjunction are unknown; however, several possible explanations have been proposed, including relictual disjunction during the Wisconsin Glaciation and relatively recent introduction by long-distance dispersal during bird migration events (Ginzburg 1992). Although the Alabama populations were discovered prior to the Texas populations, it is not known for certain which became established "first," and therefore in which direction such long-distance dispersal might have occurred.

*Croton alabamensis* forms widely dispersed but locally dense populations that appear to be in overall good health. The species is absent from many apparently suitable sites near the known existing populations. The density of local colonies suggests that the explosive dehiscence of capsules and vegetative root layering are the primary forms of reproduction (Ginzburg 1992). Both doves and quail have been observed eating *Croton* seeds (Johnston 1959) and it is likely that the fruits/seeds are eaten by other birds as well. Intact seeds passing through the birds' digestive tracts could be responsible for secondary dispersal and the establishment of new colonies (Ginzburg 1992).

At Fort Hood, populations are concentrated in two main canyons, Croton Canyon and Sycamore Canyon, with smaller populations found at several widely dispersed locations (Figure 2). Between the two main canyons lies a third canyon (Pear Canyon) wherein *Croton* is nearly absent, with the exception of a few individuals. The three canyons are quite similar in dominant vegetation, total overstory and understory cover, mean steepness of canyon walls, watershed sizes, and soil depth; the only differences are stream gradient and steepness of the creekbed, both of which are significantly greater in Pear Canyon (Aplet et al. 1994). In Travis County, populations occur primarily in deciduous forest in mesic limestone canyons dominated by similar vegetation to that found in those canyons at Fort Hood in which *Croton* is abundant (Ginzburg 1992).

#### *Species Description*

*Croton alabamensis* var. *texensis* is a relatively short-lived (20–30y), monoecious shrub, typically less than 3 m tall. Mature plants are multi-

stemmed, with stems arising from a woody base at or just below the soil level. Leaves are bright green with silvery scales above; the undersides of the leaves are covered with silvery scales dotted with conspicuous copper scales giving the plants a distinct coppery appearance during breezy conditions. Flowers are inconspicuous and bloom in early spring from buds formed in late spring of the previous year (Figure 3).

Very little is known about the pollination or reproductive biology of either variety of *C. alabamensis*. The inflorescence of var. *texensis* is a terminal 6–14 flowered raceme with 1–6 pistillate flowers near the base and 4–12 staminate flowers above. The degree of self-pollination vs. outcrossing has not yet been determined. Farmer (1962) suggested that var. *alabamensis* is wind pollinated. In var. *texensis* beetles have been found feeding on pollen, and pollen grains have been observed adhering to the beetles' bodies, suggesting they may play a role in pollination (Steed 1993). Fruits consist of capsules with 3–4 cells that are borne on pedicels and explosively dehisce in late May to early June. Seeds are 6.7–7.9 mm long, 5.2–6.0 mm wide, dark brown to almost black with white blotches and streaks, and have a yellow caruncle below a prominent white keel at the point of attachment (Figure 3).

Populations often form locally dense thickets, making it difficult to distinguish individual plants. Numerous instances of asexual reproduction have been observed among the Texas populations in which plants produce upright shoots through nodal rooting of prostrate branches (layering), which adds to the difficulty of distinguishing individuals. According to Farmer (1962), no asexual reproduction had been reported among plants of var. *alabamensis*; however, Ginzburg observed root layering in some Alabama populations (1992).

Very little information is available on germination of *Croton*. An alternating temperature regime with no pretreatment has been suggested for several related species in the Euphorbiaceae, as well as in castorbean (*Ricinus communis*), which has a similar seed morphology to *C. alabamensis* (Young and Young 1986, AOSA 1993). Removal of the caruncle to prevent fungal infection during germination has also been suggested for castorbean (AOSA 1993). Currently, no published information is available regarding the germination or seedling establishment requirements for either variety of *C. alabamensis*; nor is there any information available regarding the relative degree of inter- or intravarietal genetic diversity.

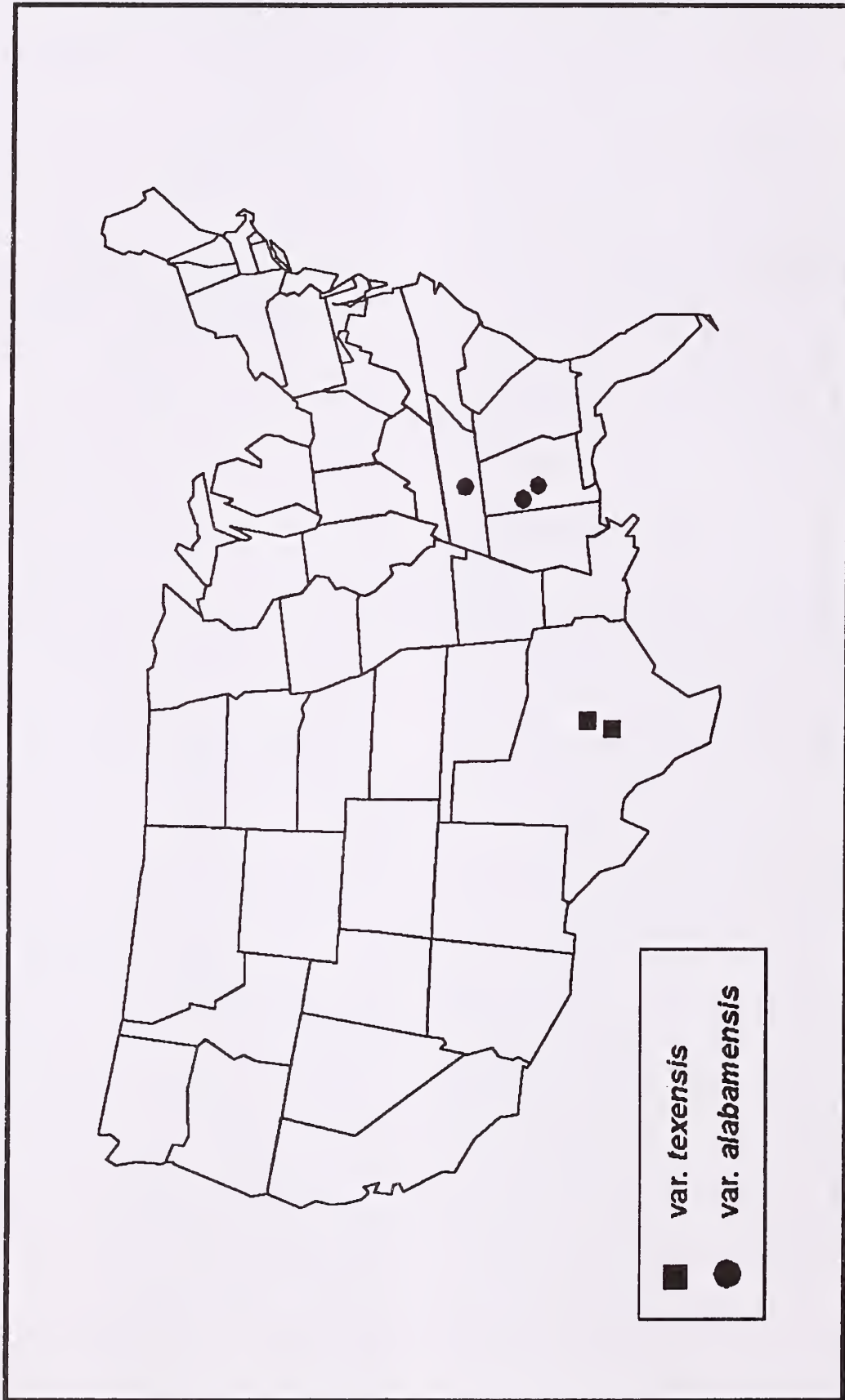


Figure 1. U.S. distribution of *Croton alabamensis*.

# Populations of *Croton alabamensis* var. *texensis* at Fort Hood, Texas

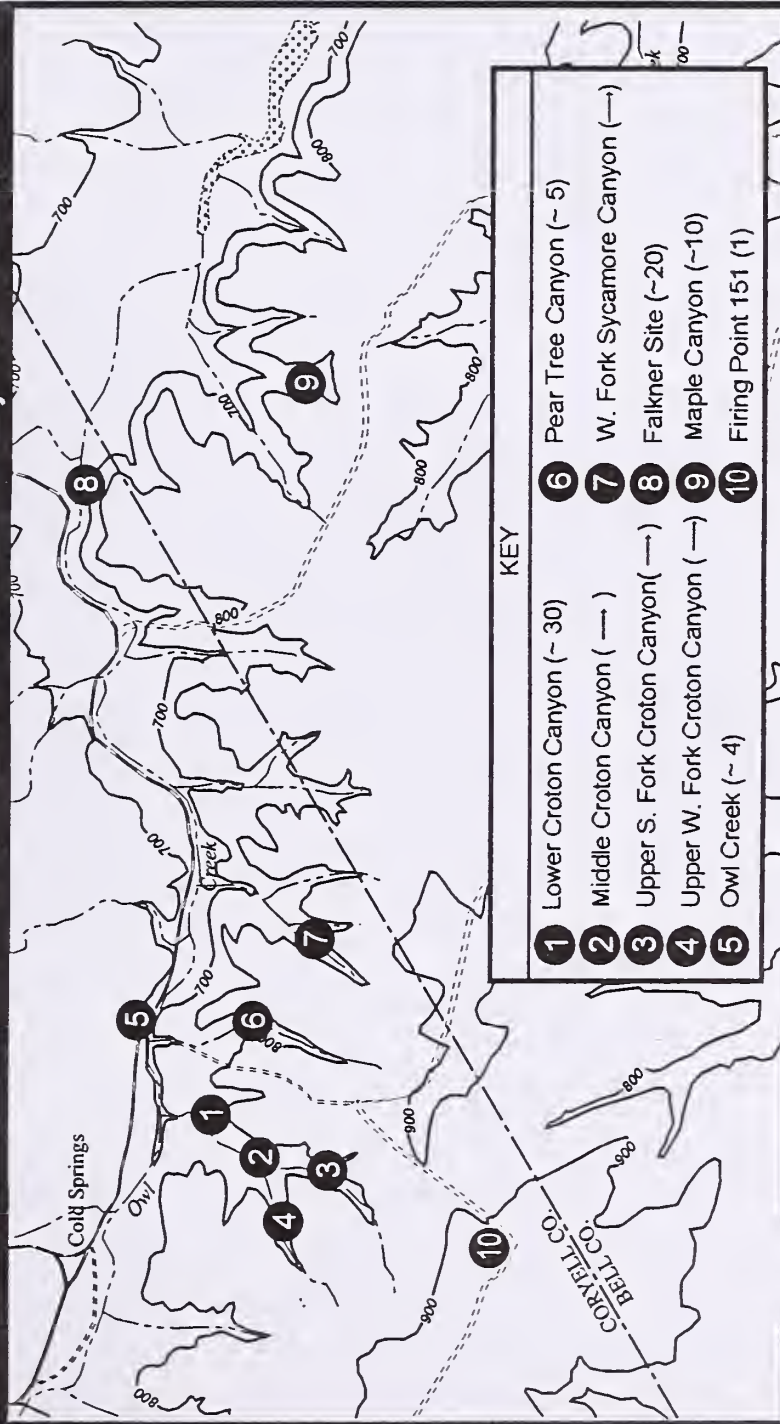


Figure 2. Distribution of populations of *Croton alabamensis* var. *texensis* at Fort Hood, Texas.

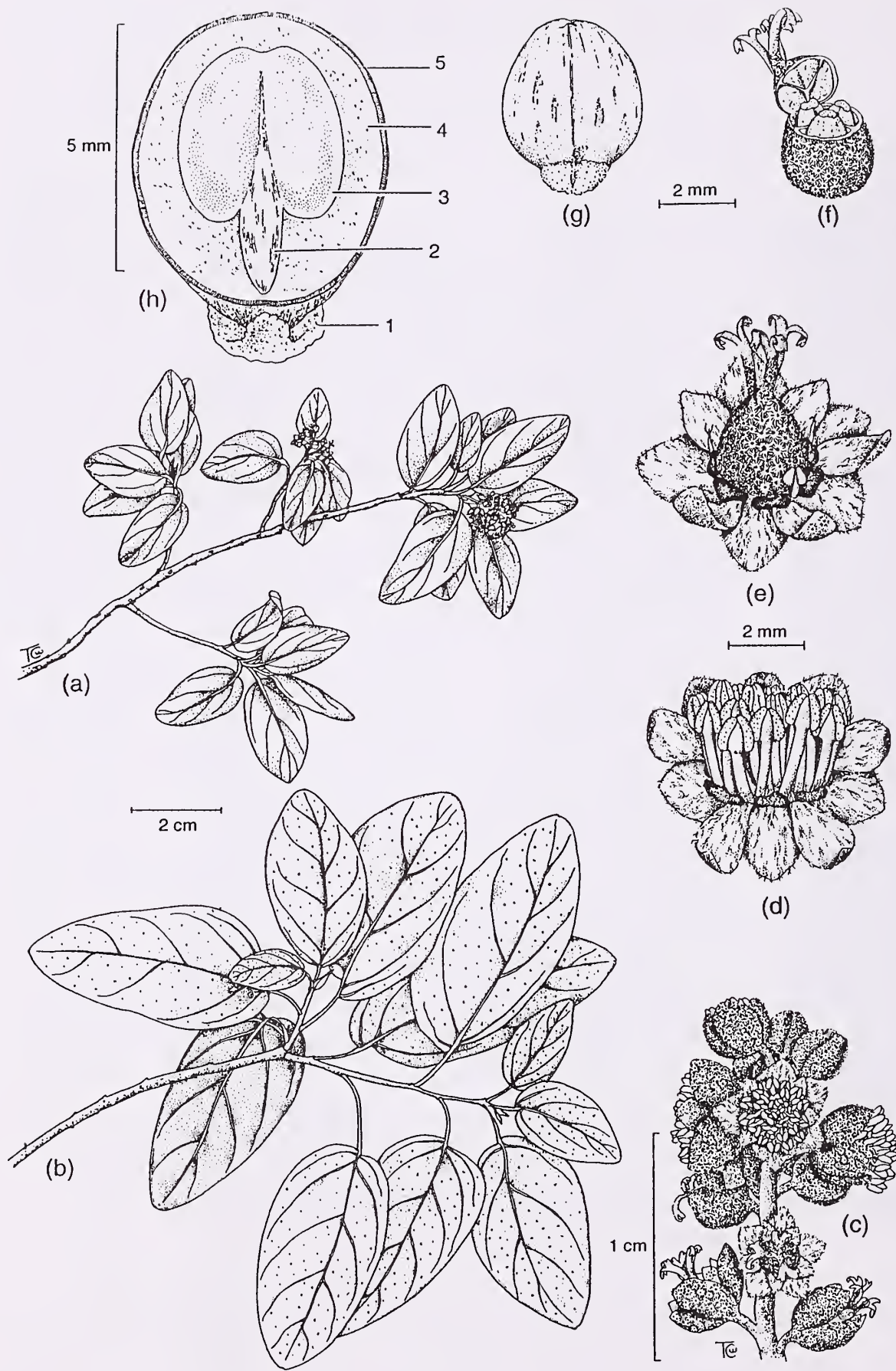


Figure 3. *Croton alabamensis* var. *texensis*: (a) stem with flowers clustered at apex of primary and secondary branches (flowers open before leaves are fully expanded); (b) stem showing fully developed leaves; (c) androgynous raceme inflorescence; (d) male flower; (e) female flower with rudimentary stamens; (f) three-celled capsule; (g) seed; (h) cross-section of seed: 1 = caruncle, 2 = embryonic radicle, 3 = embryonic cotyledons, 4 = endosperm, 5 = seed coat.

### *Importance of Croton alabamensis in the Ecosystem*

Many *Croton* species are rich in various alkaloids and terpenoids (Farnsworth et al. 1969), and several species have been utilized as sources of medicinal compounds (Webster 1993). At Fort Hood, many of the populations occur in woodland areas dominated by *Fraxinus texensis* (Gray) Sarg., *Quercus muhlenbergii* Engelm., *Quercus texana* Buckl., and *Juniperus ashei* Buchh., which meet essential habitat criteria for two rare birds found at Fort Hood, the black-capped vireo (*Vireo atricapilla*) and the golden-cheeked warbler (*Dendroica chrysoparia*) (Aplet et al. 1994). A number of other rare plant species share the canyons occupied by *Croton*, although most are also abundant in nearby canyons from which *Croton* is absent. Among these rare species is another candidate for federal listing, the orchid *Hexalectris nitida* (Steed 1993). The occurrence of several rare species in these canyons suggests the presence of unique microclimate/microhabitat parameters and a rich biological history. Due to its limited distribution, *C. alabamensis* is being considered for listing under the Endangered Species Act (ESA) of 1973, as amended. It is currently designated as a "category 2 candidate species" which is defined as having "evidence for vulnerability, but ... there are not enough data to support listing proposals at this time" (USDI 1991).

This study was designed to establish protocols for germination and seedling establishment for *C. alabamensis* var. *texensis*. Thus far, our research efforts have focused on the Fort Hood populations as part of the Department of Defense's Legacy Resource Management Program, which was established to enhance the conservation of biologically and culturally significant resources on military lands. The results of our research efforts are being used to develop conservation strategies for *C. alabamensis* in order to prevent listing of the species in accordance with ESA regulations at both the federal and state levels.

## Methodology

### *Populations Evaluated for Germination and Genetic Diversity*

To obtain an adequate sample of genetic diversity, seed capsules were collected from 8 of the 10 known populations at Fort Hood. No seeds were collected from individuals in population 1 due to its close proximity and similar habitat to population 2, and the individual reported at Firing

Point 151 (population 10) during a survey in 1993 by DLS Associates could not be located. The number of capsules collected varied considerably from population to population due to wide variation in fruiting among populations, ranging from 1 for population 9 to 159 for population 7. A random bulk sample of capsules was also collected from the most prolific populations (1, 2, 3, and 7) for use in preliminary investigations. Capsules were dried in the greenhouse for one week, at which time all mature capsules had dehisced and ejected their seeds. Capsules from individual plants were kept separate and individual drying trays were covered with mosquito netting to prevent dehiscing seeds from contaminating a different container.

### *Viability Analysis*

Seed viability was determined using standard Tetrazolium (TZ) staining methodology (ISTA 1985). Viability evaluations were conducted on seeds from the bulk sample. Embryos were extracted from seeds that had been soaked in ddH<sub>2</sub>O for 48 hours. Extracted embryos were placed in 0.1% TZ and incubated at 30°C for 4 hours, then left at room temperature overnight.

### *Germination Trials*

Germination trials were conducted on seeds from the bulk sample to preserve individual population collections for more detailed germination and genetic studies. For all germination trials, seeds were placed on top of autoclaved blotters moistened with distilled water, set within petri dishes treated with 95% EtOH, and placed in germination chambers with an alternating temperature of 30°C (8 hrs)/20°C (16 hrs) and 8 hours light/16 hours dark per 24-hour period, unless otherwise indicated. All observations were made over a 50-day period.

*Initial Evaluations:* Two samples of 20 intact seeds were plated out with no further treatment. The caruncles were removed from 10 seeds by cutting with a scalpel, and 10 seeds were placed in a 107°C oven for 30 min in an attempt to melt the wax-like caruncles. An additional 20 seeds were soaked for 48 hours. Seed coats were removed from 10 of these, leaving the seeds intact, and embryos were extracted from the remaining 10. The cotyledons were clipped on half of these, while the others were left intact. In addition, 30 seeds were soaked for 30 min in a 1.5% solution of sodium hypochlorite (dilute bleach) with constant stirring, then rinsed six times in ddH<sub>2</sub>O. Of these,

15 seeds were left intact and the caruncles were removed from the remaining 15. Seeds from these initial evaluations that developed mold during incubation were subsequently tested for viability.

*GA<sub>3</sub> Soak:* The effect of gibberellic acid (GA<sub>3</sub>) on germination was evaluated, as gibberellins are the family of growth regulators with the broadest spectrum of activity. Cytokinins were not evaluated, as they are less widely effective and often induce abnormal germination (Bewley and Black 1985). The caruncles were removed from 30 seeds. Of these, samples of six seeds received the following five treatments: (1) left intact, (2) clipped on the non-radicle ends, (3) seed coats removed, (4) cut in half longitudinally, and (5) embryos extracted. All seeds and embryos were soaked for 24 hours in 500 ppm GA<sub>3</sub> and subjected to the above germination conditions.

*GA<sub>3</sub>-moistened Blotters:* Seeds were subjected to five treatments: (1) left intact, (2) the caruncles removed, (3) the caruncles removed and non-radicle ends clipped, (4) cut in half longitudinally, and (5) embryos extracted. Samples of 10 seeds each were placed on blotter papers moistened with 500 ppm GA<sub>3</sub>, and subjected to the germination conditions described above. All subsequent re-wetting of the blotters during incubation was with GA<sub>3</sub>.

*Prechill:* Thirty intact seeds and 30 seeds from which the caruncles were removed were placed on moistened blotter papers in covered petri dishes and incubated at 4°C for varying lengths of time. Samples of 10 intact seeds and 10 seeds with the caruncles removed were plated out after 10 days, 2 weeks, and 3 weeks of prechilling, and were subjected to the germination conditions described above.

*KNO<sub>3</sub> and HCl Treatments:* For each treatment, samples of 10 seeds each from the following three treatments were soaked in either 0.2% KNO<sub>3</sub> or 0.2N HCl for 24 hours and subjected to the germination conditions described above: (1) left intact, (2) the caruncles removed, and (3) the caruncles removed and the non-radicle ends clipped.

*Dark Treatment:* Samples of 10 seeds each from the following five treatments were subjected to the germination conditions described above, except that petri dishes were placed in a sealed box inside three black plastic bags to prevent exposure to light: (1) left intact, (2) the caruncles removed, (3) the caruncles removed and the non-radicle ends clipped, (4) cut in half longitudinally, and (5) embryos extracted. Evaluations were conducted in a photographic darkroom using a green-wavelength

safelight. After 12 days in darkness, petri dishes were removed from the box and returned to the germinator.

*Boiling:* Ten intact seeds and 10 seeds from which the caruncles were removed were boiled for 5 minutes, cooled to room temperature, and subjected to the germination conditions described above.

*Greenhouse Evaluations:* Samples of 10 seeds each from the following three treatments were planted in pots containing 'Metro Mix' soilless potting medium and placed in the greenhouse: (1) left intact, (2) the caruncles removed, and (3) the caruncles removed and the non-radicle ends clipped. In addition, five intact seeds and five seeds from which the caruncles were removed were planted in pots containing sterilized sand and were placed in the greenhouse.

*After-ripened Seeds:* The caruncles were removed from four after-ripened seeds harvested in 1992, the only after-ripened seeds available, and were subjected to the germination conditions described above.

#### *Genetic Diversity Evaluations*

*Isozyme Analysis:* Leaf material from two Fort Hood populations (C1 and C2) was collected, placed on ice, and shipped overnight to the electrophoresis laboratory in the Department of Biology at the University of New Mexico. C1 consisted of 18 individuals collected from populations 2 and 3 in Croton Canyon; C2 consisted of 19 individuals collected from population 7 in Sycamore Canyon. Standard methodologies for starch gel electrophoresis were employed in the study. Fresh leaf tissue was ground in cold Tris-HCl extraction buffer according to the procedures of Soltis et al. (1983) and the supernatant was absorbed onto filter paper wicks that were inserted into 12% starch gels. Nine enzyme systems were resolved for each population (AAT, ADH, ALD, G6PDH, 6PGD, PGI, PGM, SDH, and TPI). Enzymatic assays followed the procedures of Soltis et al. (1983), except for ADH which was assayed according to the procedures of Wendel and Weeden (1989). Genetic diversity statistics were generated based on Rogers's (1972) genetic distance estimates.

*RAPD Analysis:* The same individuals evaluated for isozyme diversity were evaluated for genetic diversity using RAPD (Random Amplified Polymorphic DNA) analysis. DNA was extracted from leaf tissues according to T. Lowrey (unpublished, see Appendix A). DNA concentrations



were determined using a fluorometer and were stored at  $-20^{\circ}\text{C}$ . For RAPD analysis, a  $25\ \mu\text{L}$  reaction mixture was prepared according to the procedures of T. Lowrey (unpublished, see Appendix A). The thermal cycler program used was as follows: (1) 3 min @  $94^{\circ}\text{C}$ ; (2) 45 cycles consisting of 1 min @  $94^{\circ}\text{C}$  (denaturing), 1 min @  $38^{\circ}\text{C}$  (annealing), 30 sec @  $54^{\circ}\text{C}$  (annealing), and 2 min @  $72^{\circ}\text{C}$  (elongation); (3) 15 min @  $72^{\circ}\text{C}$ ; and (4) soak @  $4^{\circ}\text{C}$ . Four  $\mu\text{L}$  gel loading dye was added to each reaction and centrifuged for 2–3 seconds. Each reaction plus loading dye was loaded onto 1.4% agarose gels prepared with 1X TAE (Tris, NaOAc, EDTA) buffer. A molecular weight marker (100 bp ladder) was loaded into at least one well of the gel for band size comparisons. Gels were electrophoresed for 5 hours at 120 milliamps, stained with ethidium bromide for 20 min, destained with distilled water for 1 hour, and photographed under UV light. A total of 70 random primers were evaluated. No genetic diversity statistics were calculated for the RAPD data, as the analyses are considered preliminary. More extensive investigations are currently underway.

## Results

### *Viability Analysis*

All extracted embryos stained normally except in those areas that had sustained damage during the extraction process, indicating virtually 100 percent viability among the seeds evaluated.

### *Germination Trials*

*Initial Evaluations:* Attempts at germination of seeds with the seed coats intact resulted in 100 percent of the seeds succumbing to fungal infection within 48 hours. Fungal growth typically began at the caruncle, eventually covering the entire seed. Seeds from which the caruncles were removed by cutting, as well as intact seeds incubated at  $107^{\circ}\text{C}$  for 30 min, also developed fungal infections; however, onset was delayed to between 48 hours and 2 weeks. Fungal contamination among seeds from which the caruncles were removed was minimal. None of the individuals from which the seed coats were removed had germinated or become infected with fungus when the trial was terminated after 50 days of incubation. In all of the extracted embryos, the cotyledon next to the blotter turned green within 5 days. When these were inverted, the formerly upper cotyledons turned green within 2 weeks. The cotyledons expanded and appeared to be developing normally; however, no radicle development

was observed after 50 days in the germinator. None of the embryos became contaminated with fungal or bacterial infections during the duration of the study. Fungal and bacterial infections were reduced by approximately 70 and 80 percent, respectively, among seeds treated with sodium hypochlorite, and onset of infection was delayed to between 1 and 2 weeks; however, no germination was observed among any of the treated seeds during the 50-day observation period.

*GA<sub>3</sub> Soak:* All seeds that were soaked overnight in 500 ppm GA<sub>3</sub> showed extensive damage, regardless of pretreatment. All exposed regions turned brown and necrotic. All seeds from which the caruncles were removed, with no further treatment, developed bacterial infections within 2 weeks; none had germinated after 50 days. Among seeds in which the non-radicle ends were clipped in addition to removing the caruncles, 50 percent developed bacterial infections and none had germinated after 50 days. All seeds from which the seed coats were removed became severely necrotic and failed to germinate, but showed no signs of microbial contamination. After 1 week in the germinator, a radicle emerged from one of the seeds that had been cut in half longitudinally; after 50 days, none of the others showed signs of germination or microbial contamination. The seed from which the radicle emerged subsequently developed a bacterial infection, which caused the seed coat to deteriorate into a thin shell and eventually rupture. Surprisingly, within this ruptured seed coat, cotyledons were developing. The "seedling" was transferred to a pot containing soilless potting medium and placed in the greenhouse. The "seedling" failed to develop any further and died within a week of transplanting. All extracted embryos became severely necrotic, the cotyledons failed to turn green, and no further growth or development was observed during the 50-day observation period.

*GA<sub>3</sub>-moistened Blotters:* For all seeds evaluated, regardless of pre-treatment, areas in contact with GA<sub>3</sub>-moistened blotters turned brown and necrotic. Seven of the 10 intact seeds developed fungal infections and none had germinated after 50 days. Among seeds with the caruncles removed, one developed fungal infection and two became infected with bacteria; none had germinated after 50 days. Of the seeds in which the non-radicle ends and caruncles were removed, seven showed no signs of contamination or germination, two developed fungal infections and failed to germinate, and one became infected with bacteria after

which a radicle emerged, the seed coat deteriorated and ruptured, and the "seedling" was transplanted into soilless potting medium in the greenhouse where it subsequently died. Among seeds cut in half longitudinally, a radicle emerged from one seed; however, it subsequently became necrotic and no further development was observed. Of the remaining nine seeds, two became infected with bacteria and one developed a fungal infection; none of these showed signs of germination after 50 days. All extracted embryos exposed to GA<sub>3</sub>-moistened blotters turned green within 2 weeks; however, the radicles failed to develop.

*Prechill:* Fifty percent of intact seeds developed fungal infections within 1 week and 100 percent became infected within 2 weeks during incubation at 4°C. None of the seeds from which the caruncles were removed succumbed to infection during incubation at 4°C. All seeds, both intact seeds and seeds from which the caruncles had been removed (which were plated out after 10 days, 2 weeks, and 3 weeks of prechill), developed fungal infections within 1 week of being transferred to the germinator. A radicle emerged from one seed that had been prechilled for 3 weeks; however, no further development was observed at the end of 50 days.

*KNO<sub>3</sub> Treatment:* All intact seeds and three seeds from which the caruncles were removed developed fungal infections within 1 week. Two seeds with clipped non-radicle ends and caruncles removed also succumbed to fungal infection. No other development or contamination was observed after 50 days.

*HCl Treatment:* Regardless of pre-treatment, all seeds developed fungal patches on the seed coats within 1 week. The fungus was of a different type than previously observed, forming green patches on the seed coats without affecting the caruncles. All intact seeds also developed the typical fungal infection initiated in the caruncle region within 2 weeks of incubation in the germinator. No other contamination was observed and none of the seeds had germinated after 50 days.

*Dark Treatment:* When seeds were removed from the box after 12 days of darkness, no signs of germination were apparent, regardless of pre-treatment. One of the intact seeds had become infected with fungus, while no other fungal contamination was present on any of the seeds, regardless of pre-treatment. Extracted embryos were white with no growth or contamination having taken place during the 12 days of exposure to darkness. Upon exposure to light, all intact seeds developed fungal infections within 48 hours.

None of the seeds from which the caruncles were removed, neither clipped nor unclipped, had developed microbial infections after 50 days. Among seeds cut in half longitudinally, one developed a fungal infection and two developed bacterial infections. None of the dark-exposed seeds had germinated after 50 days. Three of the extracted embryos turned green within 1 week of exposure to light, while the others remained white. Radicles of all extracted embryos became necrotic and failed to develop.

*Boiling:* None of the seeds subjected to boiling developed microbial infections and none showed signs of germination at the end of the 50-day observation period.

*Greenhouse Evaluations:* None of the seeds planted in the greenhouse germinated after 50 days, regardless of treatment or planting medium.

*After-ripened Seeds:* After 50 days in the germinator, none of the after-ripened seeds had germinated. Three of the four had become slightly contaminated with fungal growth of a different type than that noted on any of the freshly harvested seeds evaluated. Embryos were extracted from all four seeds. One of the embryos, along with the endosperm, was rotten; however, the other three embryos were tested for viability. All three of the embryos stained normally, indicating that the seeds were viable despite their failure to germinate after 50 days of incubation in the germinator.

#### *Genetic Diversity Evaluations*

*Isozyme Analysis:* For the nine enzyme systems assayed, 11 putative loci were resolved and scored for variation. Six (AAT-1, ADH, G6PDH-1, 6PGD-1, SDH and TPI) were monomorphic in both populations. Three loci (6PGD-2, PGI and PGM-2) were polymorphic in one population (C1), while two (ALD and G6PDH-2) were polymorphic in both populations. Only one locus was observed for both PGI and TPI, although the number of isozymes normally present in diploids is two for both of these enzyme systems (Wendel and Weeden 1989). The mean number of alleles per locus was 1.45 and 1.18 for populations C1 and C2, respectively. This difference corresponds with the percent polymorphic loci in the two populations (0.45 for C1 vs. 0.18 for C2). The mean number of alleles per polymorphic locus was two for both populations, and no loci with greater than two alleles were observed for either population. The expected heterozygosity (H) was less in population C2 (0.05) than in population C1 (0.08). Gene diversity statistics were calculated and then aver-

aged over all loci. The total gene diversity ( $HT = 0.066$ ) was nearly identical to the gene diversity within populations ( $HS = 0.067$ ). The proportion of total gene diversity ( $Gst$ ) found among populations was 1.2 percent.

*RAPD Analysis:* Of the 70 primers evaluated, 28 showed clear, repeatable banding patterns. Sixteen (57%) of these were polymorphic in at least one of the populations.

## Discussion

### *Population Distributions*

The reported distributions of both varieties of *C. alabamensis* are controversial. Various investigators have defined both population densities and distribution ranges using different sets of criteria, and several populations have been observed by some investigators, but not others (Farmer 1962, Farmer and Thomas 1969, Ginzburg 1992, Steed 1993, Aplet et al. 1994, J. Cornelius, M. Falkner, and C. Sexton, personal communication). Despite these controversies, one interesting observation agreed upon by all investigators is the fact that *C. alabamensis* is restricted to relatively few locations, yet it is quite abundant and populations appear healthy in those few niches in which it occurs.

Populations in Texas occur in mesic areas with apparently suitable habitat for bigtooth maple (*Acer grandidentata*); however, there is only one known canyon at Fort Hood where the two species occur together. The *Croton* population in this canyon is quite small and appears to be in reproductive decline. Several theories have been proposed to explain this phenomenon including differences in shade tolerance, microclimate differences among canyons, and rare fire or drought events (Steed 1993, Aplet et al. 1994). The absence of *Croton* from both the drier westernmost canyons and from the easternmost mesic canyons in which maple is abundant, along with the presence of *Croton* and absence of maple in the intermediate canyons in between, has been speculated to be due to the interplay between fire (and/or drought) and competition with more adapted mesic species such as maple (Steed 1993). Such interactions would restrict *Croton* growth to those areas in which these two opposing forces balance each other to form a stable intermediate zone that is not too dry or too frequently burned, yet not quite mesic enough to support competing species (Steed 1993).

### *Viability Analysis*

Despite the difficulty with which seeds were prompted to germinate in growth chambers and

failure of the seeds to germinate under greenhouse conditions, Tetrazolium staining of extracted embryos indicated virtually 100 percent viability among seeds evaluated, including those that developed fungal infections during incubation and were subsequently tested for viability. This suggests a mechanical barrier to germination, such as possible interference with imbibition or the presence of germination inhibitors in the seed coat.

### *Germination Trials*

Although the results of the germination trials are considered preliminary, they suggest a potential need for fungal and/or bacterial infection of the seed to stimulate germination. Microbial attacks, as well as abrasion by soil particles, are considered important in the softening of hard seed coats, especially those that are impermeable to water, and may affect control of germination (Bewley and Black 1985). Perhaps the microclimate is not suitable for the appropriate microorganism(s) necessary to stimulate germination in *Croton* within those canyons in which the species is absent. In addition, the microorganism(s) may be very sensitive to environmental conditions; this could further explain, along with low shade tolerance among seedlings, the limited seedling production observed in shady canyons where *Croton* is abundant.

### *Genetic Diversity Among and Within Populations*

In a recent summary of allozyme variation in plant species, Hamrick and Godt (1989) found that geographic range accounted for the largest amount of genetic variation in both population and species level statistics. Taxa with narrow ranges often have less genetic variation than taxa with regional or widespread distributions (Karron et al. 1988). Our preliminary investigations on limited material of *C. alabamensis* revealed very low levels of allozyme variation compared to most other seed taxa analyzed (Hamrick and Godt 1989). The mean number of alleles per locus for population C1 is identical to the mean value for taxa with narrow ranges as reported by Hamrick and Godt (1989) and is slightly higher than that reported for endemic species. However, in population C2 the mean number of alleles per locus is lower than the mean value for endemic species, 1.39, reported by Hamrick and Godt (1989). The differences observed between populations C1 and C2 are not unexpected, considering that the individuals comprising population C1 were collected over a slightly larger, more diverse habitat range

than those comprising population C2. The values for expected heterozygosity for both populations are similar to the mean reported for endemic taxa, 0.063 (Hamrick and Godt 1989). The gene diversity statistics indicate very little differentiation between the populations, as evidenced by the low *Gst* value of 1.2 percent for the total gene diversity occurring between populations. This is reflected in the nearly identical values obtained for total genetic diversity and genetic diversity within populations, indicating a high degree of homozygosity within populations and very little inter-population genetic differentiation. The levels of polymorphism detected in the RAPD analyses were similar to those observed in the isozyme analyses. Although the genetic diversity data should be considered quite preliminary, and was obtained from a relatively small sample of individuals, the results support the theory of a relatively recent establishment of the Texas populations from a limited number of genetically similar founders. The lack of significant genetic differentiation between the populations due to drift further argues for recent establishment of the populations. We are currently evaluating genetic diversity among and within a wider range of Texas populations, as well as obtaining estimates of genetic diversity between the Alabama and Texas populations. These investigations should allow for the determination of the direction of population dispersal, as well as verify the merit of varietal status assigned to the Texas populations. Such information is important when determining population sizes in making decisions regarding listing the species in accordance with ESA regulations.

### Summary

*Croton alabamensis* has an interesting life history, being restricted to relatively few locations, yet forming abundant, healthy populations in those areas. The natural dormancy period of the seed, which prevents germination during the dry, hot summers in the regions of Texas and Alabama to which it is endemic, along with our observations that germination could only be stimulated in seeds following bacterial and/or fungal infection, suggests a possible role for microbial breakdown of the seed coat for germination to occur in the field. Such a potentially symbiotic relationship, with microorganisms being sustained in the caruncles of the seeds, could be responsible for maintaining seed dormancy. With the onset of spring rains, the increased soil moisture levels

could stimulate microbial activity in the caruncle region, ultimately leading to a breakdown of the seed coat, allowing germination to occur. The relationship could also explain the absence of *Croton* from apparently suitable habitats, if the microenvironment was not conducive to survival of the appropriate microorganism(s) needed to stimulate germination. Future research is needed to determine which fungal and/or bacterial organism(s) is/are necessary for germination, as well as to determine the microbial composition of the soils both in canyons where *Croton* is present and where it is absent. Such information is critical to obtain, since determining the optimal conditions for germination and seedling establishment are the first steps in the development of effective conservation and management plans.

### Acknowledgments

Special thanks go to Dennis Herbert, B.R. Jones, and John Cornelius, Division of Wildlife, Fort Hood, Texas, for their assistance and support during field collections; to Dixie Daniels, Department of Biology, University of New Mexico, for conducting the isozyme and preliminary RAPD analyses; to Julie Laufmann, USDA National Seed Storage Laboratory, Fort Collins, Colorado, for her assistance and encouragement during the germination trials; and to Tracy Wager for providing the botanical illustrations, as well as for preparing graphics for the slide presentation. Funding was provided by the USDA Forest Service, Rocky Mountain Forest and Range Experiment Station; and the Department of Defense Legacy Resource Management Program.

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## Appendix A

### *DNA Extraction Procedure Used in Genetic Diversity Evaluations*

Leaf material was ground with a mortar and pestle in CTAB extraction buffer containing 1% 2-mercaptoethanol, incubated for 1–3 hr at 60°C, then allowed to cool to room temperature. After centrifugation for 5 min, the aqueous layer was removed and transferred to a clean tube to which an equal volume of chloroform/octanol (24:1) was added. Tubes were inverted briskly for 2–3 min followed by centrifugation for 7 min. The aqueous layer was removed and transferred to a clean tube. The chloroform/octanol extraction process was repeated two additional times. One  $\mu\text{L}$  RNAase (10 mg/mL) per mL of volume was added to the reaction and allowed to digest at 37°C for 30 min. An equal volume of ice-cold (-20°C) isopropanol was added to the tube, which was gently rocked until the DNA precipitated. The solution was then centrifuged for 5 min and the supernatant discarded. The DNA was then placed in a mixture of 500  $\mu\text{L}$  of 76% EtOH/0.2 M Na acetate and left at room temperature for 10 min. The DNA pellet was transferred to a clean tube and allowed to dry completely at room temperature, then resuspended in 50–200  $\mu\text{L}$  TE (0.01 M Tris/0.001 M EDTA, pH 8.0), depending on the size of the pellet.

### *Preparation of Reaction Mixture Used for RAPD Analysis*

A 25  $\mu\text{L}$  reaction mixture was prepared by adding 18  $\mu\text{L}$  sterile ddH<sub>2</sub>O, 5  $\mu\text{L}$  Master Mix (500  $\mu\text{L}$  10X Reaction Buffer supplied by Boehringer Mannheim, 50  $\mu\text{L}$  each 10 mM dATP, 10 mM dCTP, 10 mM dGTP, and 10 mM dTTP, 299  $\mu\text{L}$  ddH<sub>2</sub>O, and 1.25  $\mu\text{L}$  1M MgCl<sub>2</sub>), 0.1  $\mu\text{L}$  (0.5 unit) Taq DNA polymerase, 1  $\mu\text{L}$  (5 picamoles) Primer (supplied by Operon), and 1  $\mu\text{L}$  extracted genomic DNA (20–100 ng) to a sterile 0.6 mL microcentrifuge tube. The mixture was gently vortexed and centrifuged for 2–3 seconds to collect the mixture at the bottom of the tube. The reaction mixture was covered with 50  $\mu\text{L}$  electrophoresis grade mineral oil and tubes were placed in the DNA thermal cycler wells to which a drop of mineral oil had been added.

Both appendices are from Timothy Lowrey, Department of Biology, University of New Mexico (unpublished).

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# Pollinator Lost? Reproduction by the Enigmatic Jones Cycladenia, *Cycladenia humilis* var. *jonesii* (Apocynaceae)

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**Abstract:** We studied the pollination and reproduction of the rare Jones cycladenia (*Cycladenia humilis* Benth. var. *jonesii* (Eastw.) Welsh & Atwood). Preliminary evidence suggests that Jones cycladenia is self-compatible, although it requires a pollen vector. Fruit production was low under both hand-pollinated and natural conditions, and in both cases, we observed frequent fruit abortion. The reasons for low fruit set are presently unclear. Insect visitors to Jones cycladenia flowers were uncommon, and varied greatly among years. The paucity of pollinators suggests pollinator-limited fruit production. Conversely, the frequent abortion of fruit, and a decrease in fruit set over the flowering season, suggests that resources may limit fruit production. However, water emendation did not increase fruit set. Excessive inbreeding appears to be ruled out by a companion study of the population genetics and clonal structure of Jones cycladenia. It may be that the original pollinator of Jones cycladenia has become dissociated from its host, or that pollinator visits and fruit production are episodic.

## Introduction

The federally listed Jones cycladenia, *Cycladenia humilis* Benth. var. *jonesii* (Eastw.) Welsh & Atwood (Apocynaceae), is a rare desert wildflower endemic to the Colorado Plateau. The taxon appears to be edaphically restricted, occurring only on certain members of the Summerville, Chinle, and Cutler formations in southern Utah and northern Arizona (Sipes et al. 1994, Welsh et al. 1987). Currently, the main threat to its existence is degradation of its habitat by bicycles and off-road vehicles (Sipes et al. 1994). Potential threats include mining impacts if such activities resume; most of the geologic members in which Jones cycladenia occurs have historically been mined for uranium.

Jones cycladenia is a perennial of unknown longevity that appears to persist mainly by the spreading of rhizomes. Although it flowers from April through June, sexual reproduction appears to be infrequent, based on our observations over 4 years of low fruit set and lack of seedlings (Sipes and Tepedino, unpublished data).

Its low sexual reproduction may be due in part to a paucity of visits by pollinators. Insect visits are uncommon, and of the insects that have been observed visiting the flowers from 1988 to 1993, none can be distinguished as the primary pollinator (Sipes et al. 1994). However, the fact that flowers often appear to commence fruit development, but later abort, suggests that some other factor, such as resource limitation, may be responsible for the low fruit set. Because Jones cycladenia occurs

on extremely water poor soils in a desert environment, the most probable limiting resource is water.

We address the following questions in this study: (1) Does Jones cycladenia require a pollinator for sexual reproduction? (2) Is the taxon self-compatible? (3) Is there evidence that fruit set is limited by either pollinators or water availability?

## Methods

### *Breeding System*

Our study site was near the San Rafael Reef in Emery County, Utah (Table 1). This is the largest and most accessible known population. Breeding system experiments were performed in 1991. Before implementing the experiments, we dissected flowers of Jones cycladenia to become familiar with the floral morphology. To describe the breeding system, we chose 20 discrete clumps of ramets<sup>1</sup> with at least three ramets with an unopened flower. The clumps were caged to exclude pollinators. Cages were constructed of 1 inch chicken wire covered with bags made of 1 mm nylon tulle. Calyces of flowers were marked for treatment with enamel paint (previously shown not to harm flowers), and the following treatments were applied when flowers opened: (1) Autogamy—flower unmanipulated to test for automatic self-pollination (without a pollen vector); (2) Self-Pollination—flower pollinated with its own pollen

<sup>1</sup>In a study of clonal structure using allozyme data, tightly grouped clumps of ramets usually shared the same multilocus genotype, indicating that they belonged to the same genet (Sipes et al. 1994).

Table 1. Known locations, land ownership, and estimated population size of Jones cycladenia. Within legal descriptions, section numbers in parentheses indicate estimated sections in unsurveyed areas.

Population	Land Ownership	Size*
San Rafael, UT	BLM, State, Private?	> 10,000
Castle Valley, UT	BLM	> 5,000
Onion Creek, UT	BLM	< 500
Purple Hills, UT	Glen Can. Nat. Rec. Area	2500
Circle Cliffs, UT	Glen Can. Nat. Rec. Area	< 500
Deer Point, UT	BLM	> 500
Capitol Reef, UT	Capitol Reef Nat'l Park	> 500
Pipe Springs, AZ	BLM, Private?	>5000

\*Estimated number of ramets.

or pollen from another flower on the same ramet to test for self-compatibility; (3) Cross Pollination—flower pollinated with pollen from a ramet at least 15 meters away (to increase the probability of a cross between two genets).

Flowers were pollinated by first pulling two anthers away from the anther cone to expose the stigma of the recipient flower, and applying pollen from the donor flower to the recipient stigma using a 00-size insect pin. Pollen transfer was confirmed with the aid of a 10x dissecting scope; preliminary pollinations followed by dissection and examination of stigmas showed that this technique accomplished pollen transfer. We attempted to apply pollen to as much of the stigma as possible, although pollen deposition was focused on the sticky sides of the cylindrical stigma, which we judged to be the receptive area. All flowers were hand pollinated on both the first and second day of anthesis, to increase the probability that pollination took place during the time of stigma receptivity. Newly opened flowers were chosen as pollen donors to help insure that pollen was viable. Plants remained bagged until corollas of treated flowers dried. For each treated group of ramets, a flower on an adjacent, unbagged ramet was marked for an open-pollinated control. Treated flowers were checked every few days for signs of fruit development. Any visible enlargement of the ovary was considered fruit commencement.

Breeding system experiments were repeated in 1992 and 1993. We used the same methodology except for the following changes: We chose 20 ramets with at least three unopened flowers and applied all three treatments to different flowers on

the same ramet. As flowers opened, treatments were applied in random order so as to remove any effect of flower phenology. Additionally, we chose the largest, most vigorous ramets we could find, because the low fruit set in 1991 suggested that fruit set was resource limited.

#### *Water Emendation*

In May 1992, we watered Jones cycladenia plants at the San Rafael site to test the hypothesis that fruit set was limited by water availability during the flowering/fruitletting season. Twenty clumps of ramets were chosen and randomly assigned to wet (watered) or dry (control) groups. We wanted our treatment to mimic conditions during a wet year, so water was added in an amount equal to the mean precipitation plus one standard deviation for Green River, Utah (location of closest weather station) for May (1.7 cm + 1.3 cm = 3 cm). Once a week for four weeks, one fourth of this total amount (0.75 cm) was applied to a 0.75 m circle around the center of each "wet" clump (3.3 liters water per clump per week). This diameter was chosen because it was approximately twice the diameter of the clumps, and we estimated that it would include most of the clumps' root systems. Water was applied in early evening to minimize evaporation.

#### *Natural Fruit Set*

Natural levels of fruit set were censused in 1992–1994. At each population censused, between 70 and 300 ramets were sampled (depending on population size and time constraints) using the step-point method (Bonham 1989). For each ramet, number of leaves, number of flowers, and number of fruits were recorded. In 1993 and 1994, we also recorded number of aborted fruits.

To further monitor fruit development at San Rafael in 1993, and to compare the reproductive success of plants beginning anthesis at different times, we marked three groups of 10 ramet clumps, beginning with plants from among the earliest to bloom. Five flowers on each plant were individually marked by gluing small numbers on the calyx with Elmer's glue. Flowers were monitored throughout their lifetimes for fruit development, abortion, and maturation. At 10-day intervals, groups of mid-blooming and late-blooming plants were marked in the same way. We were only able to monitor these last two groups at the end of the fruiting season, so only fruit maturation data are available.

### *Observations of Flower Visitors*

We observed and collected floral visitors to Jones cycladenia 1991 to 1993. Visitors were also collected in 1988 and 1989 (Griswold and Tepedino, unpublished data). In each of these years, collections were made at the San Rafael and Castle Valley populations. Additionally, we looked for visitors in 1993 at Circle Cliffs, Purple Hills, Capitol Reef, and Onion Creek (Table 1). Insect specimens were mounted and returned to the laboratory for identification. In 1991–1993, we observed during the evening at the San Rafael site to determine if nocturnal visitation took place.

## Results

### *Floral Morphology*

The floral morphology of Jones cycladenia is illustrated in Figure 1. The calyx, corolla, and androecium are 5-merous. Filaments are adnate to

the corolla tube, and anthers form a tight cone over the stigma. Each anther produces pollen in its upper half, and is attached on its lower half to the stigma by a thin band of tissue. The ovary is composed of two separate carpels that share a common style and stigma. As in other members of the Apocynaceae, the stigma is highly specialized, and in conjunction with the morphology of the anthers, promotes outcrossing while minimizing self-pollination. Self-pollen dehisces and collects on the top surface of the stigma, which is hard and dry. In most members of the Apocynaceae, the top of the stigma is unreceptive to pollen, serving instead as the platform of a chamber for secondary pollen presentation (Fallen 1986). The sides of the cylindrical stigma exude a sticky substance, presumably to aid in adherence of pollen grains. This is the area we judged to be the receptive region. Apparently, as an insect's proboscis is withdrawn

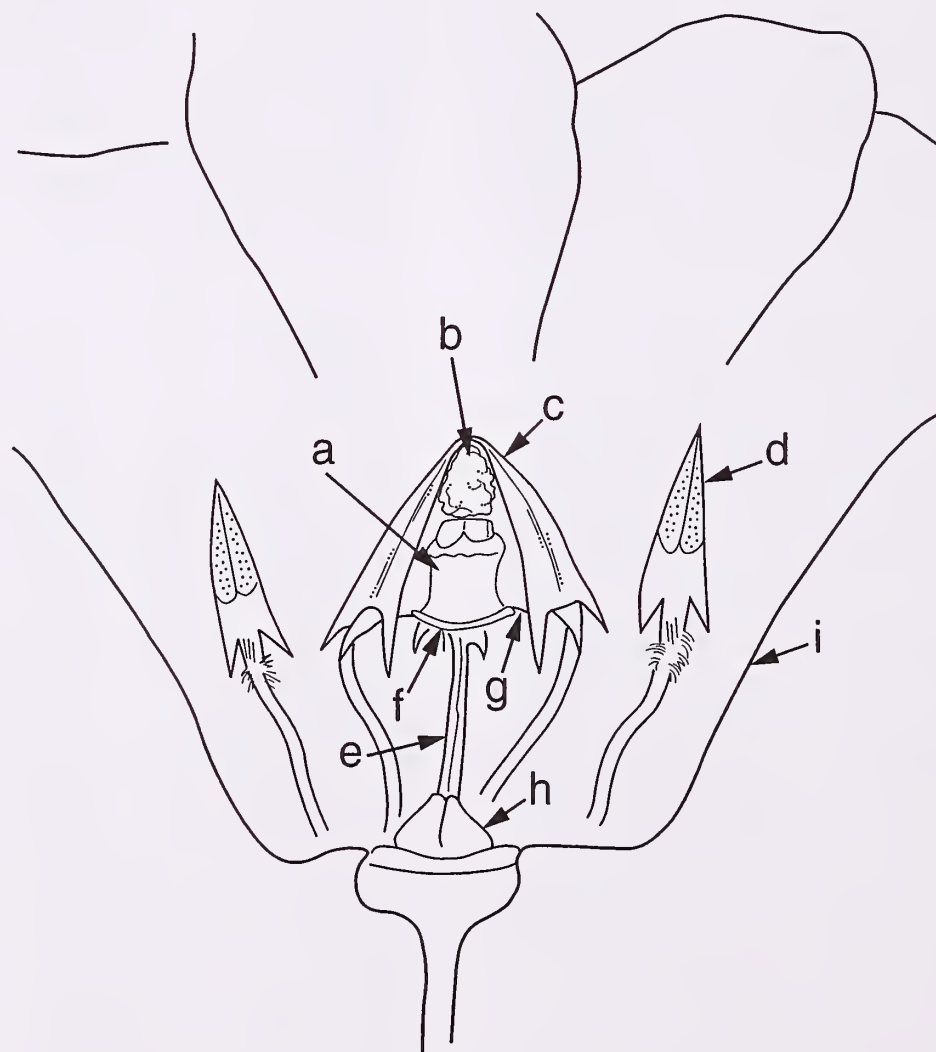


Figure 1. Floral morphology of Jones cycladenia. a = secretory sides of stigma, b = chamber for secondary pollen presentation, c = anther, d = pollen sacs of anther, e = style, f = ventral surface of stigma, g = tissue connecting anther to stigma, h = ovary composed of two partially separate carpels, i = corolla split here and spread for viewing.



after probing the flower, it is directed inside the anther cone where (1) it passes the stigmatic surface, depositing pollen from previously visited flowers, (2) it becomes coated with a glue-like exudate from the stigma, and (3) it becomes coated with dehisced pollen that has collected above the stigma. Available evidence suggests that Apocynaceous pollen is not collected by bees for provisioning of nests, but is picked up only incidentally as visitors probe for nectar (Hurd 1979).

#### Breeding System

Few fruits matured on any of the treatment flowers; no mature fruits were produced in the 1993 trial (Table 2). However, ovaries of numerous flowers expanded after pollination, suggesting that fruit had commenced development and then aborted. Assuming we adequately pollinated the treatment flowers (see discussion below), we can tentatively draw several conclusions about the breeding system of Jones cycladenia. First, it appears to require a pollen vector for pollination. No fruit commencement was observed in the autogamy treatments in any year. Second, the species appears to be at least partially self-compatible. A fruit matured on a self-pollinated flower in both 1991 and 1992, and fruit commencement is about equal between selfed, crossed, and control flowers.

Table 2. Results of 1991–1993 breeding system experiments. Data are number of fruits / number of flowers treated. Numbers in parentheses are fruits that commenced development but aborted.

	Autogamy	Selfed	Crossed	Control
1991	0/11 (0)	1/26 (11)	1/24 (8)	1/14 (2)
1992	0/24 (0)	1/20 (11)	1/20 (7)	0/23 (7)
1993	0/16 (0)	0/15 (3)	0/16 (7)	0/16 (9)

#### Water Emendation

Natural precipitation in May was above average (approx. 2.5 cm), but most of it fell after the last weekly water emendations. Because of this, natural precipitation was not subtracted from the emendation; thus, the treatments received more water than intended. Nevertheless, evidence that fruit set was limited by water availability was not forthcoming. Few fruits were produced by either the wet or the dry clumps. In the wet treatment, three clumps produced fruit (1, 1, and 7 fruits). In the dry treatment also, three clumps produced fruit (1, 1, and 3 fruits).

#### Natural Fruit Set

The proportion of ramets flowering in a particular year varied among sites from 0.36 in 1992 at San Rafael to 0.86 in 1994 at Onion Creek (Figure 2). Within populations, the proportion of ramets flowering and fruiting across years was surprisingly consistent, with the exception of 1992 at San Rafael. In each year at each site, more ramets commenced fruit development than matured fruit. Fruit production was always low; in all cases but one, fewer than 20 percent of ramets matured at least one fruit. Interestingly, although Onion Creek had the highest proportion of flowering ramets in both 1993 and 1994, it had the lowest fruit production in both years. Onion Creek is also a very small population, consisting of fewer than 15 discrete patches of ramets.

Table 3 shows the fate of the early cohort of flowers monitored in 1993. Only six flowers showed no signs of fruit development. Most fruits aborted, and did so throughout all stages of development. Only 6 of the 42 flowers matured fruit, but this percentage is much higher than the overall percent fruit set for the population (14.3% vs. 2.3%). No flowers in the mid- and late-flowering cohorts matured fruit.

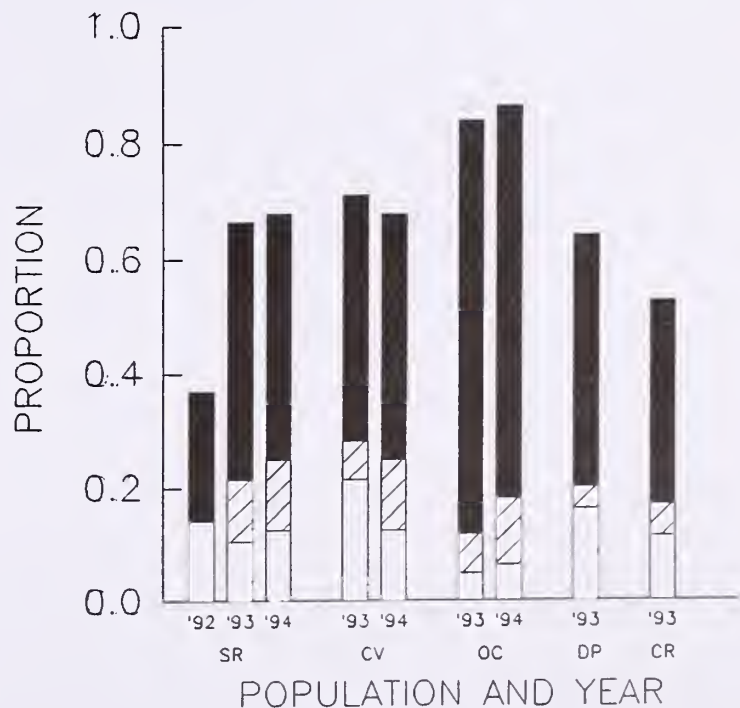


Figure 2. Proportion of ramets that flowered but did not commence fruit (black bars), commenced at least one fruit but did not mature one (striped bar), and matured at least one fruit (white bar). Data on fruit commencement not available for San Rafael 1992. SR = San Rafael, CV = Castle Valley, OC = Onion Creek, DP = Deer Point, CR = Capitol Reef.

Table 3. Fate of early cohort flowers (opened approx. 15 April 1993) at San Rafael 1993: ab = aborted; measurements indicate length of fruit when aborted. No fruits were formed from mid and late cohorts (not shown here).

Plant	No. Flws Marked	No. Fruit	Fruit ab. < 5 mm	Fruit ab. 5–10 mm	Fruit ab. 10–15 mm	Fruit ab. > 15 mm	Fruit matured
	4	2				1	1
E2	4		1			1	2
E3	2					1	1
E4	5		5*				
E5	5	1	3				1
E6	4			2	2		
E7	4	3	1				
E8	5		3		1		1
E9	4		1	1	1	1	
E10	5			2	3		
Total	42	6	14	5	7	4	6

\*Ramet died.

#### *Observations of Flower Visitors*

A variety of diurnal insects have been found to visit the flowers of *Jones cycladenia* at San Rafael and Castle Valley populations during 1988–1993 (Table 4). Our 5 years of data show great inconsistency from year to year in visitor species. For example, few bees were collected in 1988, except for the common generalists, *Lasioglossum* spp., while in contrast, a variety of small andrenid bees were the most common visitors in 1991. In the other years, visitors represented a diverse assemblage of bees, flies, and lepidopterans. Many of these insects may be rare themselves; no fewer than six undescribed species of bees and one undescribed species of wasp have been collected from the San Rafael site, five of these from *Jones cycladenia*. No nocturnal visitors were observed in 1991 or 1993. However, in 1992, we twice caught fleeting glimpses of nocturnal visitors to *Jones cycladenia* flowers, but it was too dark to positively identify the taxa. No floral visitors were observed at Circle Cliffs, Purple Hills, Capitol Reef, or Onion Creek. However, our observation time was minimal at these populations. A single visitor, a male *Agapostemon* sp., was observed in a flower at Deer Point.

In each year, various dead bees, flies, and lepidopterans were found stuck in a small proportion of flowers. Several times we watched as butterflies and bees probed the flowers, became entrapped, and struggled to free themselves. Dissections of

these flowers showed that the insects' mouthparts had become wedged between the anthers and then had apparently become glued to the stigma via the sticky exudate.

#### Discussion

##### *Breeding System*

We present the results of the breeding system experiment as preliminary findings for several reasons. First, the low fruit set obtained from our hand pollinations are insufficient to support anything other than tentative conclusions. Second, a recently published study has clarified a question that has been debated in the literature for years: where is the receptive region in the Apocynaceae stigma? Some authors have declared the top of the stigma to be the receptive region, while others have implicated the sticky, adhesive-producing sides of the stigma (Woodson 1930, Beentje 1978, Hickey and King 1981). Fallen (1986) described four classes of stigma morphology within the Apocynaceae and concluded that the receptive surface varies among these classes. In these studies, conclusions as to the receptive surface were drawn indirectly, either by speculation about the pollination mechanism, or by histological studies.

Recently, Albers and van der Maesen (1994) presented conclusive evidence, via staining of pollen tubes, that the receptive stigmatic region does indeed vary within the family, and the morphology of the stigma can be used as a predic-

Table 4. List of visitors to Jones cycladenia flowers at San Rafael and Castle Valley, from 1988 to 1993. Asterisk (\*) = observed but not collected at Deer Point only.

	1988	1989	1991	1992	1993
<b>HYMENOPTERA</b>					
<b>Andrenidae</b>					
<i>Andrena linsleyana</i> Thorp.				X	
<i>Perdita aridella</i> Timb.			X		
<i>P. holoxantha</i> Timb.			X		
<i>P. moabensis</i> Timb.			X		
<i>Perdita</i> new species # 2			X		
<i>Perdita</i> new species # 4			X		X
<b>Apidae</b>					
<i>Anthophora affabilis</i> Cr.			X		
<i>A. dammersi</i> Timb.			X		
<i>A. lesquerellae</i> Skll.			X		
<i>A. (Anthophoroides)</i> new species			X		
<i>Habropoda morrisoni</i> (Cr.)			X		
<i>Synhalonia phaceliae</i> Ckll.					X
<i>S. quadricincta</i> Timb.					X
<i>Apis mellifera</i> L.		X		X	
<i>Bombus morrisoni</i> Cr.		X			
<b>Colletidae</b>					
<i>Colletes phaceliae</i> Ckll.		X			
<b>Halictidae</b>					
* <i>Agapostemon</i> sp.					X
<i>Dufourea</i> new species		X		X	X
<i>Lasioglossum (Dialictus)</i> sp.	X	X			
<i>L. (Evylaeus)</i> sp.	X				
<b>Megachilidae</b>					
<i>Osmia crassa</i> Rust & Bohart		X			
<i>Ashmeadiella</i> new species		X			
<i>A. sonora</i> Mich.				X	
<i>A. gillettei</i> Titus		X			
<b>Masaridae</b>					
<i>Pseudomasaris</i> new species		X			
<b>DIPTERA</b>					
<b>Bombyliidae</b>					
	X	X	X	X	X
<b>Sarcophagidae</b>					
		X			
<b>Syrphidae</b>					
	X			X	
<b>LEPIDOPTERA</b>					
<b>Nymphalidae</b>					
		X		X	
<b>Geometridae</b>					
	X				
<b>Noctuidae</b>					
			X	X	X
<b>Sphingidae</b>					
				X?	

tor of this location. Based on a comparison of the stigmatic morphology of Jones cycladenia to those presented in Albers and van der Maesen (1994), it appears probable that the receptive stigmatic surface of Jones cycladenia is located on the *ventral* surface of the cylindrical stigma (see Figure 1). If this is so, then our above description of the pollination mechanism still appears to hold, except that pollen carried on the mouthparts of pollinating insects is scraped off by the lower collar of the stigma and collects on its underside, rather than adhering to the sticky sides of the stigma.

This new finding warrants a repetition of our breeding system experiments, because we are unable to determine the degree to which our hand pollination technique transferred pollen to this ventral stigmatic surface. It is possible that flowers with aborted fruit were not adequately pollinated; perhaps some ovules were fertilized, but not enough for the plant to invest in fully maturing the fruit (Lee 1988). Alternatively, ovaries may expand and appear to be developing fruit after pollination, but before fertilization (Lee 1988). We plan to address these questions in future studies.

#### *Evidence for Resource-Limited Fruit Set*

The high abortion rate and low fruit set observed in our breeding system experiment is mirrored in natural populations: three years of data collected from five populations have shown extremely low fruit set (<< 10% of flowers mature fruit), with a higher percentage (20–50%) of flowers commencing fruit set, but later aborting (Sipes et al. 1994). Resource limitation is the most common cause of fruit abortion (Stephenson 1981). The fact that fruit set dropped precipitously from the early cohort of flowers to the mid and late cohorts suggests that some resource may have become depleted during the course of the flowering and fruiting season.

The most obvious resource, water, was not implicated in fruit set limitation in this study. Water emendations, supplied at one standard deviation above normal rainfall for the month of May, had no measurable effect on fruit set. It remains possible that water is limiting, but that the critical period for its delivery and uptake is at times of the year other than spring. Further studies are needed to examine the role of time of water delivery on fruit set.

Other resources, such as soil nutrients, may limit the fruit production of Jones cycladenia. The soils in which this plant is found are extremely low in both nitrogen and phosphorus (Sipes et al.

1994). Deficiencies of either of these nutrients are known to cause low fruit set and fruit abortion in other plants (Stephenson 1981). Future studies of fruit limitation should incorporate nutrient emendations.

#### *Evidence for Pollinator-Limited Fruit Set*

Despite the suggestion that fruit set in Jones cycladenia may sometimes be limited by resources, the low visitation rates to the flowers recorded over 5 years strongly suggests that pollination rates are never high enough to challenge resource availability. Insect visitors to the flowers are uncommon and have been highly variable from year to year. No single taxon or group of taxa has consistently visited Jones cycladenia year after year. In fact, strikingly different assemblages of floral visitors have been collected each year. Additionally, the extremely low visitation rate makes it difficult to establish the relative importance of any particular visitors as pollinators. It is likely that the various insects that occasionally visit the flowers do not often pollinate them adequately and that the result is fruit abortion and low fruit set. The selective abortion of fruits with few fertilized ovules has been reviewed by Stephenson (1981) and Lee (1988). However, this argument for pollinator limitation of fruit set must confront the finding of low fruit set even in flowers cross-pollinated by hand.

The Apocynaceae is generally considered to be adapted for pollination by butterflies and large, long-tongued bees (Waddington 1976, Albers and van der Maesen 1994). However, about half of the bee species collected from Jones cycladenia were small, short-tongued species (Table 4). No long-tongued bees were abundant or consistent visitors. Additionally, few butterflies visited the flowers. These findings, gathered over 5 years, raise the possibility that the original pollinator of Jones cycladenia is no longer consistently found within the plants' distribution. Alternatively, pollination and fruit set may be facilitated by episodic appearances of migrant pollinators such as the painted lady, *Vanessa cardui* (Nymphalidae).

The entrapment of insects within the flowers poses yet another mystery in this puzzle. It is unclear what, if any, significance this intriguing phenomenon has to Jones cycladenia's reproductive success, but similar observations have been made in other members of the Apocynaceae (Leggett 1872, Osburn 1920, Woodson 1930, Waddington 1976). Although large insects, such as anthophorid bees and nymphalid butterflies, are

occasionally trapped in Jones cycladenia flowers, the most common victims are small bees (e.g., *Perdita*, *Dufourea*) and bombyliid flies. The tar and feather process of insect probosci being guided first over the sticky stigma and then through the pollen chamber is intended to promote outcrossing, but apparently goes awry when the visitor is not strong enough to pull out, or after the insect has become gummed up by multiple visits to Jones cycladenia flowers. A similar scenario has been postulated by Osburn (1920), after extensive observations of *Apocynum* spp. Great persistence may be needed to solve the riddle of what, if anything, presently pollinates Jones cycladenia.

#### *Other Possible Limiting Factors of Fruit Production*

If genets of a clonal species are very large, then most pollinator movements will be within genets, a phenomenon which could limit fruit set if species are self-incompatible or suffer inbreeding depression (Aspinwall and Christian 1992). Large self-incompatible clones were suspected to be the reason for low fruit set in the related *Apocynum sibiricum* (Waddington 1976). However, a study of the clonal structure of Jones cycladenia utilizing enzyme electrophoresis showed that Jones cycladenia clones generally do not extend further than 10 meters in any one direction, and are extensively interdigitated (Sipes and Wolf, in preparation). This suggests that pollinators will quite often move between genets and carry out cross-pollination. Additionally, genotype frequencies in seven populations of Jones cycladenia did not differ significantly from Hardy-Weinberg proportions, suggesting the populations are not inbred (Sipes and Wolf, in preparation).

Low fruit set in Jones cycladenia may be a reflection of its phylogeny and growth habit. Low fruit set and high fruit abortion have been commonly reported in members of the Apocynaceae (Woodson 1930, Waddington 1976, Anderson 1983, Fallen 1986), as well as in Asclepiadaceae, a closely related family (Broyles and Wyatt 1990, Stephenson 1981, Willson and Price 1977). Although the reasons for this pattern are not completely clear, it has been postulated that flowers in excess of those that can mature fruit serve to increase a plant's fitness through the male function (Broyles and Wyatt 1990, Stephenson 1981, Willson and Price 1977). Such an explanation of low fruit set in Jones cycladenia strains credulity. The average plant's fitness in any given year, achieved through male or female function, is minuscule.

Low sexual reproduction also appears to be the norm for clonal plants (Callaghan et al. 1992, Ellstrand and Roose 1987). Clonal plants may form dense stands where recruited seedlings cannot compete. For this reason, clonal plants may allocate more resources to vegetative rather than sexual reproduction. This is an unlikely explanation for Jones cycladenia, because total vegetative cover within populations is usually less than 5 percent, and Jones cycladenia cover is less than 1 percent (Sipes et al. 1994). Nonetheless, we have never observed any seedling recruitment in Jones cycladenia, although searches for seedlings have been conducted during spring and summer 1991-1994.

#### *Implications for Conservation*

Regardless of the cause, the low sexual reproduction observed in Jones cycladenia poses special concerns for its conservation. Because Jones cycladenia reproduces vegetatively, it may be persisting despite a loss of its original pollinator species. However, the infrequent (or nonexistent) recruitment of new individuals into populations makes each existing clone especially valuable to conservation efforts. Jones cycladenia may have a very low ability to replace individuals lost to human impacts or stochastic events. It may also have a low ability to colonize other suitable habitats. For example, in the Circle Cliffs area, Jones cycladenia inhabits only a small area, despite seemingly extensive habitat in the same geologic members with similar soil, slope, aspect, and vegetation. As with all rare species, but especially with Jones cycladenia, all known populations should be protected as insurance against unpredictable events.

#### *Acknowledgments*

Funding for this study was provided by USDA/APHIS and USDI/BLM Challenge Cost Share Program. We thank Dr. Terry Griswold (USDA) for identification of the insect specimens, as well as for his participation in numerous brainstorming sessions over the years in an attempt to understand Jones cycladenia. Dr. P.G. Wolf (USU) made many helpful suggestions during the study. We thank the field crew: W.R. Bowlin, S. DiGiacomo, Y. Wilder, M. Sipes, G. Warren, and R. Nielson, and the numerous other persons and agencies that cooperated with us, including Larry England (U.S. Fish and Wildlife), Ron Bolander (BLM), Lori Armstrong (BLM), John Spence (Glen Canyon NRA), and Norm Henderson (Capitol Reef NP).

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# Seed Germination and Pollination Requirements of Holy Ghost Ipomopsis (*Ipomopsis sancti-spiritus*)

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**Abstract:** Holy Ghost Ipomopsis is an endangered species known only from a single location in northern New Mexico. To examine its cultivation requirements and breeding system, I conducted seed germination and pollination studies. Results indicated that maximum germination occurred after at least 1 month of cold stratification. Fruit set occurred in response to application of both self and outcrossed pollen, indicating that the species has a flexible breeding system. Fruit set success from outcross pollen varied with pollen donor. This evidence and the observation that anthers often lack pollen may be responses to small population size.

## Introduction

For many rare plants, recommended conservation strategies include maintaining an ex situ collection of the species and determining autecological factors necessary for growth and reproduction (USFWS 1989, 1991, 1992). To accomplish this, botanical gardens in the consortium known as the Center for Plant Conservation have collected seeds or cuttings of rare species from the wild to house "captive" populations in their gardens. The captive populations are a resource for studying growth and reproduction requirements, which are essential for the management of the species in captivity and can aid conservation efforts in the wild.

One of the species for which ex situ and biological studies have been recommended is Holy Ghost Ipomopsis (*Ipomopsis sancti-spiritus* Wilken & Fletcher), housed at The Arboretum at Flagstaff and part of the National Collection of the Center for Plant Conservation. The species is known only from a 1.3 mile stretch along a road through mixed conifer forests of Holy Ghost Canyon in northern New Mexico, Santa Fe National Forest. *Ipomopsis sancti-spiritus* population size is estimated to be between 1250 and 2500 individuals (Sivinski 1991). It was listed as endangered in 1994 (USFWS 1994). Because the majority of the population occurs along the cut-and-fill slopes of the road, it is hypothesized that management practices, which eliminated natural disturbances such as fire and erosion, have shifted the dependence of the species to man-made disturbances for suitable habitat (Sivinski 1991). Like other members of the genus, the species requires well-drained soils and is not very competitive (Maschinski and Whitham 1989, Wilken 1996). The extremely narrow geographic distribution of the species and its lack of competitive ability make it extremely vulnerable to activities related to road maintenance and/or

activities suppressing moderate disturbance (Sivinski 1991).

Little was known about the reproduction and cultivation requirements of the species. Yet, this information is essential to conserve the species ex situ and to carry out the conservation recommendation to introduce the species to new locations (Sivinski 1991). Here I report on seed germination and pollination requirements and suggest avenues for future research.

## Methods

To determine the germination requirements of the species, seeds were exposed to six different conditions: (1) direct exposure to moisture on a mist bench in a greenhouse facility with ambient temperatures of 60–80°F without any special treatment; (2) scarification with concentrated sulfuric acid for 1 min., rinsing, and placement on a mist bench; (3) exposure to variable day/night temperatures of 55°/35°F in a growth chamber; (4) exposure to variable day/night temperatures of 76°/38°F in a growth chamber; (5) stratification at 42°F for 4 weeks; (6) stratification at 42°F for 6 weeks; and (7) stratification at 42°F for 8 weeks. Seeds from individual fruits were kept separate for germination trials so that parental origin could be tracked. In addition, the total number of seeds in 55 fruits was counted to determine average seed set. The total number of seeds used for each treatment varied between 60 and 117 in 24 to 38 replicates per treatment. Germination was checked weekly for 7 weeks after the treatment was imposed. Differences in the percentage of seeds that germinated were analyzed using analysis of variance.

To determine whether *Ipomopsis sancti-spiritus* is self-compatible or self-incompatible, 10 plants growing at The Arboretum at Flagstaff were

bagged to eliminate any avian or insect contact with flowers. Within each plant, test flowers were haphazardly selected and emasculated before pollination treatment; five flowers received pollen directly from the anthers of another plant, five flowers received self pollen, and five flowers received no pollen. Fruit set was then quantified at the end of 4 weeks.

### Results

The mean number of seeds/fruit was 4.1 in the 55 fruits measured. The percentage of seeds that germinated varied according to treatment ( $F = 5.08$ ,  $p < 0.0001$ , Figure 1) with the highest percentages occurring in the 4-week and 8-week stratification treatments, 73 percent and 77 percent, respectively. Surprisingly, the 6-week stratification treatment was not intermediate between the 4-week and 8-week treatments. Scarification had the lowest percentage (36%) of seeds germinating. These findings suggest that most germination in the wild would occur in the summer or fall when soils are moist and temperatures warm to  $>60^{\circ}\text{F}$  following the winter stratification period.

Seedlings that arose from the germination studies were transplanted to standard potting mix

(2 peat, 3 sand, 4 perlite, 4 composted bark) and then into Flagstaff basalt cinder soils. They survived well on the grounds of The Arboretum at Flagstaff. Thirty-two of 215 or 15 percent of transplants died.

Pollination trials indicated that *Ipomopsis sancti-spiritus* could set fruit when flowers received either self or outcrossed pollen. None of the flowers that received no pollen set fruit. The percentage of successful fruit set with selfed vs. outcrossed pollen varied across individuals and with pollen donor, such that 57 percent of selfing trials successfully set fruit, whereas outcrossed pollen trials had between 9.5 percent and 77 percent successful fruit set, depending on the pollen donor. Finding viable pollen for the experiment was sometimes problematic, because a small, unmeasured percentage of anthers appeared empty of pollen, being completely white instead of blue in color. Blue anthers contained pollen, whereas white ones did not.

### Discussion

Several pieces of evidence from these preliminary studies suggest that *I. sancti-spiritus* has characteristics symptomatic of the ills of small

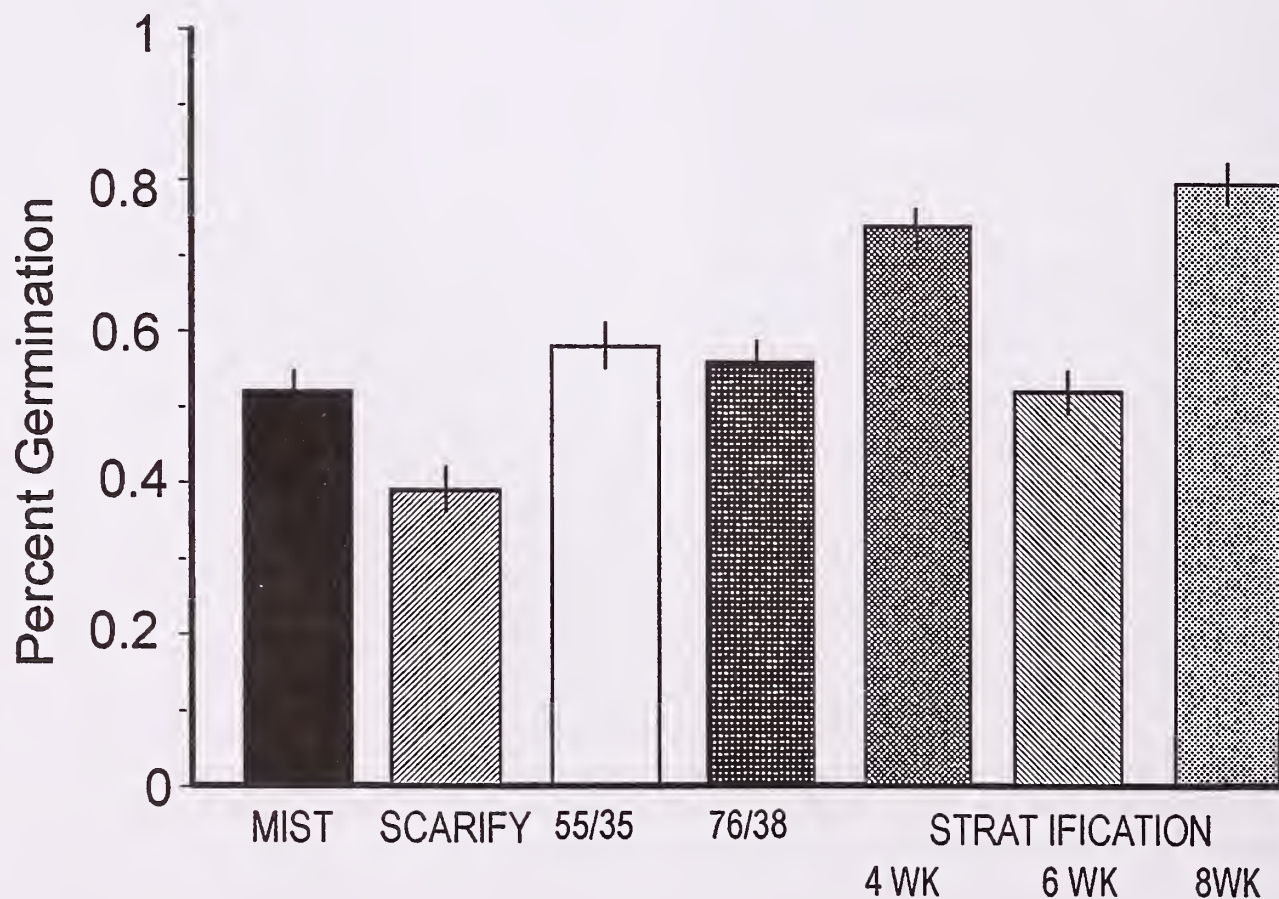


Figure 1. Percentage of seeds germinating with various treatments.



population size. With a mean of 4 seeds/fruit and 10–12 ovules per locule (Wilken and Fletcher 1988), its seed/ovule ratio is approximately 0.38. This value is equal to or below levels seen in other outcrossing species (i.e., 0.37, 0.45, 0.48) and is dramatically lower than the values of 0.92, 0.95, 0.91 measured in inbreeding species (Weins et al. 1987). Apparently, *I. sancti-spiritus* is not receiving adequate pollen at Holy Ghost Canyon to optimize seed set. Studies with the congener *I. aggregata* (Pursh) V. Grant indicated that seed set could be enhanced with pollen supplements, suggesting that the species was pollen limited (Hainsworth et al. 1985). Because the number of flowering *I. sancti-spiritus* may be less than 500 individuals in a season with clusters of <50 plants in a 30-m stretch of habitat (Maschinski, personal observation), pollinator visitation may be very low.

The results of the pollination studies suggest the narrowly distributed *I. sancti-spiritus* is apparently capable of some selfing, but is also outcrossing. In comparison, its more widespread congener, *I. arizonica*, is obligately outcrossing. It should be noted, however, that neither viability of seeds produced from selfed vs. outcrossed pollen nor survivability of offspring produced by the two methods was measured. For now it is possible to state that *Ipomopsis sancti-spiritus* apparently has a flexible pollination system, which may be an adaptation to small population size.

These findings also indicated that outcrossing success varied with pollen donor. Due to the requirement that two individuals must differ by at least one allele at the incompatibility locus (S-locus; de Nettancourt 1977) in order for a self-incompatible species to successfully set fruit, the probability of incompatibility increases in small populations (Byers and Meagher 1992, Byers 1995). Lloyd (1965) and Wyatt (1983) suggested that self-compatibility may evolve from self-incompatibility due to pressures imposed by small population size, such as small numbers of pollinators or a lack of compatible pollen. As Byers (1995) found with the rare plant *Eupatorium resinosum*, low seed set in wild populations of *I. sancti-spiritus* is most likely influenced by insufficient quantities of pollen and incompatible pollen. As population size becomes smaller, the proportion of incompatible mates increases. Further, the observation that anthers were empty of pollen suggests that the species may be experiencing inbreeding depression and subsequent reductions in gamete production.

These preliminary studies have provided a

good foundation for future studies. Among the questions that will need to be addressed are whether selfed or outcrossed progeny have equal fitness, what percentage of pollen is viable, and what the incompatibility ratio is in the population. These are essential pieces of information needed to unravel the breeding system and to determine what measures need to be taken for the conservation of the species.

### Acknowledgments

Special thanks to Meg Heim for her help with the germination studies and to Kenric Kesler for his help with the pollination studies. This work was supported by a U.S. Fish and Wildlife Service contract to The Arboretum at Flagstaff.

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# Self-Incompatibility in *Abronia macrocarpa* (Nyctaginaceae), an Endangered Texas Endemic: Comparison of Self- and Outcross Pollen Tube Growth

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**Abstract:** *Abronia macrocarpa*, large-fruited sand verbena, was listed as federally and state endangered in 1988. The plant is an herbaceous perennial characterized by large anthocarps. A study of its pollination biology by Williamson et al. (1994) revealed that *A. macrocarpa* is reliant upon hawkmoths and noctuid moths to bring about cross-pollination for successful fruit set. The study showed that flowers experimentally crossed autogamously and geitonogamously do not produce seed. Failure to set seed following self-pollination may be the result of pre-fertilization or post-fertilization barriers. This study examines the barrier to selfing present in this species. Flowers were experimentally self- and outcrossed, and then pollen germination and relative growth of pollen tubes were examined using fluorescent microscopy. Self-pollen adheres to the stigma and does germinate; however, self-pollen tubes reach lengths of only 0.07 to 0.1 mm. Self-pollen tubes do not penetrate into the style, and therefore self-fertilization is precluded.

## Introduction

*Abronia* is a western North American genus in the Nyctaginaceae or four o'clock family. Sixteen species occur in the United States (Galloway 1975). *Abronia ameliae* Lundell, *A. angustifolia* Greene, *A. carletonia* Coult. & Fish., *A. fragrans* Hook., and *A. macrocarpa* Galloway occur in Texas (Figure 1). *Abronia ameliae* and *A. macrocarpa* are Texas endemics (Correll and Johnston 1970).

*Abronia macrocarpa*, commonly called large-fruited sand verbena, is known to occur in three counties (Freestone, Leon, and Robertson) in the Post Oak Savannah Woodlands region of east-central Texas (Figure 1). When the taxon was originally described in the early 1970s (Galloway 1972), only one population was known. The type locality is a sand-dune area, approximately 9 miles northwest of Normangee, Texas. Nine additional populations have recently been identified: three more in Leon County, one in Freestone County, and five in Robertson County (Yantis and Williamson, unpublished).

The plant appears to be restricted to deep sandy soils of the Arenosa and Padina series. The Arenosa series, which formed in deep beds of sand, consists of deep gently sloping to undulating, somewhat excessively drained soils on uplands; the solum ranges from 80 to more than 100 inches in thickness (U.S. Department of Agriculture 1989). The Padina series consists of deep, gently sloping to moderately steep, well-drained soils on uplands; they formed in thick beds of

sandy material with the solum ranging from 70 to more than 80 inches in thickness (U.S. Department of Agriculture 1989).

*Abronia macrocarpa* was listed as a federally endangered species on September 28, 1988 (U.S. Fish and Wildlife Service 1988) and received listing as an endangered species by the state of Texas on December 30, 1988 (U.S. Fish and Wildlife Service 1992). Potential threats to the species include oil exploration, resort and residential development, off-road vehicle use, the agricultural industry, browsing by deer and other wildlife, and fire suppression (U.S. Fish and Wildlife Service 1992).

*Abronia macrocarpa* is characterized by thin-walled, papery anthocarps (structure in which the fruit proper is united with the perianth) that are larger than those of other species (Galloway 1972). The plant is an herbaceous perennial with a long taproot, glandular-pubescent leaves, flowers grouped into a head, and achene fruits (Figure 2). Plants form a rosette of leaves in October and overwinter in this stage. Increase in height coupled with the onset of anthesis begins in February, with peak flowering typically occurring in March. The aboveground parts of the plants die back in late April and May, with a few lasting into June. The plant survives the hot, dry summer with the shoot apex and taproot buried in the sand.

The inflorescence is a capitulum or head typically composed of 25–48 individual flowers. The average number of flowers per inflorescence is 31 (Williamson et al. 1994). Five bracts subtend the

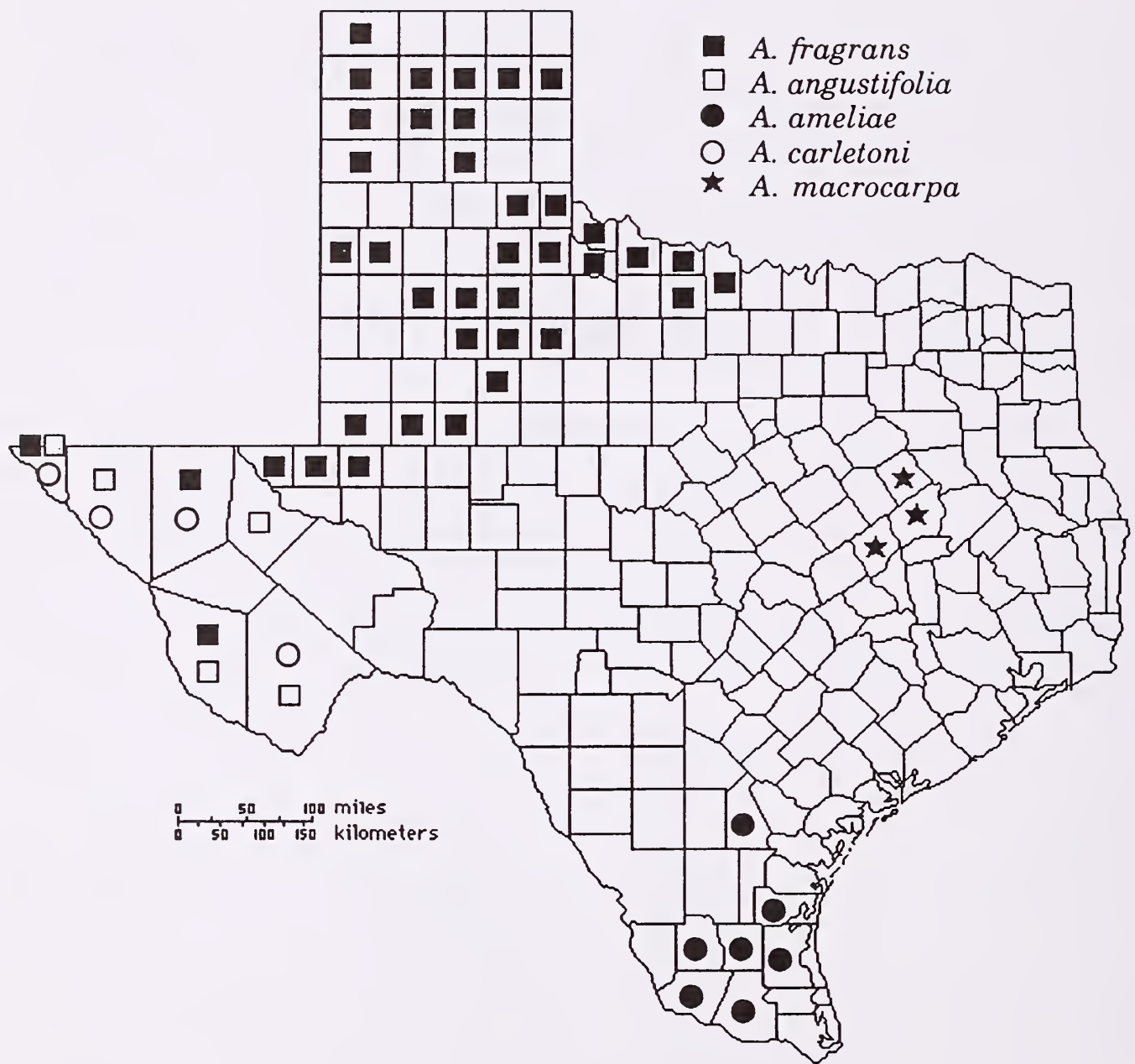


Figure 1. Map of Texas showing distribution of *Abronia ameliae*, *A. angustifolia*, *A. carletonia*, *A. fragrans*, and *A. macrocarpa*. All known populations of *A. macrocarpa* occur on private lands in Freestone, Leon, and Robertson counties.



Figure 2. Line drawing of *Abronia macrocarpa* (scale bar = 0.5 cm) showing the plant habit, inflorescences, and anthocarps (C). Note the flowers (scale bar = 1 cm) showing positions of anthers (A) and stigma (S).

inflorescence (Figure 2). The perianth consists of five pink sepals fused around the ovary and extending to form a narrow floral tube averaging 2.0 cm in length (Figure 2). The flower is apetalous. Nectar is secreted at the base of the floral tube. Sugar content of the nectar varies from 25 to 29 percent (Williamson et al. 1994). The stamens are adnate to the floral tube. Anthers are positioned approximately 1.0 cm above the stigma (Figure 2). The gynoecium consists of a single carpel with one basal ovule. The ovary is superior. The stigma is linear, 0.1–0.2 cm long (averaging 0.14 cm), covered with papillae, and wet when receptive (Williamson et al. 1994). Flowers open at 3:00–4:00 pm and close at approximately 9:00–10:00 am (Williamson et al. 1994). A sweet floral odor is present when the flowers begin to open, which increases in intensity towards dusk and remains strong during the night.

A study of its pollination biology by Williamson et al. (1994) revealed that *A. macrocarpa* is reliant upon hawkmoths and noctuid moths to bring about cross-pollination for successful fruit set. Williamson et al. (1994) also examined the breeding system by experimentally crossing plants autogamously, geitonogamously, and xenogamously, then monitoring for development of fruit. Flowers that were not manipulated served as controls. Development of achenes signified that fertilization resulted from a particular type of pollination. The study showed that the plant is self-incompatible since fruit developed only from xenogamous crosses (49% fruit set). No fruit was set in any cross involving autogamy, geitonogamy, or in the controls. Failure to set seed following self-pollination may be the result of pre-fertilization or post-fertilization barriers. This study examines the barrier to selfing present in this species.

## Materials and Methods

To ascertain that hand-pollination, performed in experimental crosses, was carried out at the peak of stigma receptivity, the timing of receptivity was tested using the peroxidase enzyme test (Zeisler 1938, Kearns and Inouye 1993). This test is based on the assumption that enzyme presence reflects stigma receptivity.

Peroxidase tests were conducted on stigmas of flowers on the first, second, and third day of blooming. Stigmas were examined hourly from the time the flowers opened (approximately 3:00–4:00 pm) until 10:00 pm and again the following morning (approximately 9:00–10:00 am). Intact pistils were excised, placed in depression microscope

slides, and immersed in a solution of 3% hydrogen peroxide. At each time interval, stigmas were observed for approximately 5 minutes using a dissecting microscope at 200x magnification. The occurrence and relative extent of bubbling action on the stigma was used as an indication of peroxidase activity and inferred stigma receptivity (Zeisler 1938, Arnold 1982, Macior 1986, Herrera 1987, Osborn et al. 1988, Kearns and Inouye 1993).

Flowers of five greenhouse grown plants and 29 bagged plants growing in the field were hand-pollinated. Field plants with developing floral buds were covered with bags constructed of a double layer of bridal veil material. The plants were enclosed in tomato cages with the bags placed over the cages and secured at the base to exclude potential pollinators. Bagging was not deemed necessary for greenhouse grown plants since potential pollinators are excluded from the greenhouses.

The following three types of experimental crosses were made:

- Autogamous cross: Plants were self-pollinated by transferring pollen from the anther to the stigma of the same flower.
- Geitonogamous cross: Plants were pollinated by transferring pollen from one flower to the stigma of another flower on the same plant.
- Xenogamous cross: Plants were cross-pollinated by transferring the pollen from one plant to the stigma of a different plant.

It was necessary to emasculate the flowers to preclude self-pollination and thereby control the type of cross. This was accomplished by removing the portion of the floral tube to which the anthers are adnate prior to anther dehiscence. Then pollen from the same or a different flower, as indicated above, was smeared onto the receptive stigma. Following pollination, flowers were fixed for use in studying pollen germination and pollen tube growth.

Flowers were fixed at 1, 2, 3, 6, 12, 24, 48, and 72 hours and at 5 and 7 days following hand-pollination in Carnoy's solution (Smith 1991, Waser and Price 1991) or in 70% ethanol (Mulcahy and Mulcahy 1982, Aizen et al. 1990). Fixed tissues were treated with 1N NaOH at room temperature for approximately one hour to soften and clear the styles (Kho and Baër 1968). A 0.01% decolorized aniline blue solution was prepared by dissolving aniline blue dye in  $K_2HPO_4$ , which after one or two hours at room temperature becomes colorless (Currier 1957). Tissues were rinsed in water and

stained for 24 to 48 hours in the decolorized aniline blue. Then tissues were mounted in a drop of the stain on a microscope slide and squashed with a cover slip. Tissues were viewed under a Zeiss epifluorescent microscope using a blue excitation, yellow transmittance filter. Pollen grains and pollen tubes with callose plugs, deposited periodically as the pollen tubes grow down the style, fluoresce a yellow color using this particular filter combination. The number of pollen grains adhering to the stigma, the number germinating to form pollen tubes, the lengths of pollen tubes, and the region of the carpel where the tubes stop growing were recorded. Photomicrographs were taken using a Minolta X700 35 mm camera with Kodak Ektachrome tungsten film (ASA 64).

## Results

Peroxidase enzyme tests revealed that stigmas are most receptive 4–5 hours after flowers open on the first day of blooming. The relative amount of bubbling action on the stigma is indicative of the level of receptivity. The extent of bubbling action was greatest from 7:00 to 9:00 pm. The amount of bubbling decreased around 10:00 pm. Very little bubbling activity was observed by 8:00 am. Flowers closed at approximately 9:00 am. The intensity of bubbling action was again high between 7:00 and 9:00 pm the second day a flower opened. Stigmas are most receptive near dusk, which corresponds to high activity of the crepuscular moths serving as pollinators.

Fluorescent microscopic examination of stigmas following experimental crosses indicates that both self- and cross-pollen readily adheres to the stigmatic surface (Table 1). The mean number of self-pollen grains present on the stigma ranges from 43 to 98. A mean number of 61 pollen grains are present on the stigma as long as 7 days after self-pollination. The mean number of cross-pollen grains present on stigmas ranges from 7 to 96. The lower mean numbers in certain xenogamous crosses may be the result of smaller sample sizes.

Both self- and cross-pollen grains germinate forming pollen tubes. As soon as one hour following hand-pollination, 63 percent of self-pollen and 49 percent of cross-pollen have produced pollen tubes (Table 1).

Initial rates of pollen tube growth are similar for self- and cross-pollen (Figure 3). However, growth of self-pollen tubes then ceases. The self-pollen tubes are capable of achieving lengths no greater than 0.07 to 0.1 mm even when given one week in which to grow (Table 1, Figure 3). Cross-

Table 1. Pollen tube growth in experimental crosses of *Abronia macrocarpa*. Mean numbers and standard deviation (SD) of pollen grains adhering to the stigma, pollen tubes penetrating the carpel tissue, and lengths (mm) of pollen tubes. The time interval at which flowers were fixed following hand-pollination is indicated with the type of cross. S = self-pollination, C = cross-pollination.

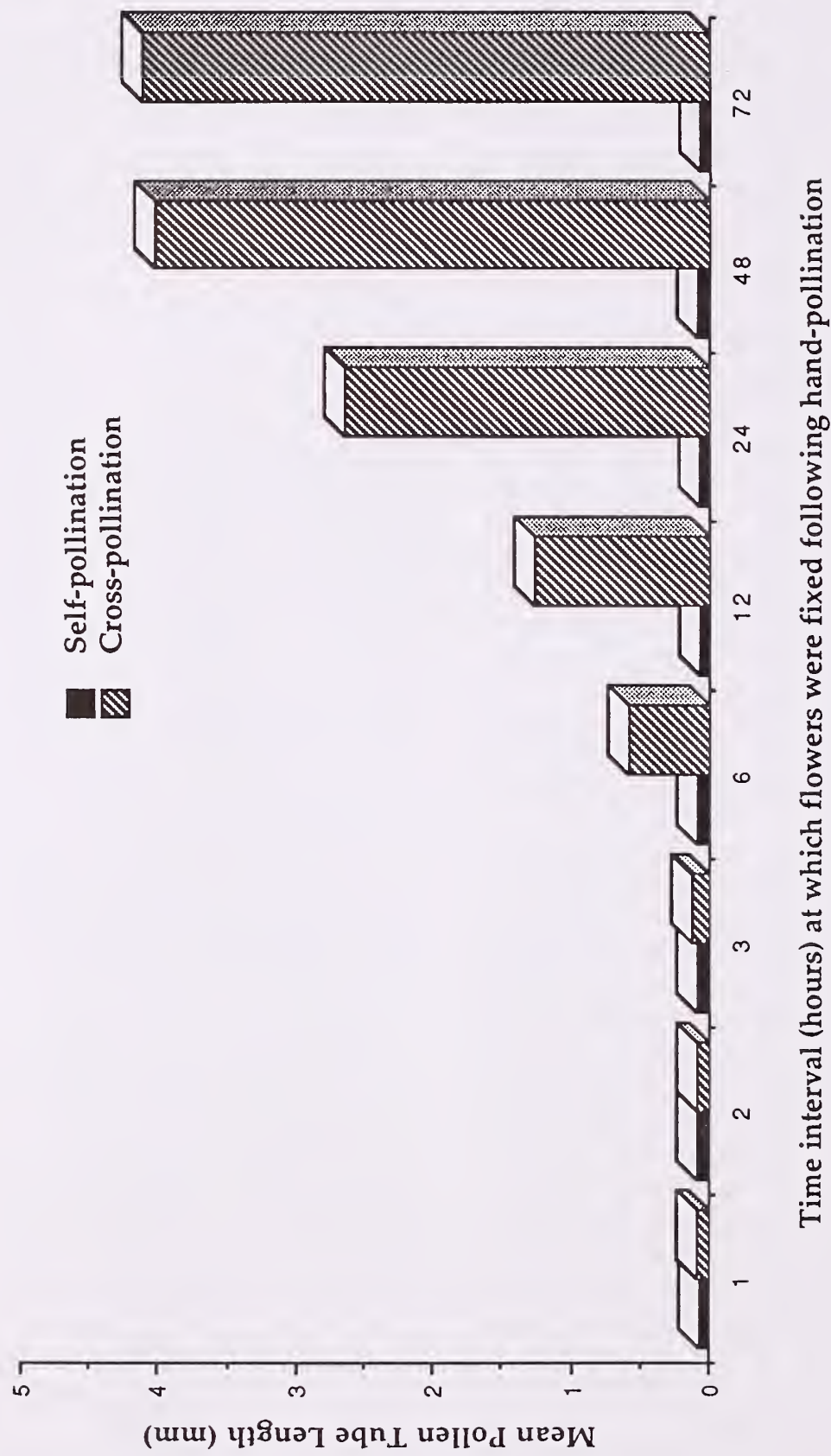
Cross	No. of Grains on Stigma	No. of Pollen Tubes	Length of Pollen Tubes
S-1 hr (N = 10)	43 (19)	27 (17)	0.07 (0.03)
S-2 hr (N = 10)	57 (37)	40 (26)	0.09 (0.02)
S-3 hr (N = 8)	62 (31)	38 (24)	0.09 (0.02)
S-6 (N = 4)	78 (31)	55 (16)	0.09 (0.02)
S-12 (N = 5)	98 (55)	45 (23)	0.08 (0.01)
S-24 (N = 10)	58 (26)	29 (17)	0.07 (0.03)
S-48 (N = 9)	61 (31)	36 (25)	0.10 (0.01)
S-72 (N = 8)	50 (31)	35 (22)	0.08 (0.02)
S-120 (N = 2)	70 (28)	35 (18)	0.08 (0.01)
S-168 (N = 6)	61 (15)	29 (12)	0.10 (0.01)
C-1 (N = 13)	39 (28)	19 (16)	0.10 (0.03)
C-2 (N = 3)	26 (1)	15 (1)	0.10 (0.02)
C-3 (N = 2)	7 (4)	6 (4)	0.13 (0.04)
C-6 (N = 2)	26 (25)	17 (16)	0.58 (0.00)
C-12 (N = 1)	96 (0)	27 (0)	1.26 (0.00)
C-24 (N = 4)	58 (2)	46 (4)	2.65 (0.62)
C-48 (N = 4)	41 (38)	34 (37)	4.03 (0.02)
C-72 (N = 1)	26 (0)	13 (0)	4.11 (0.00)

pollen tubes, on the other hand, continue to grow extending into the mid-stylar region with a mean length of 2.65 mm within 24 hours and are long enough (mean pollen tube length of 4.11 mm) to reach the ovule within 72 hours (Table 1, Figure 3).

## Discussion

Outcrossing results in greater genetic variability than does selfing. Flowering plants have developed mechanisms to promote outcrossing and prevent self-fertilization. Examples include dioecy, protogyny, protandry, and self-incompatibility systems. Post-fertilization barriers to seed set in self-pollinated flowers, such as embryonic lethality, are also known. The floral morphology of *A. macrocarpa* allows self-pollination to occur. The flowers are bisexual and pollen dehiscence occurs during the time of stigma receptivity. Since seed does not result through self-pollination, it is apparent that either a pre-fertilization or post-fertilization barrier is present in the species.

Pollen in experimental self-pollination of *A. macrocarpa* flowers does adhere to the stigma and



Time interval (hours) at which flowers were fixed following hand-pollination

Figure 3. Mean pollen tube length (mm) of self- and cross-pollen at various time intervals following hand-pollination of *Abronia macrocarpa* flowers.



does germinate, but growth of self-pollen tubes is blocked and self-fertilization does not take place. A self-incompatibility system apparently acts as a pre-fertilization barrier, preventing the production of seed following self-pollination.

Self-incompatibility may result from a variety of mechanisms. Self-pollen may not adhere to the stigma, may adhere but not germinate, or may germinate but be unable to penetrate the carpel or grow down the style (Richards 1986). Two types of self-incompatibility systems, sporophytic and gametophytic, are generally recognized. The two systems differ in site of inhibition and genetic control.

Although considered unrelated, both systems are thought to have been derived from proteins produced in plants that are involved in defense against pathogens (Dickinson 1994). These proteins, through a process of selection, also identify and reject self-pollen. Typically, self-incompatibility systems are under the control of one multiallelic locus known as the S-locus. The different S-alleles in a population are designated as S1, S2, S3, etc.

The incompatibility reaction with the female tissue is mediated by the genotype of the diploid anther that produced the pollen grain in sporophytic systems (Kearns and Inouye 1993). The system involves proteins produced by the anther tapetal cells and deposited on the developing pollen grains. Rejection usually occurs at the stigma-pollen interface. The pollen may not adhere to the stigma, may not germinate, or pollen tubes may not be able to penetrate the stigmatic tissue.

In a gametophytic incompatibility system, the incompatibility reaction with the female tissue is mediated by the genotype of the haploid pollen grain (Kearns and Inouye 1993). Two S-alleles are expressed by the stigma and style and one by the male gametophyte. If the pollen S-allele matches one of the two female S-alleles, rejection occurs. The incompatibility reaction results in inhibition of pollen tube growth within the style (Kearns and Inouye 1993).

Plants exhibiting gametophytic systems typically have flowers that produce binucleate pollen grains with long viability and wet stigmatic papillae, whereas plants with sporophytic systems (except in heteromorphic sporophytic species) are characterized by trinucleate pollen with short viability and dry stigmas covered with a cuticle (Kearns and Inouye 1993). These characters have yet to be fully analyzed in *A. macrocarpa*.

It is important to note that this paper reports only preliminary data. A more complete study of pollen tube growth is underway. Further analysis of pollen grains and the stigma will be necessary to determine the type of self-incompatibility system operating in this taxon.

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# Reintroduction of Texas Wildrice (*Zizania texana*) in Spring Lake: Some Important Environmental and Biotic Considerations

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**Abstract:** Texas wildrice (*Zizania texana* Hitchc.), an endangered macrophyte, is endemic to the San Marcos River and Spring Lake in Hays County, Texas. Its population declined dramatically between 1940 and 1967. In a reintroduction program that was initiated in 1992, plants were transplanted into five microhabitats at a density of 10 plants/m<sup>2</sup>. Seasonal monitoring identified an initial increase in plant size followed by high mortality in three of five sites. Data suggest that stem density is a good indicator of future transplant success and that current velocity is an important environmental factor associated with transplant success. However, herbivores have continually clipped reproductive culms just below the water surface. This limits transplants to clonal reproduction.

## Introduction

Texas wildrice (*Zizania texana* Hitchc.) is an emergent macrophyte in the family Poaceae. It has long ribbon-like submerged leaves and reproductive culms that arise from the base of the plant. Each culm has an emergent terminal inflorescence, allowing for wind pollination. Culms root at the nodes, producing tillers, which is a form of clonal reproduction.

*Zizania texana* is endemic to the first 2.4 km of the San Marcos River and Spring Lake, Hays County, Texas, the headwaters of which are springs arising from the Edwards Aquifer. The springs are located in Spring Lake. Historically, *Z. texana* was abundant in Spring Lake, the San Marcos River, and adjoining irrigation ditches (Watkins 1930, Silveus 1933, Devall 1940). During the first half of this century, *Z. texana*, like many macrophytes, was considered a pest species and efforts were made to control the growth of the plant (Watkins 1930, Emery 1967). Between 1933 and 1967 the population declined significantly, and after 1977 *Z. texana* occurred only in the first 2.4 km of the San Marcos River (Emery 1977).

*Zizania texana* is now listed as an endangered species by both the U.S. Fish and Wildlife Service and the Texas Parks and Wildlife Department (U.S. Fish and Wildlife Service 1985). Factors threatening the survival of *Z. texana* include reduced spring flow from the San Marcos springs due to overpumping of the Edwards Aquifer, reduced water quality in the San Marcos River from nonpoint source pollution, competition and predation by nonnative species such as nutria (*Myocaster coypus*), absence of sexual reproduction,

and alteration of sediments in the river bottom (U.S. Fish and Wildlife Service 1984). Alteration of the river sediments includes deposition of fine organic and inorganic particles due to reduction in frequency and magnitude of historic flooding cycles, and coarse gravel deposition from soil erosion in the immediate water shed.

Twice during the past 20 years *Z. texana* has been transplanted from captive populations on the Southwest Texas State (SWT) campus into the San Marcos River in attempts to increase the naturally occurring population. There were no long-term survivors in either case (W.H.P. Emery, personal communication, Fonteyn and Power 1990).

Monitoring a plant population concerns the quantitative assessment of the population over time using data derived from individual plants, which allows managers to detect important changes in health, vigor, and reproductive potential, as well as identify demographic trends such as adult survivorship and seedling recruitment. These measurements are vital in evaluating the long-term success of a reintroduction program (Palmer 1987, Pavlik and Barbour 1988). The objective of this project, which will be completed in 1996, is the reintroduction of *Z. texana* into different microhabitats in Spring Lake, and the monitoring of the status of the transplants in each microhabitat. The outcome of the study will provide managers with important information necessary for decisions with respect to San Marcos River and watershed management, as well as insights into appropriate environmental conditions for more successful transplantation into the San Marcos River.

## Methods and Materials

Spring Lake is an impoundment formed by a dam originally constructed across the San Marcos River in 1849, approximately 600 m downstream from the San Marcos Springs. As recently as 1940, *Z. texana* occupied a relatively large area of the lake (Devall 1940), but it was extirpated from the lake by 1986, due, in part, to bottom plowing of Spring Lake (Silveus 1933, Emery 1977). Spring Lake was selected as a study site because it is part of the historic habitat of *Z. texana*, there is limited public access, and a variety of microhabitats are present. Five sites were selected in the lake (Figure 1). Each site differed with respect to water depth and current velocity (Table 1). Water depth was defined as shallow (<1m), moderate (1–2 m), and deep (>2 m). Current velocity was the average of measurements taken at 20, 60, and 80 percent depth, and was defined as slow (<0.04 m/s), moderate (0.04–0.1 m/s), and fast (>0.1 m/s). Two sites were partially shaded by overhanging trees. Sediment texture was similar in all sites. The Wetland (WT) site was shallow, had no detectable current, and was in full sun; the Transition (TR) site was shallow, had slow current velocity, and was in full sun. Water depth at the Riverine (RV) site ranged from shallow to deep, current velocity ranged from slow to moderate, and the site was in partial shade. The Springs (SP) site was within 15 m of spring openings. Water depth was deep, current velocity was slow, and the site was in full sun. Water depth at the Spillway (SW) site varied from shallow to moderate, current velocity ranged from moderate to fast, and the site was in partial shade.

Plants used for reintroduction were grown from seed in 10 cm peat pots filled with mud collected from ponds on the SWT campus. Plants were grown in slow-flowing water, in an outdoor cement raceway on the SWT campus. When plants had 3–5 stems and leaves were approximately 0.75 m long, they were transported in ice chests to Spring Lake. Field assistants in scuba gear, using a 1 m<sup>2</sup> quadrat, planted transplants at a density of 10 plants/m<sup>2</sup>.

Sixty transplants were planted in WT, TR, and RV in winter 1992 as part of a habitat study (see Rose and Power 1992). One hundred and fifty plants were added to RV during fall 1992, 500 were planted in SP during fall 1993, and 160 were planted in SW during winter 1995.

Monitoring of *Z. texana* transplants began in winter 1994. Belt transects, 0.25 m x 10 m, were randomly placed parallel to the current in each

site. Initially, two transects were employed; during summer 1995, the number of transects was increased to three based on Eberhardt and Thomas (1991). The total number of plants intersecting the transect was recorded. From these data, plant density was calculated. Four plants intersecting the transect were randomly selected for measurement. The lengths of 5–6 leaves were recorded. Stem density was measured by placing a three-sided, 10 cm x 10 cm frame at the base of each plant and counting the number of stems within the frame. The distinction was made between submerged leaves and reproductive culms. For each culm in the frame, the number of nodes with roots was recorded. The presence of an inflorescence or signs of herbivory were also recorded.

## Results

Transplants at WT, TR, and RV were planted in January 1992 (Table 2). Plants at WT survived one growing season, very few plants survived at TR until 1994, and RV plants have survived to the present. A few plants survived at SP but were lost by summer 1995. The plants at SW, planted in January 1995, appear very healthy at this time.

Plant density dropped from an initial density of 10 plants per m<sup>2</sup> in all sites. In RV, plant density has fluctuated between approximately 2.5 and 3.5 plants per m<sup>2</sup> since fall 1994 (Figure 2). Plant density at SW also dropped after transplanting, but it remains to be seen the extent to which plant density will fluctuate in the future (Figure 3).

Leaves in all sites decreased in length during spring, summer, and fall 1994 (Figure 4). Plants in TR did not recover from the loss, and eventually died. Plants in RV and SP recovered during winter 1995, but again showed a loss in leaf length during spring and summer 1995. Plants in SP were lost after spring 1995.

Plants in all sites showed an increase in stem density after transplanting at an initial density of 3–5 stems (Figure 5). Plants in TR, RV, and SP showed a decrease in stem density during summer and fall 1994. Plants at TR did not recover. Plants at RV and SP recovered from the loss of stem density, but showed a second decline during spring and summer 1995. Plants remaining at SP were lost during summer, 1995.

Plants at RV and SW produced reproductive culms and tillers (Table 3). Plants at RV produced more culms and tillers than plants at SW. Over half of all reproductive culms at RV and 8.3 percent of reproductive culms at SW were clipped by

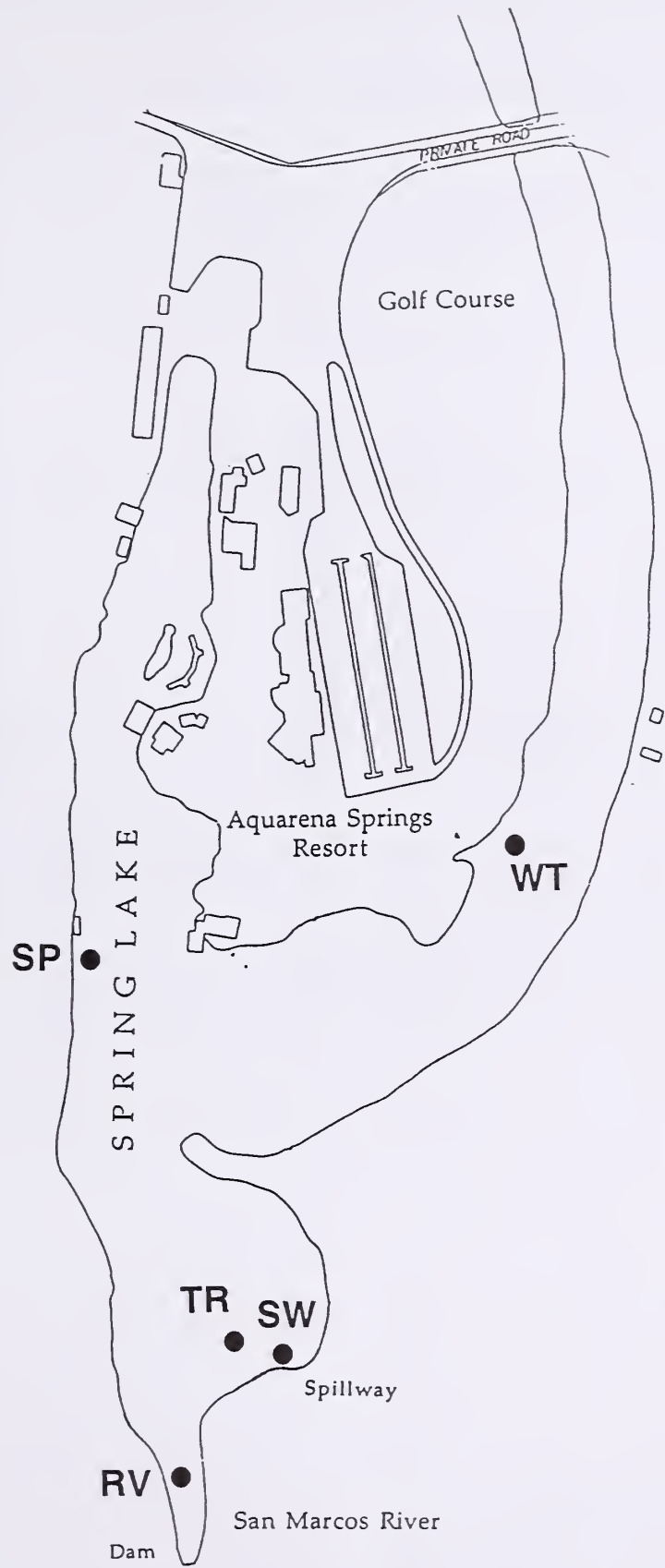


Figure 1. Spring Lake, San Marcos, Hays County, Texas. *Zizania texana* was reintroduced into Spring Lake in five sites between 1992 and 1995. Sites were Wetland (WT), Transition (TR), Riverine (RV), Springs (SP) and Spillway (SW).

Table 1. Ranges for current velocity, water depth, light, and soil characteristics found in *Zizania texana* reintroduction sites in Spring Lake. Observations and measurements were collected between 1992 and 1995; + = presence; 0 = absence.

	WT	TR	RV	SP	SW
Light	Full sun	Full sun	Partial sun	Full sun	Partial sun
Sediment	Soft mud	Soft mud	Soft mud	Soft mud	Mud/sand/gravel
Water depth (m)	0.31	0.76	0.91–2.04	2.3–4.3	0.70–1.13
Current velocity (m/s)	0	0.027	0.038–0.079	0.030–0.038	0.088–0.345
Survivorship	0	0	+	0	+

Table 2. *Zizania texana* in five reintroduction sites in Spring Lake, Hays County, Texas; + = presence, 0 = absence.

Year	WT	TR	RV	SP	SW
1992	+	+	+		
1993	0	+	+		
1994	0	0	+	+	
1995	0	0	+	0	+

Table 3. The data below represent reproductive activity by *Zizania texana* grown in two sites in Spring Lake during summer 1995. Culms represent sexual reproductive activity; tillers represent asexual reproductive activity. Data were collected from three transects in each site; SD in parentheses.

	No. Culms/ Plant	Percent Clipped Culms	No. Emergent Inflorescences	No. Tillers/ Plant
RV	3.44 (1.073)	53 (31.4)	0	3.58 (1.843)
SW	1.25 (1.146)	8.3 (14.4)	0	0.42 (0.723)

herbivores. Culms that were not clipped were all well below the water surface. There were no emergent inflorescences at either site.

### Discussion

Monitoring plants has identified some important relationships between environmental factors and health and vigor of *Z. texana* transplants. For example, plants in sites with no detectable to slow current had high mortality, while plants in sites with moderate to fast current velocity had survivors. Rose and Power (1994) examined the relationship between current velocity and culm production, and suggested that culm production may increase in flowing water either by mechanical stimulation of the meristem or physiological

enrichment. In contrast, there was no consistent relationship between survivorship and water depth, suggesting that water depth does not play as critical a role in transplant success (Table 1).

During 1994 and 1995, losses in leaf length and stem density were followed by periods of recovery in some sites (Figures 4 and 5). Plants in TR failed to recover from losses of plant material in 1994 and plants in SP failed to recover in 1995. Damage to leaves and stems was probably due, in part, to floating and drifting mats of aquatic vegetation, which is mechanically cut upstream by Aquarena Springs, a nature theme park. Despite attempts to harvest cuttings, some cuttings drift downstream, form mats that tend to accumulate in spring and summer during peak growing periods, and u en

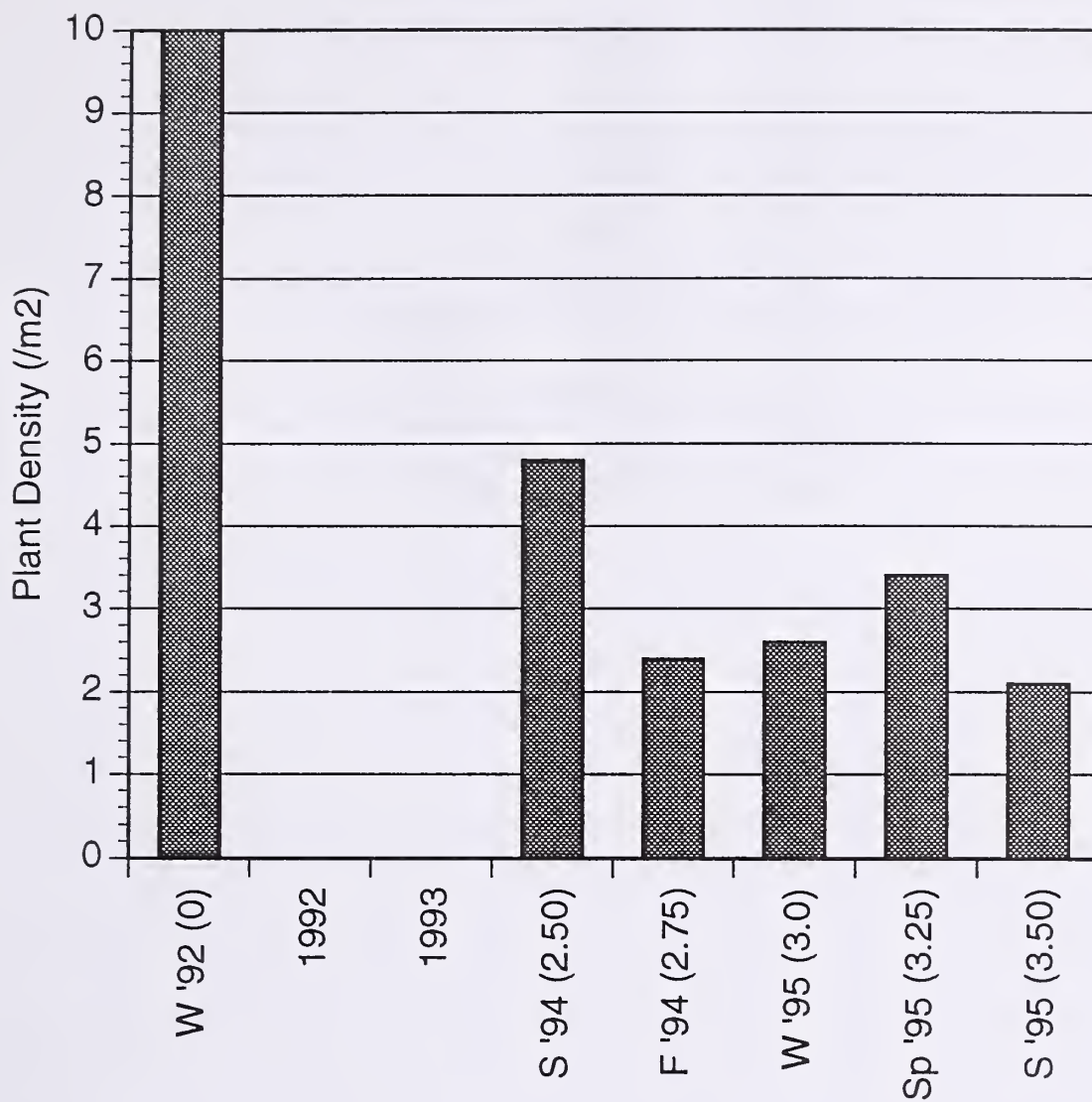


Figure 2. Plant density of *Zizania texana* transplants in Spring Lake at RV site between winter 1992 and summer 1995. No data are available for 1992 and 1993. Time since transplanting in parentheses.

break up during fall and winter. The mats, composed primarily of *Ceratophyllum demersum*, become entangled in *Z. texana* leaves, shredding them. The mats block sunlight and slow current velocity (Rose and Power 1995). *Zizania texana* plants appear to recover from the damage attributed to the mats during fall and winter.

In summer 1995, plants in SW and RV produced reproductive culms and asexual tillers (Table 3). It is unknown at this time whether the lower number of culms and tillers in SW compared with RV is attributable to the age of transplants or environmental conditions.

Reproductive failure has been noted many times as a serious threat to the survival of *Z. texana*, but there has been no documentation of the cause of reproductive failure (Emery 1967, 1977, Terrell et al. 1978, U.S. Fish and Wildlife Service 1984). In this study we identified herbivory as an

important contributor to reproductive failure. Over half the culms at the RV site were clipped and 8.3 percent of culms were clipped at the SW site. All culms close to the surface were clipped, precluding seed production. Field observations suggest that some important herbivores include crawfish, near the base of the plant; nutria, which was first introduced to Hays County after 1960 (Texas Agricultural Extension Service 1987); and resident waterfowl, including swans, mallards, and muscovy ducks, which are encouraged by Aquarena Springs employees with supplemental feed to remain throughout the year for the amusement of Aquarena Springs guests. With the loss of all inflorescences by herbivory, transplants are limited to clonal reproduction.

Will *Z. texana* persist in Spring Lake? Historically, emergent reproductive culms were a common occurrence in the San Marcos River and

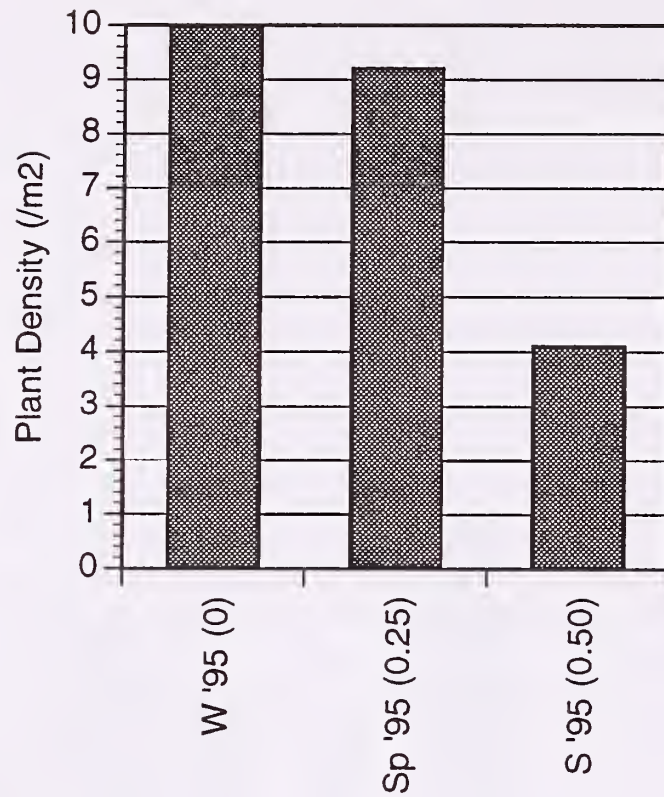


Figure 3. Plant density of *Zizania texana* transplants in Spring Lake at SW site between winter 1995 and summer 1995. Time since transplanting in parentheses.

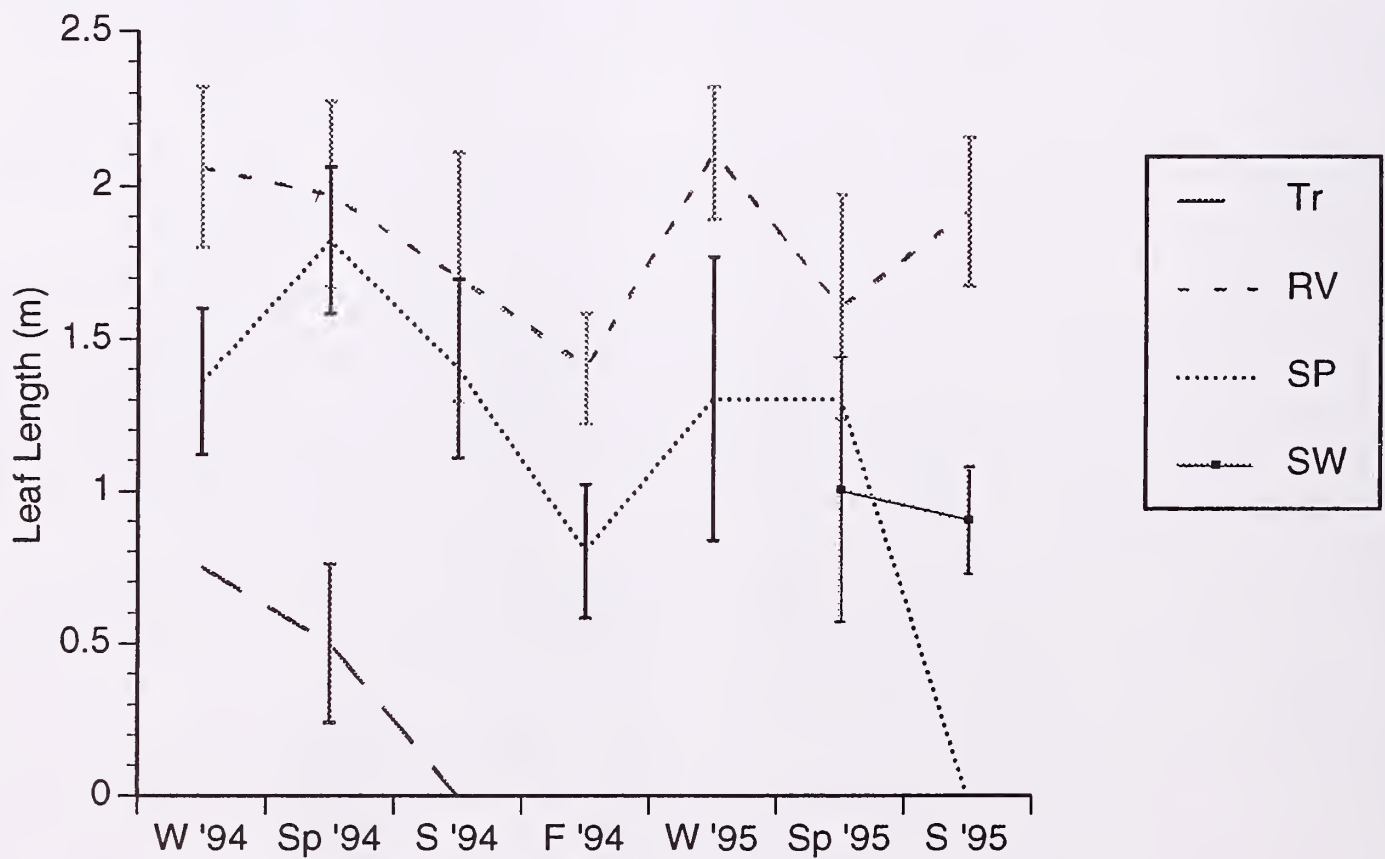


Figure 4. Leaf length of *Zizania texana* transplants grown in TR, RV, SP, and SW sites in Spring Lake.



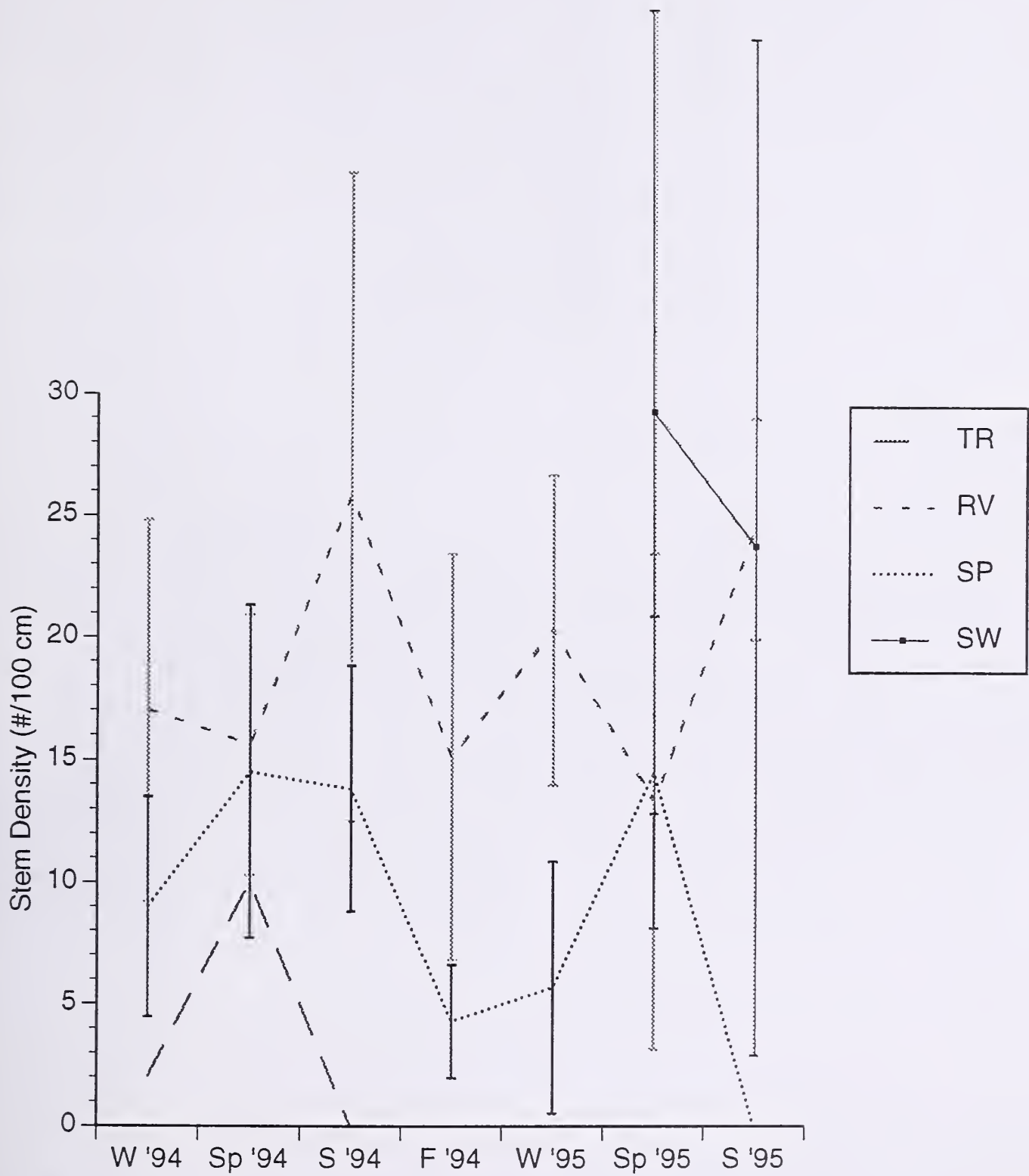


Figure 5. Stem density of *Zizania texana* transplants grown in TR, RV, SP, and SW sites in Spring Lake.

Spring Lake (Devall 1940, Silveus 1933, Watkins 1930). Today, however, reproductive culms are under an onslaught of herbivore activity, and there is no new recruitment by seed. In addition, literally tons of drifting aquatic vegetation become entangled in *Z. texana* plants, blocking sunlight, slowing current velocity, and damaging leaves. *Zizania texana*'s existence in Spring Lake is further jeopardized by reduced spring flow from the Edwards Aquifer and possibly competition for space and light from introduced macrophytes. *Zizania texana* has persisted in RV and SW despite these insults. Monitoring these sites through 1996 will provide the additional data necessary to draw conclusions about the long-term status of *Z. texana* in Spring Lake.

### Acknowledgment

Special thanks to Mr. R. Richter, Mr. R. Perry, Mr. M. Apodakis, Mr. C. Wood and Mr. D. Solanik for assistance with field work.

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# Cattle and Elk Herbivory on Arizona Willow (*Salix arizonica*)

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**Abstract:** Nearly 62 percent of Arizona willow, *Salix arizonica* Dorn, populations in Arizona have experienced reduced size and vigor due to herbivory, yet the identity of the herbivores has been unknown. Ninety Arizona willows were planted at The Arboretum at Flagstaff on the inside and outside of an enclosure surrounded by a 10-foot deer fence to determine whether cattle and/or wild ungulates ate them. The plants inside the deer fence were either protected from herbivory as controls or were exposed to cattle, while those outside were subject to herbivory by elk, *Cervus elaphus*, mule deer, *Odocoileus hemionus*, pronghorn, *Antilocapra americana*, and a variety of rodents and lagomorphs. The results indicate that both cattle and wild ungulates browse the willows. Qualitative observations suggest that elk browse the willows more intensely than either deer or antelope. An analysis of mean plant height, mean total shoot biomass, mean branch length, and total number of branches before and after the experimental time frame concluded that all parameters were lowest in the wild ungulate group, intermediate in the cattle group, and highest in the control. Duration of exposure to herbivory in the wild and cattle groups contributed to the amount of tissue lost during the course of the study.

## Introduction

Arizona willow, *Salix arizonica* Dorn, is protected by the Arizona Native Plant Law and is included on the list of Forest Service Sensitive Species for the Intermountain and Southwestern regions. (See Prendusi et al., this volume, for a complete description of the species status.) Habitat loss, degradation, and other human-related activities are believed to have changed the fragile, high-elevation riparian areas in which the plant lives as well as threaten the long-term status of the species (Arizona Willow Interagency Technical Committee [AWITC] 1995). Herbivory by both cattle and wild ungulates has been implicated as a threat to the species. Associated herd movements also contribute to degradation of the species' habitat, i.e., stream bank erosion, loss of colonization sites, increased stream sedimentation, and trampling of small plants (AWITC 1995). Nearly 62 percent of Arizona willow (*Salix arizonica*) populations in Arizona have experienced reduced size and vigor due to herbivory, yet the identity of the herbivores has been unknown.

Both wild and domestic ungulates can greatly impact riparian areas and the species that live there. In the Southwest, riparian areas form narrow corridors, where plants and animals concentrate because of the need for water (AWITC 1995). Due to the large numbers of animals using riparian areas, impacts can be severe. For example, domestic livestock inhabit 307 million acres of federal land and 212 million acres of private land

in 11 contiguous western states (Armour et al. 1994). Large concentrations of animals can adversely affect plant density and vigor. Sites where cattle grazing is common show significantly lower willow densities than ungrazed sites (Bryant and Wieland 1985).

Although factors such as dry weather, lowered water tables, and changes in hydraulic patterns are thought to cause declines in willow communities, herbivory and trampling by native ungulates also have been documented as adversely impacting willows (Singer et al. 1994). Activities associated with wild ungulates, such as trampling and rubbing antlers on the plants, can cause severe root dieback (Medina 1991). It appears that willow herbivory is common in nature, but the magnitude and timing of the herbivory are the critical factors that influence willow populations (Galeano-Popp 1988, Granfelt 1989, AWITC 1995).

Because both wild ungulates and cattle utilize riparian areas in Arizona willow habitat, the impacts of the various species are difficult to separate. Galeano-Popp (1988) stated that Arizona willow was palatable to both elk and cattle, but she did not know whether they were preferentially selected or to what degree this food source was selected; however, she believed the effects of grazing by both wild and domestic ungulates were additive. We established a controlled experiment at The Arboretum at Flagstaff to examine the separate effects of wild and domestic ungulate browsing on Arizona willow growth.

## Materials and Methods

The study was conducted during late July and early August 1995. Arizona willow plants were propagated from stem cuttings taken from parent plants growing in the White Mountains. In August 1994, after plants achieved adequate root mass, they were planted along the Sinclair Wash drainage inside and outside an existing 10-foot fence that was used to exclude the wild ungulates. Plants outside the fence were protected with caging until November 1994, in order to allow them to become established before being exposed to herbivory.

Ninety plants were established that were randomly assigned to one of three groups. Control plants were selected by placing plant numbers into a hat and having a non-biased observer extract the numbers. The three groups constituted 30 control plants that were caged or fenced from herbivory, 30 cattle plants that were exposed to cattle herbivory but not wild ungulate herbivory, and 30 wild plants that were exposed to wild ungulate herbivory but not cattle herbivory.

Populations of this willow on the Apache-Sitgreaves National Forest are exposed to wild ungulates all year and to domestic ungulates on a rotating basis for 10–30 days under current management. To imitate the natural exposure plants have to herbivory, plants in the wild ungulate group were exposed to herbivory for 10 months and plants in the cattle group were exposed for 10 days.

The area outside the 10-foot fence is visited by elk, *Cervus elaphus*, mule deer, *Odocoileus hemionus*, pronghorn, *Antilocapra americana*, and a variety of rodents and lagomorphs. These animals are seen on a regular basis in the large meadow where the experimental Arizona willows were planted. During the course of the experiment, tracks and scat found in this area were noted to attempt to identify any wild animals feeding on Arizona willow.

To expose these plants to a controlled level of cattle herbivory for a controlled duration, three cows were contained in approximately 3 acres with 30 exposed Arizona willows for ten days. The study plot was designed to mimic the environment encountered by the cows in their natural grazing areas. A 3-acre pen was constructed using barbed wire and T-posts, enclosing an area with ample amounts of shade from ponderosa pine trees, a watering trough near the willows to

provide an optimal supply of water, and ample vegetation for browse. Besides Arizona willows, other plants found in the study plot included smooth brome (*Bromus inermis* Leyss), Arizona fescue (*Festuca arizonica* Vasey), deergrass (*Muhlenbergia rigens* (Benth.) Hitchc.), ponderosa pine (*Pinus ponderosa* Lawson), *Carex* spp., and a variety of herbaceous plants. After caging the control plants within the pen, three cows were put into the pasture. The cows were all females, and one was a calf that had not been completely weaned; therefore, there were effectively two and a half cows.

Plant height, total number of branches, and total branch lengths were measured in October 1994, both before the arrival of the cows on July 21, 1995, and after 10 days of exposure to cattle herbivory. Total shoot biomass was calculated by dividing total shoot lengths per plant by 6.25, the mean shoot length of 1 gram of willow (Maschinski, unpublished data). Throughout the cattle visit, we noted the different plants that were eaten and approximate order of preference of other exposed plants. Data were statistically analyzed using a repeated measures analysis of variance.

## Results

Both cattle and wild ungulates browsed Arizona willows under the experimental conditions imposed. Plant height was equal in all groups in August 1994, but was significantly decreased in the wild group by June 1995 and in both the wild and cattle groups by July 1995 ( $F = 11.94$ ,  $p < 0.0001$ , Figure 1) after exposure to both cattle and wild ungulates.

Similarly, the total number of branches was equal across groups in August 1994, but was significantly decreased in all groups by June 1995 ( $F = 3.83$ ,  $p = 0.005$ , Figure 2). By July 1995, the cattle and wild groups had 64 percent and 57 percent fewer branches than controls, respectively (Figure 2). While control plants increased mean branch length from August 1994 to June 1995 and maintained equal branch length until July, mean branch length decreased by a significant 19 percent in the cattle group and 16 percent in the wild group by July 1995 ( $F = 5.29$ ,  $p < 0.0005$ , Figure 3).

Total shoot biomass varied across measurement time and group ( $F = 7.80$ ,  $p < 0.00001$ , Figure 4) significantly. At the beginning of the study, in August 1994, control plants had 15 percent less total shoot biomass than the cattle group, while the wild group had slightly, but not significantly less total shoot biomass than the cattle group.

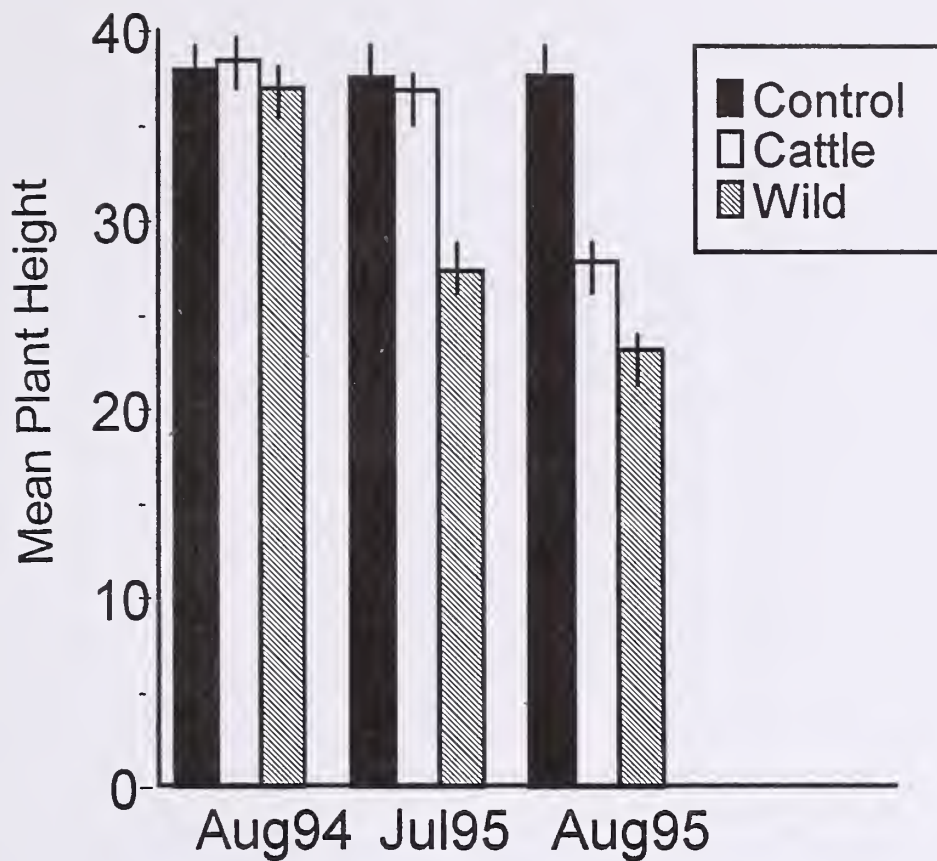


Figure 1. Mean plant height of Arizona willow plants in control, cattle, and wild groups measured at three different times. Note that cattle group plants were exposed to cattle herbivory for 10 days between July 95 and Aug 95 readings, whereas wild group plants were exposed to wild ungulate herbivory from November 1994 to August 1995.

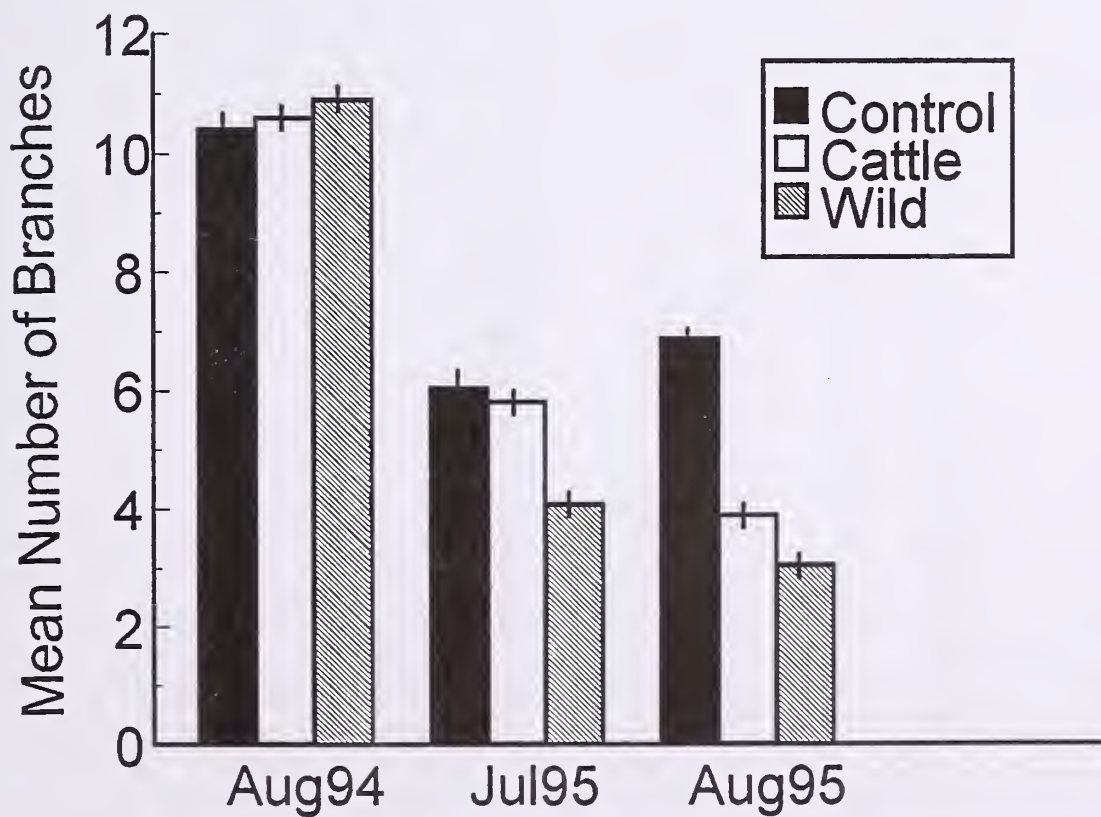


Figure 2. Mean number of branches per Arizona willow plant in control, cattle, and wild groups measured at three different times. Note that cattle group plants were exposed to cattle herbivory for 10 days between July 95 and Aug 95 readings, whereas wild group plants were exposed to wild ungulate herbivory November 1994–August 1995.

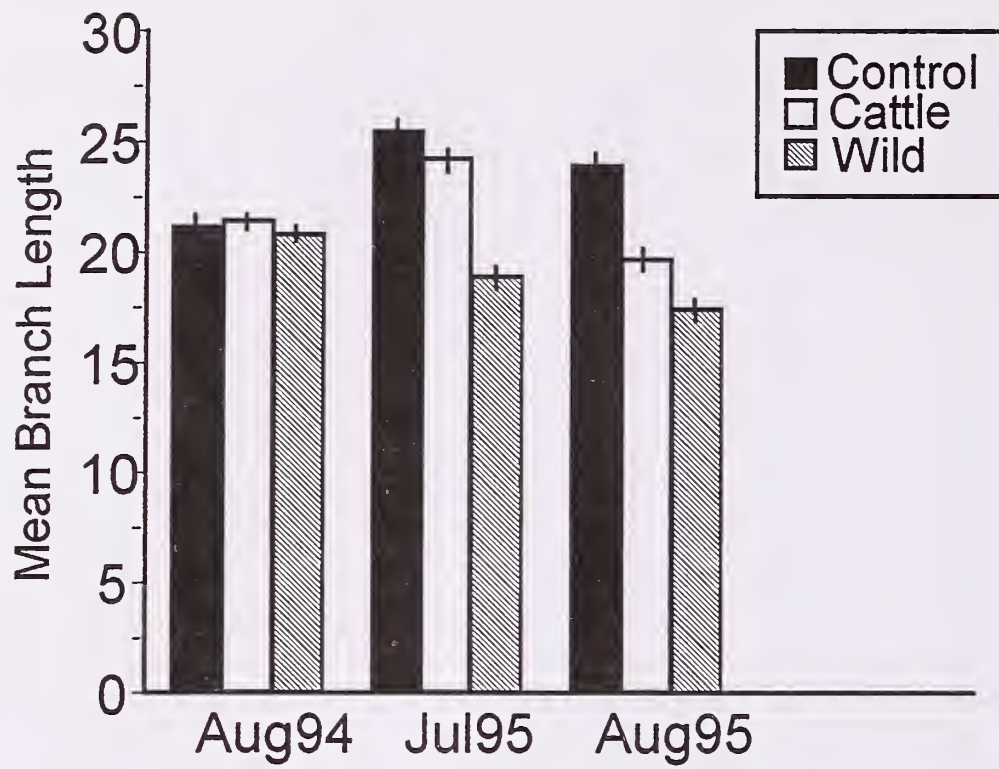


Figure 3. Mean branch length per Arizona willow plant in control, cattle, and wild groups measured at three different times. Note that cattle group plants were exposed to cattle herbivory for 10 days between July 95 and Aug 95 readings, whereas wild group plants were exposed to wild ungulate herbivory from November 1994 to August 1995.

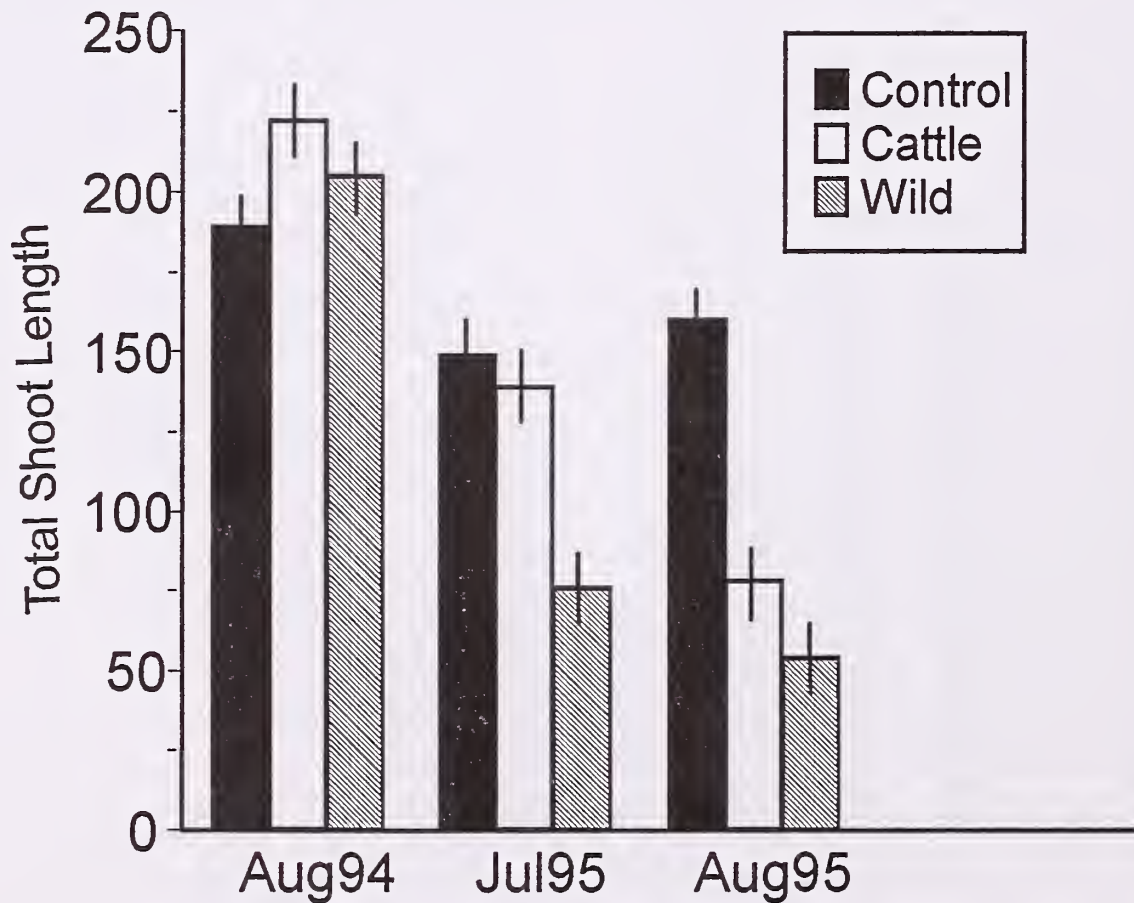


Figure 4. Total shoot biomass per Arizona willow plant in control, cattle, and wild groups measured at three different times. Note that cattle group plants were exposed to cattle herbivory for 10 days between July 95 and Aug 95 readings, whereas wild group plants were exposed to wild ungulate herbivory from November 1994 to August 1995.

However, following winter freezing, all groups experienced a decline in total shoot biomass. In the period between August 1994 and June 1995, the control group lost 22 percent of total shoot biomass, the cattle group lost 37 percent of total shoot biomass, and the wild group lost 63 percent of total shoot biomass. In the period between June 1995 and July 1995, when cattle group plants were exposed to cattle herbivory, total shoot biomass decreased by 43 percent and 29 percent in the cattle and wild groups, respectively, while controls increased by 8 percent in this 10-day period (Figure 4).

Note that the final plant measurements were generally less in the wild group compared to the cattle group and that this is probably due to the amount of time plants were exposed to herbivores: willows in the wild group were exposed to 10 months of herbivory, whereas willows in the cattle group were exposed to 10 days of herbivory.

## Discussion

This study showed that both wild ungulates and cattle browsed the Arizona willow plants. The effects of the herbivory were severe; height, the total number of branches, average branch length, and total shoot biomass all significantly declined when plants were exposed to herbivory.

Several observations indicated that cattle did not preferentially browse Arizona willows in this experimental setting. First, the cattle ate the willows only after all other desirable vegetation had been eaten. Desirable vegetation included: sweet clover (*Melilotus officinalis* (L.) Lam.), deergrass, and Arizona fescue. It wasn't until the sixth day of the experiment that the willows were tried. Secondly, the cows actually would pick grasses out from between the willows or between branches before they would eat the willows. This indicates that Arizona willows in this setting are not the preferred food, but would be selected after the other foods were eaten. Further, these studies indicate that in pastures where cattle remain more than 6 days, willow use will increase.

Observations by the first author suggest that elk are the primary wild herbivores feeding on Arizona willow in this experimental setting. There were elk hoof tracks and feces near the Arizona willows. Personal observations of elk were noted in this area nearly every morning. In contrast, the same evidence of deer or antelope was not found.

This experiment was conducted in an area in which *Salix* spp. are an uncommon food source to both elk and cattle. This may be construed as an artificial environment, but the study was conducted to determine whether cattle and/or elk eat willows. We conclude that cattle do indeed eat willows, but not as a preferred food source. Elk seem to be eating the willows, but we have not captured concrete evidence of the herbivore in action. Evidence of this nature needs to be gained by photography and/or video.

The exposure of Arizona willow to elk and cattle in its native habitats most certainly reduces plant vigor and probably reproductive capacity as well. Here we have documented reduction in four parameters related to plant vigor. In the next 2 years of this study we will be able to document the impact of herbivory in one year on reproduction in subsequent years and we will be able to determine if Arizona willow can compensate for tissue lost to herbivores. Our studies support the management efforts that have been initiated since the signing of the Arizona Willow Conservation Agreement (AWITC 1995), which reduce and/or eliminate the exposure of Arizona willow populations to elk and cattle on the Apache-Sitgreaves National Forest in Arizona and the Fishlake and Dixie National Forests in Utah.

The broader implications of these studies go beyond the "single species" perspective. Negative impacts on one species in fragile riparian zones could inevitably have lasting impacts throughout the ecosystem and through numerous and distant taxa (Armour et al. 1994). As an example, Taylor (1986) showed that there were greater numbers of passerine birds using an undisturbed willow riparian area in comparison to an extensively grazed area. Such ecosystem-level ramifications are possible, but as yet undocumented in Arizona willow habitat. By observing and recording data on the Arizona willow, correlative impacts on a variety of organisms within the ecosystem will be possible in the future.

## Acknowledgments

We would like to thank George Fischer for loaning us the cattle used in this study. Kenric Kesler and Jeff Hines assisted with measurements, and with plant and cattle care. This study was supported by the Arizona Department of Agriculture through Section 6 funding.

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# The Effects of Prescribed Burning on the Rare Plant *Chrysothamnus molestus*

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**Abstract:** We examined the effects of fall and spring prescribed burning on the rare plant, *Chrysothamnus molestus* (Blake) L.C. Anders. Plant growth and flower production increased after fall burning, compared to control plants, indicating that *C. molestus* is tolerant of fall burning. There was a differential effect of fall burning on mature plants and seedlings. We did not observe any mortality in mature plants, but seedling establishment was reduced 12-fold compared to control plots that were not burned. Considering that seedling establishment in *C. molestus* is probably rare, burning during a year of sexual recruitment would likely have long-term negative effects on population dynamics. We also found strong seasonal differences caused by burning. The spring burn, which occurred during the growing season, led to 25 percent mortality of adult plants, a three-fold reduction in volume of surviving plants, with little or no compensation in growth or flower production. We conclude that *C. molestus* is not a fire-dependent species, but is tolerant of prescribed burns implemented after the growing season. We propose that prescribed burns should never be implemented during the growing season or at least in the early part of the growing season. Fall prescribed burns can be used as a management tool without negatively impacting *C. molestus*, but should not be implemented in a year when seedlings are beginning to establish.

## Introduction

Only recently has prescribed burning been used by government agencies as a management tool to enhance ecosystem productivity and promote reproduction and growth of native plant species. Although there is a wealth of information on plant responses to fire for particular habitats, such as the North American chaparral, Australian mallee, and South African fynbos (Crutzen and Goldammer 1993, Zedler 1995), much less is known about other ecosystems that are not as fire adapted. Furthermore, little work has been conducted on how rare species respond to natural or prescribed fires. At least in certain habitats, some rare plant species are typically found in disturbed areas (Jacobson et al. 1991), suggesting that they may require some type of disturbance such as fire to survive in these communities. Prescribed fires have been conducted in order to understand to what degree the abundance of rare plants has been affected by human resource policies such as fire suppression (Thomas and Goodson 1992). Schemske et al. (1993) note that 4 out of 98 plant species for which recovery plans have been developed are endangered primarily due to the suppression of natural fires. There is also some evidence to suggest that the rarity of *Polemonium pauciflorum* S. Wats ssp. *hinckleyi* (Standl.) Wherry, *Gentianella wislizeni* (Englm.) J. Gillett, and *Pediocactus paradigminei* B.W. Benson in Arizona is due to fire suppression (Peter Warren, personal communication).

Regardless of whether any rare plant may depend on some type of fire regime for continued existence, it is important to understand how rare plants respond to prescribed burning as well as fulfilling other management needs such as increasing productivity or reducing unwanted species.

*Chrysothamnus molestus* (Blake) L.C. Anders. is a perennial prostrate shrub or sub-shrub that is found only on the Coconino Plateau in northern Arizona. It grows on limestone-derived soils where there is a thin layer of topsoil (Bill Hevron, personal communication), ranging in elevation between 1800 and 2100 m. This species is classified as a category 2 candidate for listing by the U.S. Fish and Wildlife Service due to its limited geographical range, patchy distribution, and because it is heavily used or impacted by cattle and elk. Previous studies have reported little or no recruitment in populations of *C. molestus*, and very little was known about its population biology. It was unknown what effect prescribed burning would have on the population dynamics of *C. molestus*. Savage and Swetnam (1990) have shown for ponderosa pine forests that livestock grazing has played a significant role in the reduction of natural fires. *Chrysothamnus molestus* typically grows in open pinyon-juniper grasslands where periodic natural fires would be expected to occur every 15–30 years (Kilgore 1981).

Due to the lack of recruitment observed in

natural populations of *C. molestus*, we proposed that some type of natural disturbance such as fire may promote seedling establishment and possibly increase plant performance. Therefore, the objectives of this study were to examine the effects of prescribed fall and spring burning on the plant performance and population dynamics of *C. molestus*. This study includes the effects of two burning treatments on a *C. molestus* population that has been protected from grazing for 40 years. Initially the study included a cattle-grazed area in one of the two burning treatments (fall burn). Since plant responses to fall burning were comparable between cattle-grazed and cattle-free areas, we only report the results of the burning in the cattle-free area. Specific effects of grazing on *C. molestus* will be dealt with in a later paper (Cobb et al. unpublished).

## Methods

### *Plot Description*

The study site is located in the Kaibab National Forest (T24N, R4E, northwest area of section 11) at an elevation of 2095 m. Annual precipitation in the area is 40 cm and annual mean temperature is 9.4°C (Lavin and Johnson 1977). *Chrysothamnus molestus* is a dominant plant species at the study site, along with various grasses and *C. nauseosus* (Pallas) Britt. The study site, 8.1 hectares, has been fenced and protected from cattle grazing for 40 years (Barbara Phillips, personal communication). Grazing by native vertebrate herbivores has occurred in the plot, although we never observed any indication of vertebrate herbivory (i.e., chewed leaves or stems) on *C. molestus*.

### *Spring Burn*

To examine the effect of prescribed spring burning on *C. molestus* survival and performance, one-third of the plot was burned on April 30, 1992 (spring burn). For both burn treatments fires were set with drip torches. Only 53 percent of the plants in the burn treatment actually burned, and of those plants that burned 25 percent died. For the treatment group, we only used plants that had burned and survived ( $n = 42$ ). Control plants were selected to create a comparable number of plants that were similar in plant characteristics to the treatment group before the burn.

We performed t-tests on plant characteristics to test for pre-treatment differences in the year before the spring burn, between burned (treatment) and unburned (control) plants. We performed a repeated measures ANOVA to test for

treatment effects in the 2 years following the spring burn. This analysis tests for two main effects: differences between groups (TYPE) among years and differences within groups over time (TIME), and the interaction effect of differences between groups over time (TYPE  $\times$  TIME). The latter addressed the question of whether one group changes over time in a significantly different way than the other group.

### *Fall Burn*

To examine the effect of prescribed fall burning on *C. molestus* survival and performance, a third of the plot was burned on November 18, 1992 (fall burn). Fuel loads were much greater in this burn treatment than in the spring prescribed burn, and the entire section burned uniformly. The control group ( $n = 113$ ) used to compare with the burned plants ( $n = 96$ ) consisted of all unburned plants in the plot, including those in the spring burn plot that were not burned. We did not have to select specific plants as controls in this comparison, as we did in the spring-burn analysis, since there were no significant differences between the treatment group and all of the other plants in the plot that did not burn. Fifty-five of these control plants were also used as control plants in the spring-burn analysis.

To test for effects of the fall burn, we initially performed a repeated measures ANOVA for the 2 years before the burn, to ensure that treatment and control groups were comparable. We then performed a t-test on the same variables measured after the fall burn to test for effects of the burn treatment.

### *Plant Measurements*

Individual plants were randomly selected from all plants occurring in 5 m<sup>2</sup> subplots established throughout the study site. These plants were tagged and monitored from 1991 to 1993. Plants were measured in early September, during the flowering period after annual growth appeared to be complete. For each plant we collected the following measurements: (1) plant length in cm, (2) plant width in cm, (3) plant height in cm, (4) growth rate—length of 5–11 current-year shoots in mm, (5) number of current-year shoots per quarter plant, and (6) number of flower heads per plant. We estimated plant canopy volume (decimeters<sup>3</sup>) using the height, length, and width measurements.

Seedling recruitment only occurred in 1993; we did not observe any seedlings in 1991 or 1992. Seedling production was estimated by counting all

the seedlings in randomly selected 5 m<sup>2</sup> subplots. Twelve subplots were examined in each of the control, spring burn, and fall burn sections.

### Results: Effect of the Spring Burn

The spring burn on April 30, 1992 only burned 53 percent of the tagged plants in the plot, due to inadequate fuel loads. The plot had 40 years to accumulate fuel in the absence of cattle. The burn occurred during the middle of an extended 3-year El Niño event, when annual precipitation was above normal, which should have resulted in relatively high biomass accumulation. It is possible that because of the El Niño event, plant moisture was high, leading to reduced fire intensity. However, where there appeared to be adequate fuels, vegetation including shrubs were burned. This indicates that even if spring prescribed fires promoted plant performance, spring prescribed burning would not be a feasible management practice in habitats where *C. molestus* occurs.

There were no significant differences in plant performance traits between the spring burned and unburned (control) plants for the year preceding the burn (pre-burn). We therefore presumed any differences in the years following the spring burn (post-burn) resulted directly from the burn treatment. Since seedlings did not emerge anywhere on the study site during the year preceding the burn, we did not have a pre-burn comparison.

#### *Plant Growth and Size*

Shoot growth was 35 percent greater in burned plants over the 2-year period following the burn (Figure 1A). However, this difference was due to a 45 percent increased shoot growth in 1992 in burned plants compared to controls. Shoot growth in burned plants was only slightly greater than control plants in 1993. Growth in both plant groups decreased dramatically from 1992 to 1993, although burned plants showed a relatively greater decrease in growth compared to control plants, indicated by the significant interaction term in the repeated measures analysis.

Shoot production for burned plants that survived the fire was significantly lower than control plants (40%) in the year following the fire (Figure 1B). Burned plants did recover to a certain degree 2 years following the fire, but their shoot production was still only 56 percent of shoot production in control plants. For both groups, shoot production was significantly greater in 1993 compared to 1992. The relative decrease in overall shoot production after the spring burn was greater than

the relative increase in shoot growth, leading to overall reduced (66%) annual biomass production in burned plants compared to control plants. This reduction of biomass production is reflected in plant volume, which was drastically reduced by three-fold in the year following the fire (Figure 2A), and was still only half that of control plants 2 years after the burn.

#### *Flower Production*

Flower production per plant was reduced three-fold as a result of the spring burn (Figure 2B) in the year following the burn. The difference in flower production per plant was less noticeable in the second year following the fire but was still only half that seen in the control plants. The reduced flower production of burned plants was probably due to a reduction in plant size in burned plants. We found no difference between the groups when we examined flower production on a per plant volume basis.

#### *Plant Survivorship and Seedling Density*

The most dramatic effect of the spring burn was that 25 percent of the marked plants died, whereas there was no mortality in the control plots. Since we did not observe any seedlings until 1993, we assume that seedling establishment occurred after the 1992 census in September. Therefore we don't know what the direct effect of the spring burn would have been on seedlings. Seedling density in 1993 in the burned plots was comparable to unburned subplots (Figure 3). The lack of seedlings in 1992 indicates that spring burning does not promote the establishment of seedlings and the results of 1993 indicate that spring burning has no effect on seedling establishment in ensuing years.

### Results: Effect of the Fall Burn

The fall burn differed from the spring burn in several respects: there was adequate fuel to include the grazed area, the fire burned evenly throughout the designated area, there was no adult plant mortality, and plant growth and reproductive effort were stimulated.

As was the case for the spring burn, there were no significant differences in any plant performance attribute between the fall burned and unburned (control) plants for the 2 years preceding the burn (pre-burn). We therefore presumed any differences in the year following the fall burn (post-burn) resulted directly from the burn treatment. Since seedlings did not emerge anywhere on

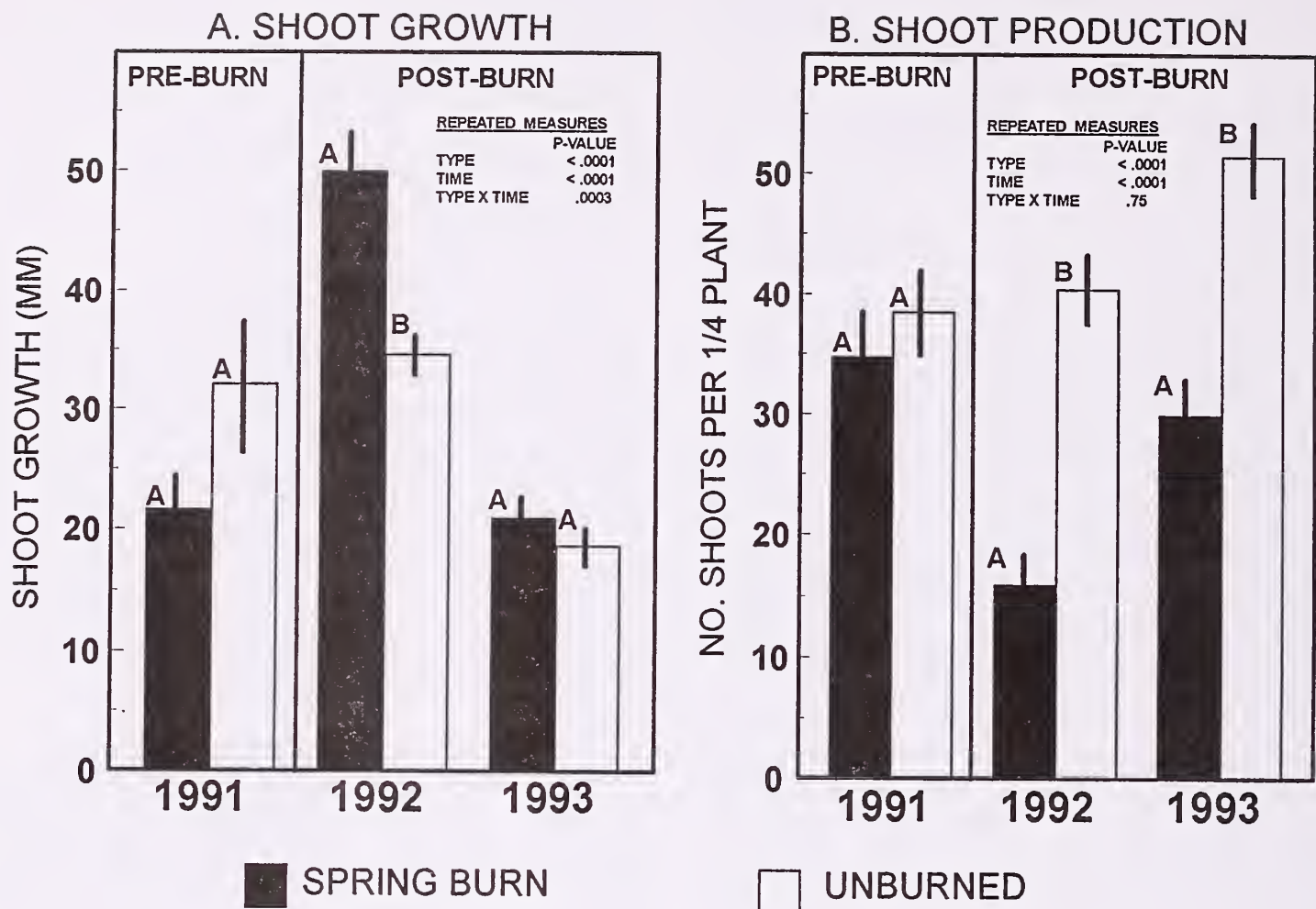


Figure 1. (A) Mean current-year shoot growth ( $\pm 1$  SE) and (B) shoot production for burned and unburned (control) plants in the spring burn experiment. P-values from a repeated measures ANOVA are presented in the upper right-hand corner, denoting significance of the main effects of plant type and time and the interaction effect. Letters above the bars denote significant differences between groups at  $P = .05$ , based on t-test.

the study site for the 2 years preceding the burn, we did not have a pre-burn comparison.

#### *Plant Growth and Size*

Shoot growth in burned plants increased over two-fold in comparison to control plants (Fig 4A). Shoot production was reduced 50 percent in the burned plants compared to controls (Figure 4B), but this was offset by the increase in shoot growth (4A). If one estimates biomass production on a per plant volume basis by multiplying number of shoots and shoot growth, annual biomass production would be 48 percent higher in the burned plants. Despite this estimated increase in annual biomass per volume of plant canopy, burned plants exhibited a 25 percent decrease in plant volume compared to control plants (Figure 5A).

The fact that plant volume in burned plants was reduced but estimated biomass production was not, indicates that these burned plants were producing a greater amount of biomass per volume of plant than control plants.

#### *Flower Production*

Reproductive effort, as measured by flower production, was stimulated by the fall burn. Flower production per plant was 69 percent higher in burned plants compared to control plants (Figure 5B) after the fall burn.

#### *Plant Survivorship and Seedling Density*

We did not observe any mortality of any adult plants in either the burn or control areas. However, seedling production was nearly 15-fold less

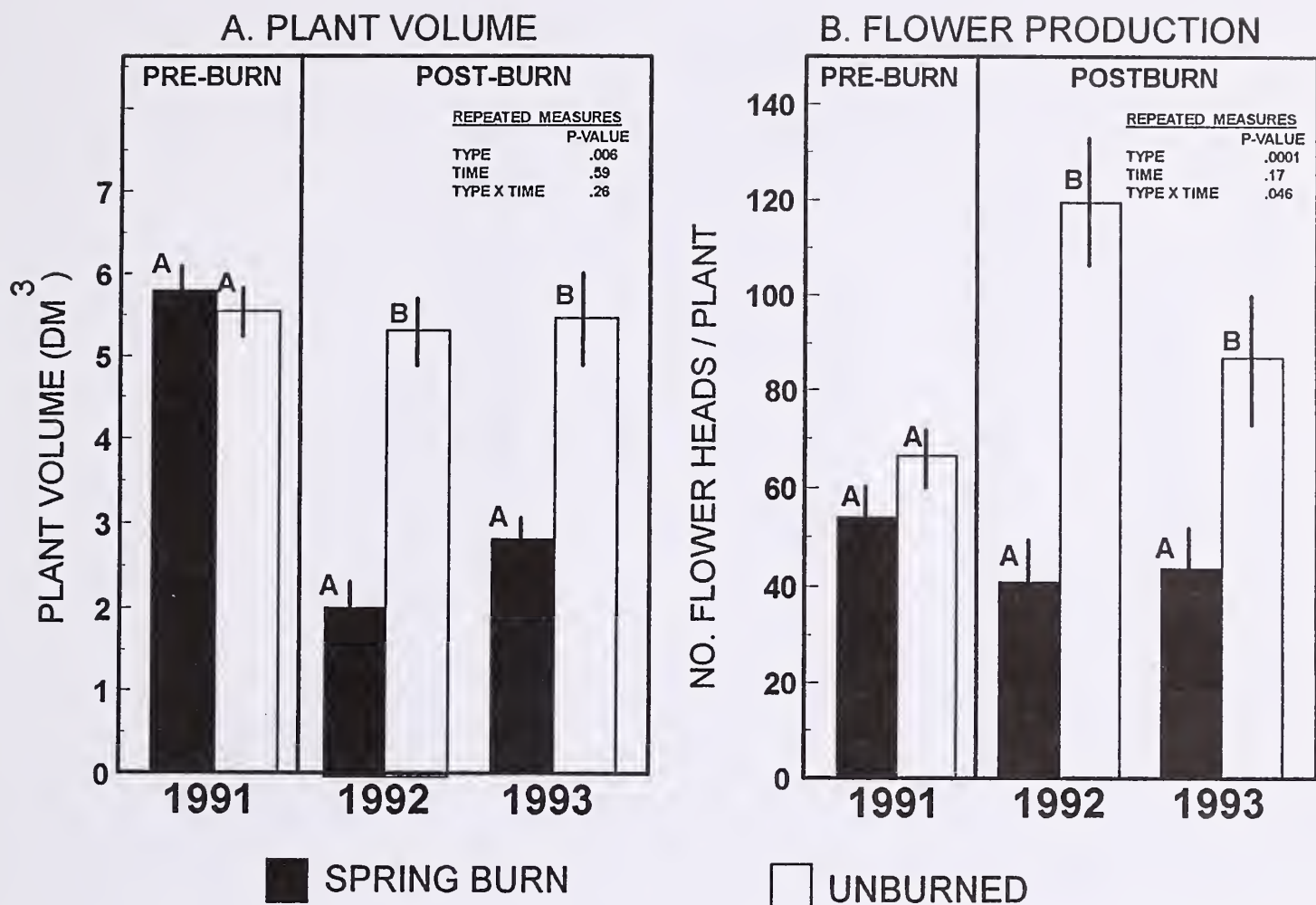


Figure 2. (A) Mean plant volume ( $\pm 1$  SE) and (B) number of flower heads for burned and unburned (control) plants in the spring burn experiment. P-values from a repeated measures ANOVA are presented in the upper right-hand corner, denoting significance of the main effects of plant type and time and the interaction effect. Letters above the bars denote significant differences between groups at  $P = .05$ , based on t-test.

in the burn treatments for the burn plots (Figure 3). This indicates that fire differentially affects distinct life stages of *C. molestus*, stimulating growth and reproductive effort in reproductively mature plants but killing new seedlings.

### Discussion

Two major conclusions from this study are that *Chrysothamnus molestus* responds differentially to prescribed burning depending on the time of year of the burn—the overall effect of the spring burn on plant performance was negative, but the effect of the fall burn was not (Table 1); and that *C. molestus* may be a fire-tolerant species as indicated by the compensatory growth and reproductive effort seen in fall burned plants, but it clearly is

not a fire-dependent species as evidenced by reduced seedling establishment (Table 1).

#### Mature Plant Mortality

The spring prescribed burn caused significant plant mortality (25%) and reduced overall plant performance in surviving plants. This strongly indicates that *C. molestus* is not adapted to fire exposure during the early part of the growing season. Plant mortality in shrublands is positively correlated with reduced frequency and predictability of fires in shrubland habitats (Christensen 1985). Most likely, naturally ignited fires in the area occurred predominantly during the monsoon period (July–September), and one would expect that *C. molestus* would not be adapted to tolerate a spring prescribed burn. Other perennial compos-

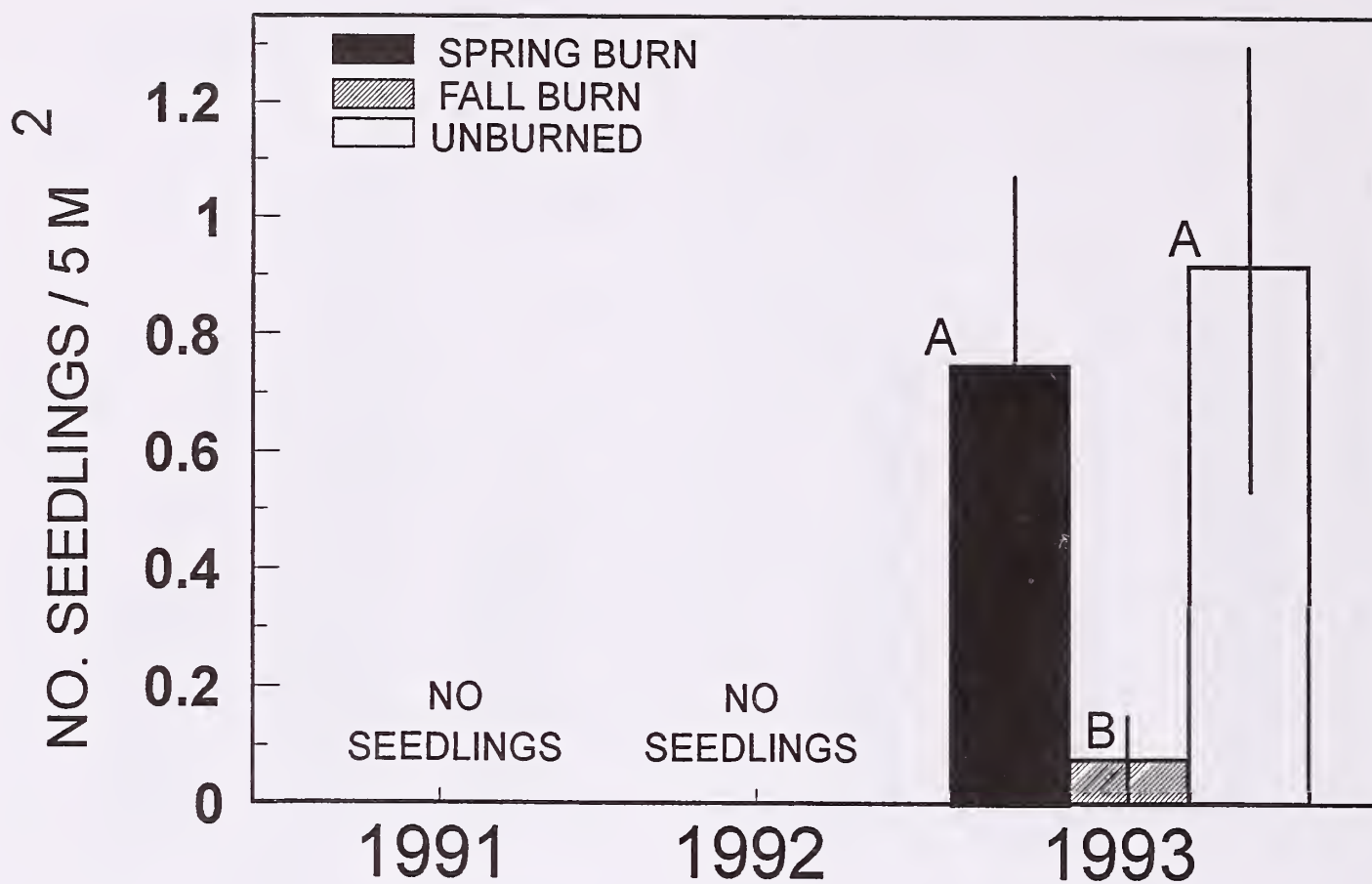


Figure 3. Mean seedling production ( $\pm 1$  SE) for burned and unburned plots in the fall and spring prescribed burns in 1993. Seedling production was not observed in the other 2 years of the study. Letters above the bars denote significant differences between treatment (burned) and control (unburned) groups at  $P = .05$ , based on t-tests.

ites also suffer high mortality when burns occur in the growing season. Humphrey and Everson (1951) found that snakeweed (*Gutierrezia sarothrae* (Pursh) Britt. & Rusby) and burroweed (*Isocoma tenuisectus* Greene) experienced 95 percent mortality or greater after a July control burn and White (1969) found continued high mortality in larchleaf goldenweed (*Ericameria laricifolia* (Gray) Shinn.) 2 years after a wildfire.

We did not observe any plant mortality as a result of the fall burn, suggesting that *C. molestus* has evolved tolerance to fires that occur after plants senesce. Although the probability of a lightning fire in November is low, the fall prescribed burn is close enough to the peak natural fire season that *C. molestus* would have experienced in the past. Brewer and Platt (1994) concluded that most positive responses of plants to fire occurred in the season when natural fires would normally occur.

#### Plant Growth and Reproductive Effort

The increase in shoot growth in burned plants compared to controls during the first post-burn year was more than negated by the decrease in shoot production for both post-burn years, which led to overall reduced biomass production in burned plants. Although shoot growth in spring burned plants was overall 35 percent greater than control plants, shoot production was overall only half that observed in control plants. Shoot production did increase significantly 2 years following the spring burn, but it was still only 56 percent as great as in control plants, whereas shoot growth was comparable.

Unlike the spring burn, the fall burn stimulated overall biomass production and reproductive effort. Shoot production in the fall burn was 33 percent less in burned plants compared to control plants, but there was over a two-fold increase in

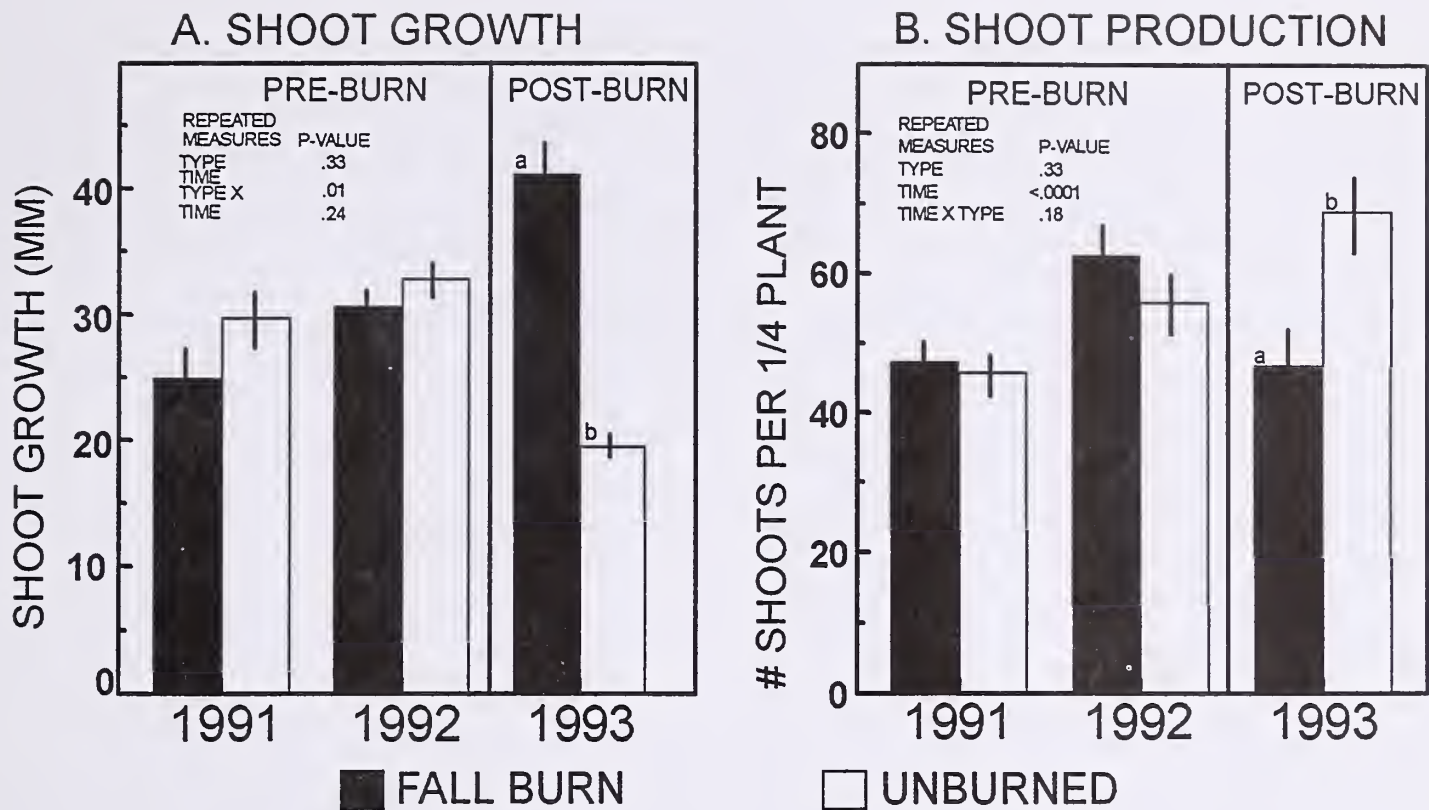


Figure 4. (A) Mean current-year shoot growth ( $\pm 1$  SE) and (B) shoot production for burned and unburned (control) plants in the fall burn experiment. P-values from a repeated measures ANOVA are presented in the upper left-hand corner, denoting significance of the main effects of plant type and time and the interaction effect before the burn (pre-burn). Letters above the bars denote significant differences between groups at  $P = .05$ , based on t-test to determine the effect of the burn (post-burn).

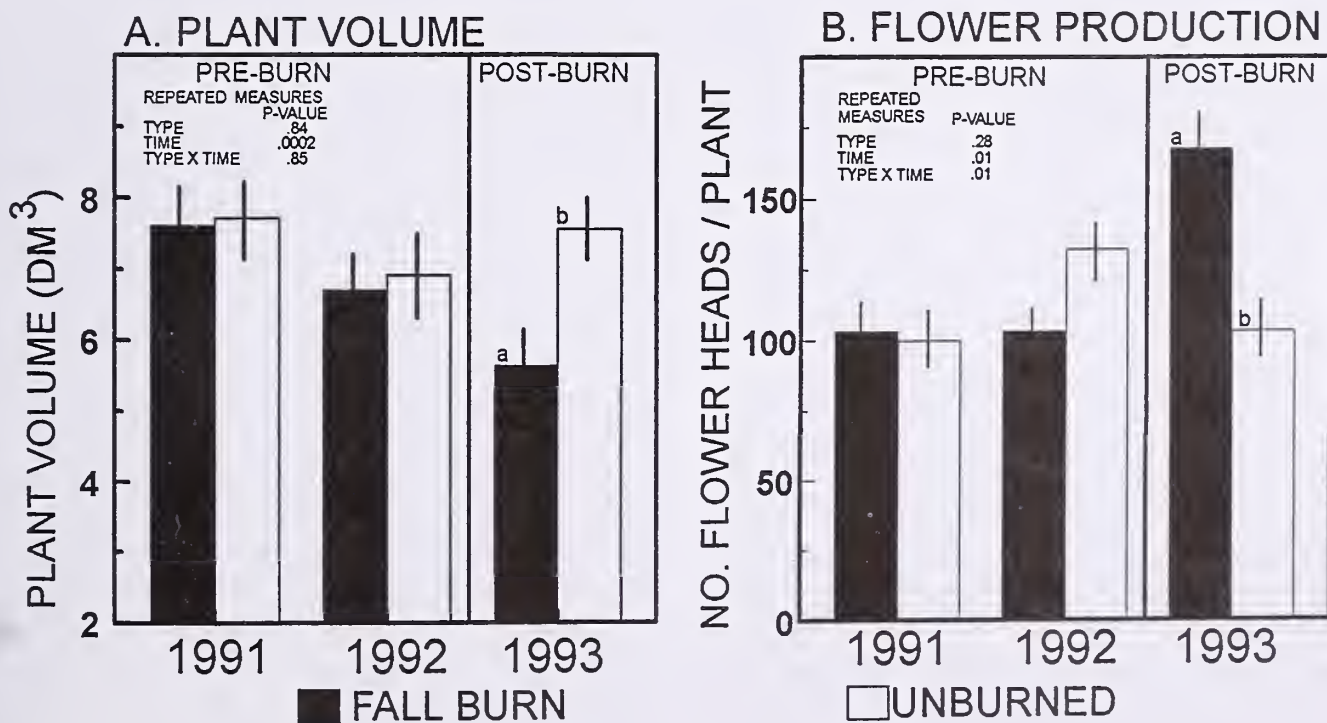


Figure 5. (A) Mean plant volume ( $\pm 1$  SE) and (B) flower production for burned and unburned (control) plants in the fall burn experiment. P-values from a repeated measures ANOVA are presented in the upper left-hand corner, denoting significance of the main effects of plant type and time and the interaction effect before the burn (pre-burn). Letters above the bars denote significant differences between groups at  $P = .05$ , based on t-test to determine the effect of the burn (post-burn).

Table 1. Summary of the relative performance of burned plants in response to the spring and fall prescribed burns. Values of relative performance in burned plants are listed as a percentage of the mean values for unburned plants, except for mature plant mortality. Mature plant mortality is listed as the percentage of plants that died as a result of the spring burn; we did not observe any mortality in any control plots and fall burn plot. There were 2 years of measured plant response in spring burned plants; we also include the mean response over the 2-year period. NA = not applicable since no seedlings were observed on the study site in 1992.

	Spring			Fall
	1992	1993	Mean (1992-93)	1993
Shoot growth	145	117	135	221
Shoot production	40	56	49	67
Biomass production <sup>1</sup>	58	65	66	148
Flower production	34	49	41	169
Plant volume	38	51	44	75
Seedling production	NA	82		6
Mature plant mortality	25	0	0	

<sup>1</sup>Biomass estimated per volume of plant as mean shoot growth x mean shoot production.

shoot growth of burned plants compared to control plants. Likewise, overall flower production was reduced by three-fold in spring burned plants, but was 70 percent higher in the fall burned plants despite fewer number of shoots and significantly smaller plants for burned plants. Stimulated growth and reproductive effort following fire has been well documented (Christensen 1985, Hartnett 1991, Crutzen and Goldammer 1993, Lamont and Runciman 1993, Brewer and Platt 1994). Stimulated growth and reproductive effort has been ascribed to increased soil nutrient availability and retranslocation of resources to aboveground growing meristems (Christensen 1985, Crutzen and Goldammer 1993).

Our results indicate that dieback from established woody stems in response to burning was much greater for spring burned plants compared to the fall burn. Less living woody stem tissue would lead to reduced shoot production. Increased dieback of woody stems could be due to a higher fuel load during the time of the spring burn, creating a hotter fire. However, since the fuel load appeared to be much greater for the fall burn (exemplified by the fact that the plot was more uniformly burned), it is likely that the increased negative effect of spring burning on *C. molestus* vegetative growth was due to plant phenology at the time of the two burn treatments. In the spring burn buds were just emerging, whereas before the

fall burn all of the foliage had senesced. This suggests that plants are not able to replace young growing meristems that have been destroyed by fire.

We do not know the long-term response of surviving plants for either burn treatment. Other *Chrysothamnus* species (i.e. *C. viscidiflorus* (Hook) Nutt., *C. nauseosus*, *C. bloomeri* Greene, and *C. puberulus* D.C. Eaton) have shown similar negative results in the first 3 years after a fire, after which production increases dramatically (Wright 1972). *Chrysothamnus viscidiflorus* cover was 4 to 9 times greater on 8 to 18 year old burns (Chadwick and Dalke 1965), rabbitbrush production in Idaho tripled in 12 years after burning (Blaisdell 1953), and production in *C. bloomeri* (i.e., *Haplopappus bloomeri* Gray) in northern California doubled 5 years after a fire (Countryman and Cornelius 1957). At least some of these species also sprout from basal buds (Whisenant 1986), which would account for a time lag in their response. We never observed any basal sprouting in *C. molestus* but did observe basal sprouting in *C. nauseosus* in the burned plots. In a review of the literature Christensen (1985) notes that sprouting species are more tolerant of fire and respond more vigorously. This suggests that *C. molestus*, a non-sprouter, may respond very differently than other sprouting *Chrysothamnus* species and thus not show long-term positive responses to prescribed burning.



### Seedling Establishment

Seedling production was not reduced in the spring burn plots, but was significantly reduced in the fall burn plots. The greater numbers of seedlings in the spring burn sub-plots suggests that the seedlings we observed in 1993 emerged in 1992 after the spring burn but before the fall burn. The overwintering seeds or seedlings then experienced significant mortality as a result of the fall burn. Other studies have also shown a differential effect on seedlings versus established plants in response to fire. Fisher (1947) found that 43–100 percent of seedlings are killed by fire, while only extremely hot fires killed mature plants, and Lamont et al. (1993) showed increased seedling mortality in several species exposed to fire in a sclerophyll shrubland.

Several studies have shown that fire increases the probability of germination and seedling establishment after fires in different habitats (Gratkowski 1961, Christensen 1985, Roy and Sonie 1992, Lamont and Runciman 1993). Clearly the 1992 spring burn did not promote seedlings. Although we did not quantify the effect of fall burning on seedling establishment that may have occurred after 1993, we did not observe any new seedlings (i.e., seedlings that may have emerged after 1993) in a casual inspection of the plots in August, 1995, indicating that prescribed burning does not promote seedling establishment in *C. molestus*. All of the seedlings we observed in 1993 were found growing in the open. Increased seedling establishment has been ascribed in large part to the opening of vegetation by fire (Christensen 1985). This suggests that prescribed fires in this open pinyon-juniper grassland do not provide open environments long enough to promote seedling establishment in *C. molestus*. This is consistent with our observation that vegetation in the burned plots recovered quickly after the burns.

It is possible that any burning, whether it is performed in the spring or fall, detrimentally affects seedling establishment if the burn occurs in the year seedlings are establishing. To determine the importance of these phenomena, one needs to know the cyclical nature of seedling establishment in *C. molestus* including when germination occurs during the year. The latter will indicate whether burns affect seedlings or the seed bank. This can only be obtained by long-term studies greater than 10 years in duration, considering that successful reproduction may be rare in *C. molestus*.

### Management Considerations

Two conclusions relevant to management considerations are that spring burning significantly increases plant mortality and reduces plant performance whereas fall burning does not affect mature plant mortality and does stimulate biomass accumulation and flowering; and that burning in a year when sexual recruitment occurs negatively affects seedling survival. Both conclusions have important management implications. First, prescribed burns should be conducted in the fall, not in the spring. Second, all prescribed burns should be conducted after dry winter-springs (i.e., La Niña years) and not during years with wet winter-springs (i.e., El Niño years), in order to approximate a more natural fire cycle (Swetnam and Betancourt 1990). In addition, an assessment of seedling recruitment should preclude any prescribed burns to prevent seedling mortality that would occur as a result of a prescribed burn.

### Acknowledgments

We would like to thank Marybeth Foushee for assistance in the field and Barbara Phillips, Ron Auler, and Bill Hevron for providing important background information. We are very grateful to the Kaibab National Forest for conducting the prescribed burns. H. David Hammond, Catherine Gehring, and Greg Martinsen provided valuable comments on the manuscript. This study was funded primarily by a challenge cost share (No. 07-09-91) through the Kaibab National Forest and the Arizona Nature Conservancy. Additional funding was provided by the U.S. Department of Energy (94ER61849), the National Science Foundation (DEB 9408009) and the U.S. Department of Agriculture (95-37302-1801).

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# An Ecophysiological Analysis of Shade Effects on *Clematis hirsutissima* var. *arizonica*

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**Abstract:** Net photosynthetic rates and water potentials of adult *Clematis hirsutissima* var. *arizonica* were compared among four canopy density environments (open, medium, closed, experimentally closed) in a *Pinus ponderosa*-dominated forest in northern Arizona on four dates in summer 1994. Daytime water potential was lower (greater water stress) in open versus shaded environments, with little difference in pre-dawn water potential among environments. Net photosynthetic rate was higher in open versus shaded environments on most dates. The results indicate that shading ameliorated daytime water stress and that net photosynthetic rate was more strongly limited by low light intensity in shaded environments than daytime water stress in open environments. Regulation of *Clematis* physiology by canopy density is discussed in relation to habitat requirements for regeneration and population growth.

## Introduction

*Clematis hirsutissima* var. *arizonica* (Heller) Erickson (referred to as *Clematis* hereafter) is considered a sensitive species by the U.S. Forest Service and is a candidate for federal protection under the U.S. Endangered Species Act. The species is an herbaceous perennial shrub with pinnately compound leaves, fine leaflets, and solitary, purple, bell-shaped flowers (Kearney and Peebles 1960). The stem dies back in the fall after seeds are shed. Populations of *Clematis* grow at high elevations (2300–2800 m) near Flagstaff, Arizona, the Chuska Mountains of Arizona, and the Zuni Mountains of New Mexico. The known populations have a patchy distribution and are geographically rare.

One of the major anthropogenic factors hypothesized to have limited the distribution of *Clematis* in northern Arizona is the removal of overstory shade by timber harvesting. Concerns over the detrimental effects of harvesting overstory *Pinus ponderosa* Lawson on *Clematis* are substantiated by recent surveys indicating that approximately 85 percent of all *Clematis* populations in northern Arizona occur in habitats where average light levels are below 50 percent full sun (Maschinski 1989), and by measurements of lower bud mortality, greater seed set, greater production of viable seed, and greater rates of seedling establishment in shaded versus open habitats (Maschinski et al. in press). These data suggest facilitation of *Clematis* reproduction by shading from the *Pinus ponderosa* canopy. Because *Clematis* grows in a geographic region characterized by low water

availability, high evaporative demand, and high light intensity, shade may be beneficial to *Clematis* reproduction and seedling survival by moderating water stress, as has been documented for understory or herbaceous species in other regions (e.g., Knapp et al. 1989, Knapp and Smith 1990, Schultz and Adams 1995). In addition, shade may stimulate *Clematis* reproduction and survival by reducing limitations on net photosynthesis by water stress. In this paper, we test the hypothesis that shading ameliorates water stress of *Clematis* in northern Arizona. We also examine the role of water stress and light intensity in regulating net photosynthesis of adult *Clematis* under field conditions.

## Methods

In 1991, we located and surveyed *Clematis* populations near Lower Lake Mary approximately 25 km southeast of Flagstaff, Arizona. All populations occur in a forest dominated by *Pinus ponderosa*. We categorized these populations into three environments based on the average percentage of full sun penetrating to the forest floor (see Maschinski et al., in press, for additional details): (1) open environments with light levels greater than 75 percent ("open"), (2) medium environments with light levels between 50 and 65 percent ("medium"), and (3) closed environments with light levels less than 40 percent ("closed"). In 1992, we created a fourth environment ("experimentally closed") by covering half of the open environments with wooden lattice structures to reduce light penetration to the forest floor. The lattice

reduced average light intensity from 76 percent to 5 percent.

In 1994, we measured net photosynthetic rates and water potentials on fully expanded foliage of reproductively mature plants in one population of each of the four environments on the following dates: May 26, June 24, July 11, and August 8. The populations used for these measurements were representative samples of the four environments at the Lake Mary Site. Weather conditions varied among the four measurement dates. In May, the sky was overcast. In June and July, the sky was free of clouds. In August, intermittent clouds occurred.

For May, June, and July, we measured water potentials on one leaf from each of five representative plants per environment using a pressure bomb (PMS, Corvallis, OR) at predawn (0600 hours), mid-morning (AM = 0800–1000 hours), and early afternoon (PM = 1300–1500 hours) time periods. We measured net photosynthetic rate on the same plants during the AM and PM periods using a 0.25 liter cuvette and portable, closed-loop photosynthesis system (LI-6200, Li-cor, Lincoln, NE). All measurements of photosynthesis were conducted over 30 seconds under near-ambient conditions ( $\pm 10\%$ ) of temperature, light intensity, and relative humidity. We measured the area of each leaf used for photosynthesis measurements in the laboratory using an Ag-Vision imaging system (Decagon Devices, Pullman, WA). We then measured the weight of each leaf following drying for 24 hours at 60°C. The measurement procedure in August was identical to that for other dates except that persistent afternoon thundershowers prevented any PM measurements. During the photosynthesis measurements, we also measured the following environmental parameters: photosynthetic photon flux density (PPFD) assessed 0.5 m above the target plant with an 0.8 m-long PAR Ceptometer (Decagon Devices, Pullman WA); leaf and cuvette temperatures assessed with fine wire thermocouples; and cuvette relative humidity assessed with a Vaisala humidity sensor (Li-cor, Lincoln, NE). Leaf-to-air vapor pressure deficit (VPD) was calculated based on leaf and cuvette temperature and cuvette relative humidity.

Physiological and environmental data from each date were analyzed by ANOVA using SAS-Statistics software (Cary, NC) with canopy environment (open, medium, closed, experimentally closed), measurement time (AM, PM, pre-dawn for water potential), and their interaction as

factors. Mean comparisons were made using the Student–Newman–Keuls test at the 0.05 level.

## Results

Light levels were highest for the June and July measurements (clear sky), intermediate for August (intermittent clouds), and lowest in May (cloudy conditions) (Figure 1). Environment  $\times$  time was a significant ( $p < 0.05$ ) source of variation in June and July, reflecting inconsistent differences in light intensity between AM and PM among environments. Light intensity differed significantly ( $p < 0.0001$ ) among canopy environments at all dates with the highest intensities occurring in the open environment and the lowest intensities occurring in the closed or experimentally closed environments (Figure 1). Light intensity did not differ significantly between closed and experimentally closed environments on any date.

Vapor pressure deficit (VPD) was greatest in June and July, intermediate in August, and lowest in May (Figure 2). Vapor pressure deficit differed significantly ( $p < 0.005$ ) among environments at all dates and was greater in open compared with other environments. The medium canopy environment had significantly ( $p < 0.05$ ) greater VPD than the closed and experimentally closed environments in July and August, but not on other dates. Vapor pressure deficit was generally similar for closed and experimentally closed environments. Differences in VPD among environments primarily resulted from differences in leaf temperature, which often differed by 7–10°C between open and shaded environments, rather than differences in relative humidity, which differed by only 1–3 percent. Measurement time was a significant ( $p < 0.0001$ ) source of variation in VPD for May, June, and July, resulting from consistently greater VPD for the PM versus the AM period. Environment  $\times$  time was a significant ( $p < 0.02$ ) source of variation in June and July due to differences in VPD response over measurement times among environments.

Pre-dawn water potential in all environments decreased from May to July, suggesting depletion of soil water in the rooting zone from early to mid summer (Figure 3). Canopy environment, measurement time, and environment  $\times$  time were significant ( $p < 0.02$ ) sources of variation in water potential for all dates. Average water potential was greatest at pre-dawn, intermediate in the AM, and lowest in the PM for all dates. Although statistically significant, variation in May water

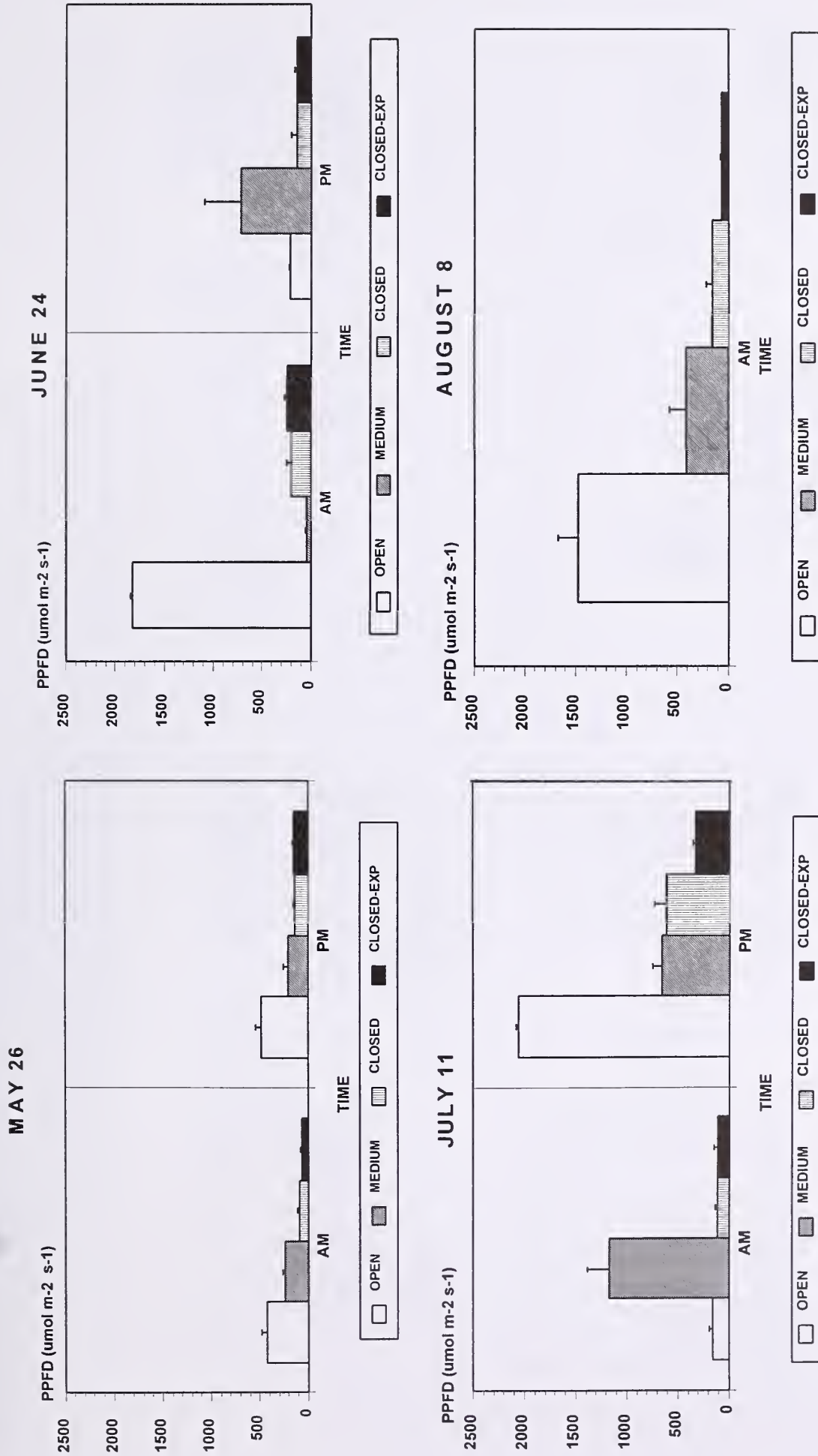


Figure 1. Photosynthetic photon flux density (PPFD) for four environments (open, medium, closed, experimentally closed) in the morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean.

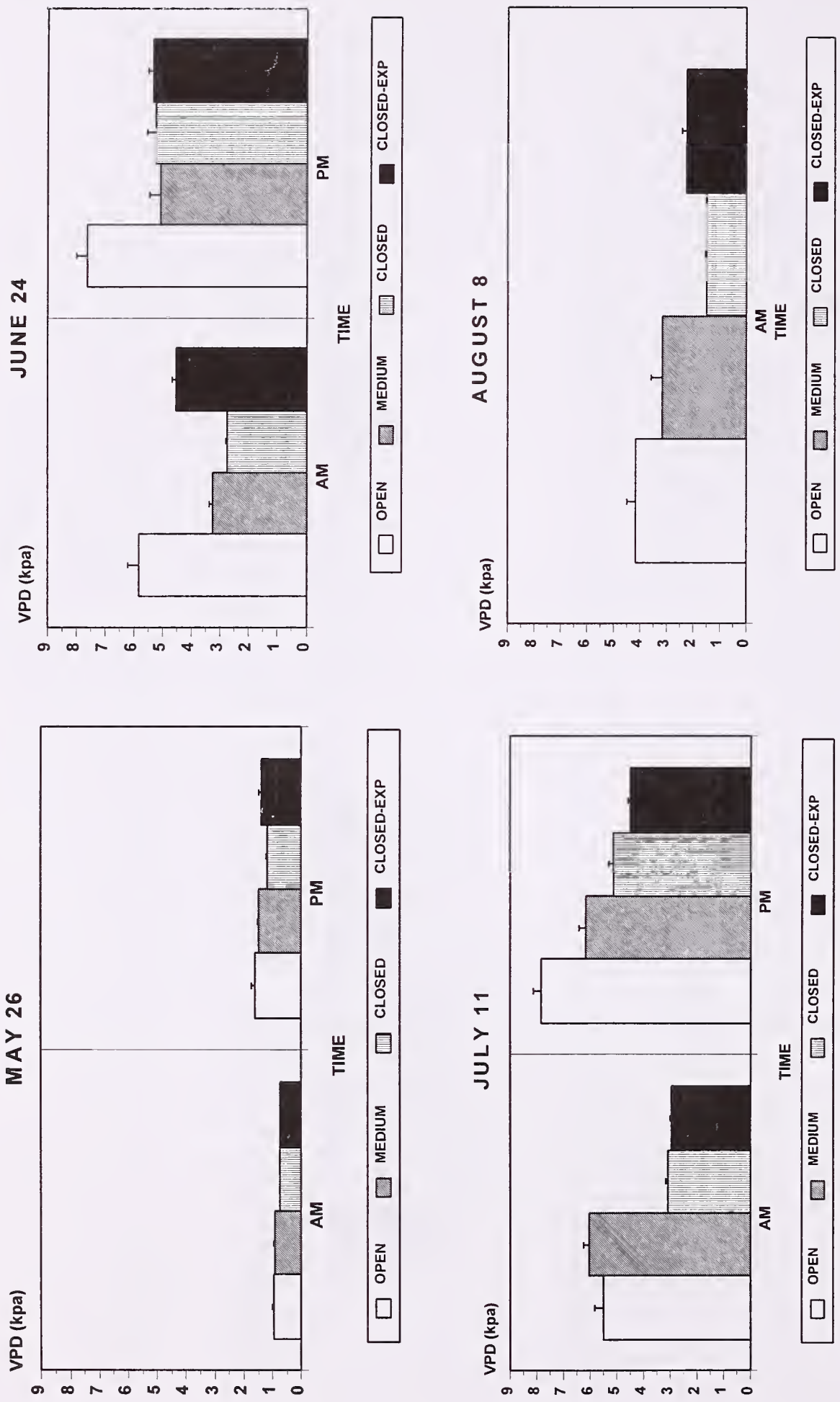


Figure 2. Leaf-to-air vapor pressure deficit (VPD) for four environments (open, medium, closed, experimentally closed) in the morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean.

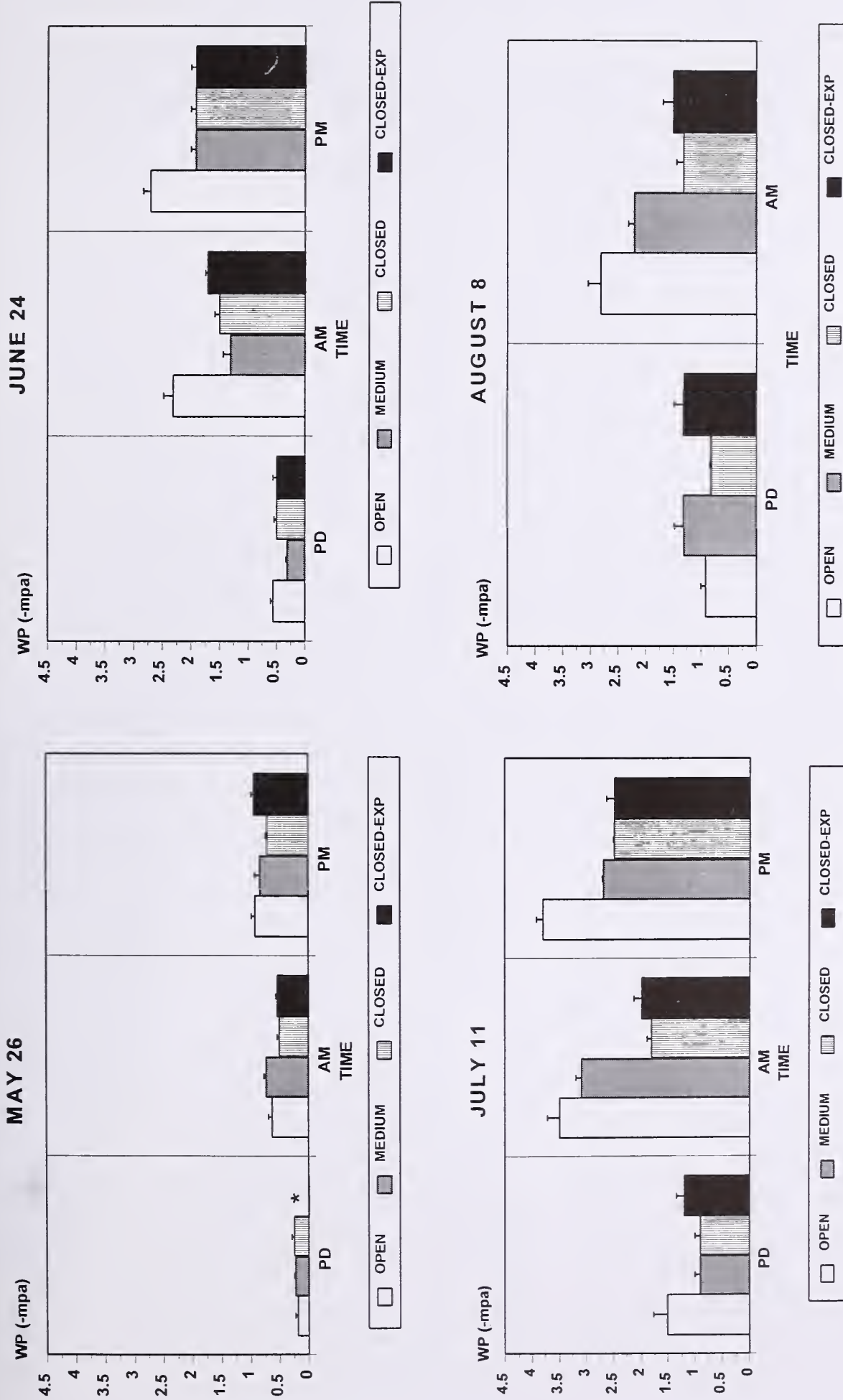


Figure 3. Foliar water potential for four environments (open, medium, closed, experimentally closed) at pre-dawn (PD, 0600 hours), morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean. The asterisk (\*) for May 26 indicates missing data for the experimentally closed treatment.

potential among environments was small at all times of the day, consisting of maximum differences of 0.25 MPa. In June, differences in pre-dawn water potential among environments were again small in magnitude and not statistically significant. However, June AM and PM water potential was significantly lower in open compared with other environments. In July, water potential at all times of the day was significantly lower in open compared with other environments. There were no significant differences in July water potential among medium, closed, and experimentally closed environments except in the AM, where water potential in the medium environment was lower than in the closed and experimentally closed environments. August pre-dawn water potential did not differ significantly among environments, while AM water potential was significantly lower in open and medium compared with closed and experimentally closed environments.

Net photosynthetic rate (leaf area basis) differed widely among dates in the open environment with the highest rates in June and the lowest rates in May and July (Figure 4). In contrast, there was little variation in net photosynthetic rate among dates for the shaded environments. Net photosynthetic rate differed significantly ( $p < 0.0001$ ) among environments in May, June, and August, but not July. On those dates where significant differences among canopy environments occurred, both AM and PM net photosynthetic rates were significantly higher in open versus shaded environments. On most dates, maximum daily values of net photosynthetic rate were greater in medium compared with closed and experimentally closed environments, but these differences were not statistically significant. Differences in net photosynthetic rate between closed and experimentally closed environments were small and not significant on all dates. The time of day that measurements were taken was a significant ( $p < 0.04$ ) source of variation in net photosynthetic rate in June and July, and environment  $\times$  time was significant ( $p = 0.03$ ) in June. Net photosynthetic rates in both June and July were generally greatest in the AM versus the PM period, the only exceptions being the medium environment in June and the experimentally closed environment in July. Differences in net photosynthetic rate among dates, environments, and measurement times calculated on a leaf dry weight basis (data not shown) were similar to differences calculated on a leaf area basis.

Correlations between net photosynthetic rate and water potential in May, June, and July were not significant (Figure 5). However, in August net photosynthetic rate was significantly and negatively correlated with water potential. In contrast, net photosynthetic rate was significantly and positively correlated with light intensity at the time of measurement in May, June, and August, but not July (Figure 6).

## Discussion

Our results support the hypothesis that shading from the *Pinus ponderosa* canopy ameliorates daytime water stress in adult *Clematis*. Morning and afternoon water potentials were consistently lower in open compared with shaded environments in June, July, and August, indicating greater cumulative water stress in the open environment. Differences in morning and afternoon water potentials among environments were predominantly the result of greater transpiration in open versus shaded environments driven by higher leaf-to-air vapor pressure deficit and higher stomatal conductance in open environments (data not shown) rather than differences among environments in availability of soil moisture. Pre-dawn water potential is a sensitive measure of soil water availability in the rooting zone (Kozlowski et al. 1991). The similarity of pre-dawn water potential among canopy environments in May and June indicates similar availability of soil water in the rooting zone in early summer when soil moisture is primarily supplied by residual winter and spring precipitation. Differences in pre-dawn water potential among environments in early July suggest lower water availability in open compared with shaded environments following depletion of residual winter precipitation but prior to the start of summer monsoonal rains. In early August following the start of the monsoonal rains, there was again no evidence of differences in availability of soil water among environments. Thus, on most dates availability of soil water in the rooting zone was similar among environments.

Net photosynthetic rate of *Clematis* was more strongly limited by shading than by water stress. Differences in net photosynthesis among environments were strongly related to light intensity in May, June, and August, indicating maximum carbon assimilation in the open environment. In July, the relationship between net photosynthetic rate and light intensity was still positive, but weaker than in other months perhaps due to se-



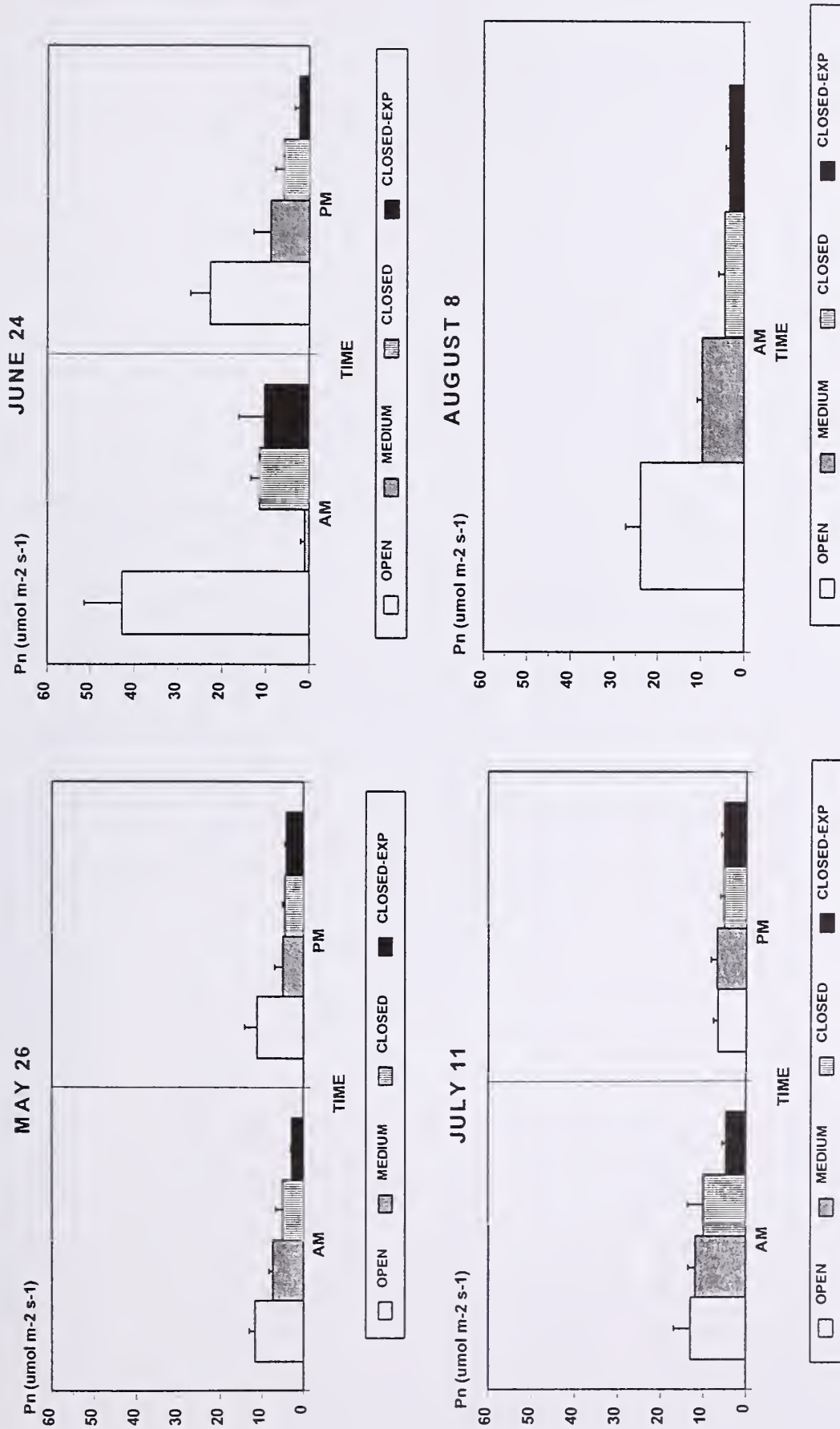


Figure 4. Net photosynthetic rate for four environments (open, medium, closed, experimentally closed) in the morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean.

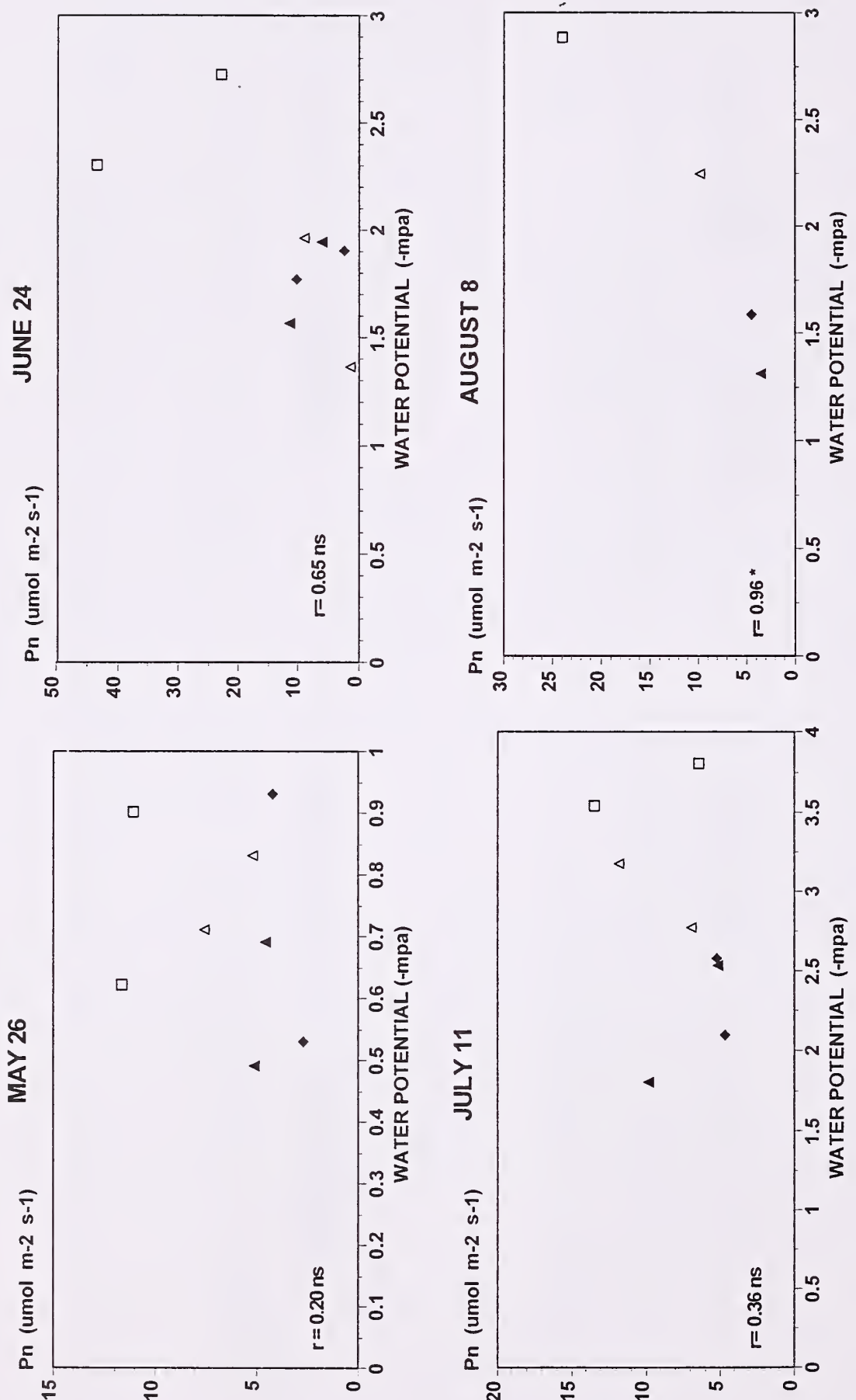


Figure 5. Relationship between net photosynthetic rate and foliar water potential for four environments on four dates. Values for each date and environment are means for morning and afternoon measurements. Environments are coded as: open = white square, medium = white triangle, closed = black triangle, experimentally closed = black diamond.

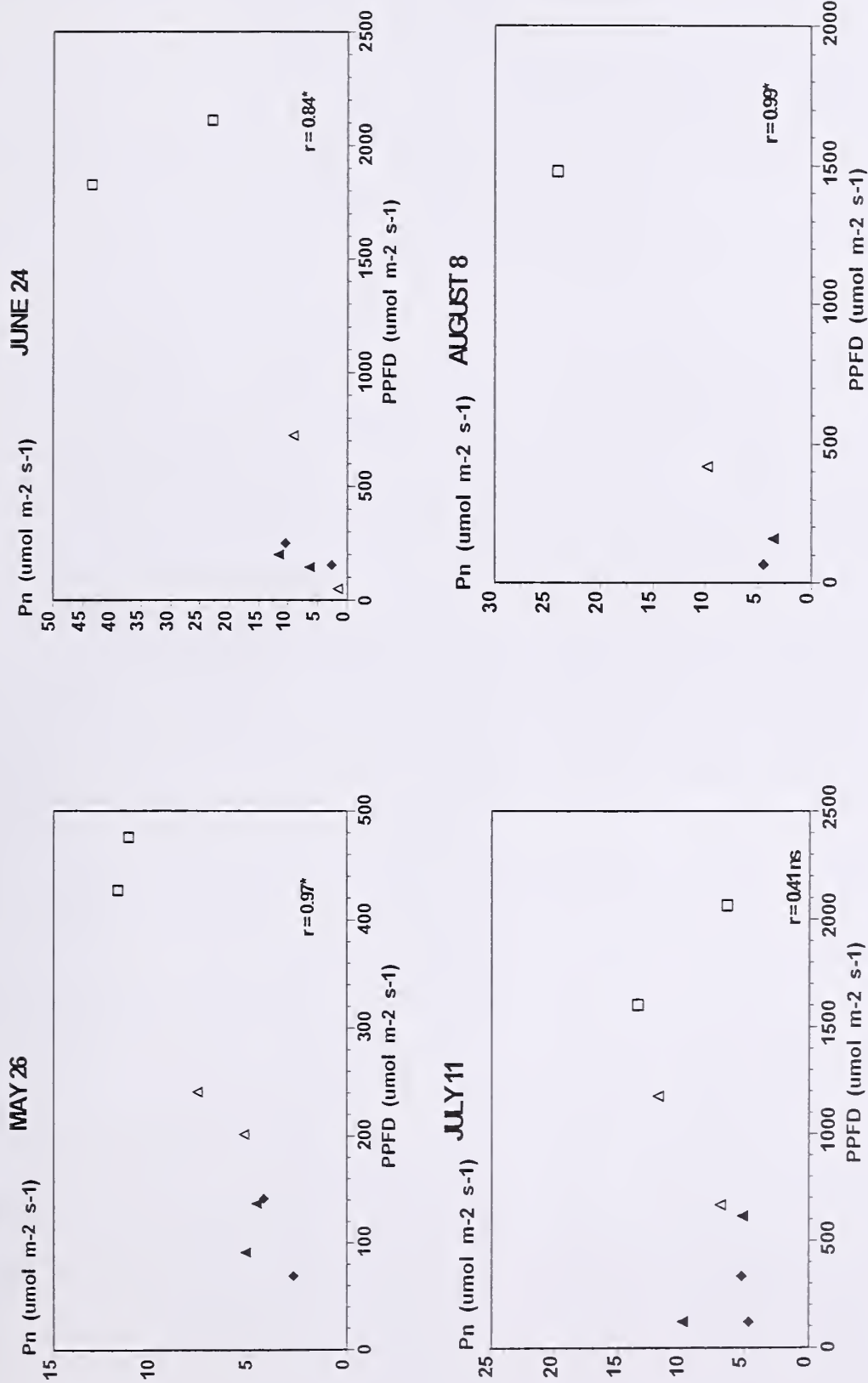


Figure 6. Relationship between net photosynthetic rate and photosynthetic photon flux density (PPFD) for four environments on four dates. Values for each date and environment are means for morning and afternoon measurements. Environments are coded as: open = white square, medium = black triangle, closed = black triangle, experimentally closed = black diamond.

vere daytime water stress in the open environment (water potentials  $< -3.5$  mPa) which limited net photosynthesis in this environment to a greater degree than in the more shaded environments. In May, June, and July, net photosynthetic rate was not significantly related to water stress. The only significant relationship between net photosynthetic rate and water stress occurred in August where high rates of photosynthesis were associated with high levels of water stress (more negative water potentials), which apparently resulted from high transpiration rates associated with high rates of stomatal conductance in the open environment. Adult *Clematis* in northern Arizona are capable of maintaining high rates of net photosynthesis in open environments despite the occurrence of high evaporative demand and severe daytime water stress. This physiological characteristic is likely an adaptation to montane environments of the southwestern United States, which are characterized by high light intensity, low humidity, and prolonged periods of low soil moisture during the growing season.

There was no apparent direct linkage between effects of canopy cover on the *Clematis* net photosynthetic rate measured in this study and reproductive success as measured in previous studies. Although net photosynthesis of adult *Clematis* was highest in open environments, reproduction and population growth are favored by shadier environments. Previous research on *Clematis* in northern Arizona (Maschinski et al. in press) has indicated lower seedling establishment in open versus more shaded environments. Plants growing in environments with average light levels greater than 75 percent full sun suffered from higher bud mortality, lower seed viability, lower seedling establishment, higher mammalian herbivory, and greater exposure to competition from other vegetation than plants growing in more shaded environments (Maschinski et al. in press). For *Clematis*, environments with intermediate levels of canopy cover characterized by frequent sunflecks may offer the best combination of exposure to direct sun, which stimulates photosynthesis but induces daytime water stress, and shading, which depresses photosynthesis but ameliorates daytime water stress through stomatal closure. Our research suggests that timber harvesting activities

that completely remove overstory shade will be detrimental to *Clematis* populations in northern Arizona, because of high levels of water stress, which likely damages flowers and young seedlings by desiccation. However, harvesting activities that maintain intermediate levels of shading (i.e.,  $>50\%$  canopy cover), where vegetation receives filtered light with frequent sunflecks, should not be detrimental.

### Acknowledgments

This study benefited from the efforts of many hands and minds. For their field assistance, we thank Edward Smith, Barbara Phillips, Cecily Criminale, Matt, Sean, Tess, and Ger Ryan, Laurie, Ruth, and Rob Brooks, Sandra and Roseanne Elliott, John Obst, Ron Bauman, Matt Caouette, Matthew Smith, Deborah Stern, Jenn Starr, Scott Killian, Bud White, Mike Manrowe, Scott Wiet, Jan Busco, Rick Paladino, Amy Gibbons, and Karin Doerr. Heather Green and Greg Goodwin provided administrative and logistic support for this project. The manuscript was improved by an early review by Shelly Fenney.

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# Conservation Assessment and Strategy for *Cimicifuga Arizona* Watson (Arizona Bugbane) on the Coconino and Kaibab National Forests

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Abstract: *Cimicifuga arizonica* Wats. is a rare endemic found in moist, forested areas near perennial or intermittent streams in four population areas located in the Mogollon Rim area of central Arizona. The Conservation Assessment and Strategy Plan addresses management of Arizona bugbane within the context of its ecosystem. The habitat of this species is a subset of larger scale habitats that support a diversity of species such as Mexican spotted owl, black bear, and deer. Continuing Level 1 and Level 2 monitoring will enable us to study the demography of Arizona bugbane, impacts, and population trends.

## Introduction

The goal of *Cimicifuga arizonica* Conservation Assessment and Strategy for the Coconino and Kaibab National Forests 1995–2005 (USDA 1995) is to develop a course of action that will contribute to the management and conservation of Arizona bugbane in Arizona. The Plan is to ensure that the species does not become threatened or endangered as a result of Forest Service activities. "Rare plants are often sensitive indicators of the overall health of an ecosystem; their persistence, resilience, and stability are clues to the general health of the larger system, as well as events in the biology of single population and species" (Falk and Warren 1994).

*Cimicifuga arizonica* Watson is a Forest Service sensitive species and is presently a category 1 candidate (58 Federal Register 51144; September 30, 1993) for listing as threatened or endangered under the Endangered Species Act of 1973, as amended. Final approval of the Arizona bugbane conservation agreement represents a commitment by the U.S. Forest Service through the Coconino, Kaibab, and Tonto national forests and by the U.S. Fish and Wildlife Service to manage sensitive plant resources in a manner consistent with each agencies' policies in order to ensure that species do not become threatened or endangered.

Monitoring, which provides valuable information, is an important component of the plan. Arizona bugbane's habitat is a subset of larger scale habitats that support a diversity of species such as Mexican spotted owl, red-faced warbler, American redstart, black bear, and deer. Level 1 and Level 2 monitoring will enable us to study Arizona bugbane's demography, impacts, and population trends within the context of its ecosystem.

## Species Background

*Cimicifuga arizonica* is an herbaceous perennial plant in the Ranunculaceae. Vigorous plants average 3–6 feet in height with large, long-petioled lower leaves and small sessile upper leaves. The blades are ternate and each segment is divided with ultimate segments toothed, jagged, and more or less three-lobed. The flowering stalks are long with slender racemes positioned above the large leaves. Small white-stamened, petal-less flowers are clustered on the flower stalk, making the plant very conspicuous when in flower. Sessile follicle fruits are produced.

*Cimicifuga arizonica* is the only species of *Cimicifuga* occurring in Arizona. It may be closely allied to *Cimicifuga elata* Nutt., which grows in Oregon, Washington, and British Columbia (Compton 1994). Detailed descriptions of *C. arizonica* can be found in Phillips et al. (1982). It was first collected on Bill Williams Mountain, Coconino County, Arizona, in August of 1883 by H.H. Rusby. Mr. and Mrs. J.G. Lemmon also collected it on August 25, 1884, in a ravine on the northwest slope of the same mountain. It was described by Sereno Watson in 1885.

*Cimicifuga arizonica* can be easily confused with *Actaea rubra* (Ait.) Willd. ssp. *arguta* (Nutt.) Hult., another ranunculaceous plant. They are vegetatively very similar and grow in similar habitats. The primary difference is in the fruits: *Actaea* has a red or white berry, while *C. arizonica* has a follicle (Phillips et al. 1982). The raceme is also much shorter in *Actaea*. In early spring, sprouting *C. arizonica* can be confused vegetatively with the Umbelliferae, *Osmorhiza* spp.

Studies of several species of *Cimicifuga* have

been completed by numerous researchers on various topics including anatomy, pollination ecology, seed germination, taxonomy, population genetics, and pharmacology (Kaye and Kirkland 1994). There are 15 species of *Cimicifuga* worldwide, with six in North America, four of which are local or regional endemics. The fact that there are several species worldwide with different degrees of endemism provides opportunities for many evolutionary and ecological studies.

The genus is noted as a medicinal herb in Kearny and Peebles (1969). Bugbane (*Cimicifuga* spp.) has been used to treat rheumatism, uterine disorder, and snakebite. The rhizomes of some *Cimicifuga* species are important plant tissues for medical use.

### Biology/Ecology

*Cimicifuga* seedlings develop rhizomes, which after a few years elongate horizontally through the soil. The adult rhizome produces a few scaly leaves and foliar leaves and terminates in an erect stem with an inflorescence. Each of the scaly leaves protects an axillary bud; one or more of these buds elongates the next year with a terminal erect stem (Kumazawa 1931). It is often difficult to determine individual plants because the underground relationships of the stems and rhizomes cannot be determined without excavation.

Each inflorescence elongates from its base toward the apex (acropetally) and when it is branched, the shorter, lateral racemes flower so that the end of anthesis is virtually synchronous over all racemes. The primary raceme, which is 4–18 inches (10–45 cm) long, has about 3.5 inches (9 cm) (ca. 35 flowers) in bloom on the raceme at any one time (Pellmyr 1985). Two staminodes occur per flower (Compton 1994), but there is no nectar produced (Pellmyr 1985).

The pollination biology of *Cimicifuga arizonica* was investigated by Olle Pellmyr in 1983. His report (Pellmyr 1985) emphasized the value of the workers of three bumblebee species as pollinators: *Bombus occidentalis*, *Separatobombus morrisoni*, and *Probombus huntii*. Self-pollination also occurred. Typically, the flowers bloom during the monsoon season (July–mid September), with peak flowering around August 1, which matches the time of peak abundance of its three species of bumblebees (Thorp et al. 1983, cited in Pellmyr 1985, Pellmyr 1985). The pollinators also visited several simultaneously flowering species: *Phacelia magellanica* (Lam.) Cov., *Asclepias speciosa* Torr., *Aquilegia*

*chrysantha* Gray, *Geranium richardsonii* Fisch. & Trautv., and the introduced *Trifolium repens* L., as well as several other species (Table 4, Pellmyr 1985). A plant species exhibiting a sequential flowering pattern may be at a disadvantage compared with nectar-producing species. Under normal conditions Arizona bugbane flowers receive a very limited number of visits (Pellmyr 1985). Therefore, it is essential to maintain a diversity of plant species pollinated by these bees in order to attract them so that the bees will be in the area when *Cimicifuga* is flowering.

The dehiscent fruits are produced in August–September. The dates of flowering and fruiting depend on yearly temperatures and precipitation. In late fall (mid-September to October), the stems and leaves turn tan and senescent as their chlorophyll deteriorates and the plant goes dormant for the winter.

Evidence of insect activity has been noted on *Cimicifuga arizonica* populations. Larvae of *Celextrina ladon* (Lycaenidae, gossamer-winged blue butterflies) have been observed affecting flowers and seeds lightly to severely on a number of occasions in many of the populations (Phillips et al. 1982, Pellmyr 1985, Gobar 1991 in Gobar 1992). Warren (1991) noted small-insect damage on approximately 10 percent of the fruit produced in the Bill Williams population, but did not observe impacts on the leaves or stems. Aphid infestations and weak stems and die-back due to unknown causes have been noted in some populations (Phillips et al. 1982).

### Distribution and Habitat

*Cimicifuga arizonica* is only known to occur in the Mogollon Rim area of central Arizona. To date, the distribution is limited to localities on the Kaibab, Coconino, and Tonto national forests. The geographic distribution extends from Bill Williams Mountain on the northwest to the Sierra Ancha Mountains on the southeast. All of the known Arizona bugbane populations and sites are found in four main population areas (PAs). Two PAs occur on Coconino National Forest. The Oak Creek PA includes most of the major tributaries of Oak Creek, including the West Fork of Oak Creek. The second PA is located along West Clear Creek. The third PA occurs on Bill Williams Mountain on Kaibab National Forest. The fourth PA is along Workman Creek in the Sierra Ancha Mountains, Tonto National Forest.

Arizona bugbane occurs within moist, forested

## Monitoring Methods

The monitoring plan identified in the action plan of the assessment and strategy has two general objectives: (1) establish a formal monitoring strategy for the populations in Oak Creek, West Clear Creek, and Bill Williams Mountain to determine trends in population size and reproductive condition; and (2) identify potential threats to the populations and recommend mitigation actions regarding management activities.

The two types of monitoring being used for this species, Level 1 and Level 2, exhibit different objectives. Level 1 monitoring identifies individual plants by location within a monitoring transect. It involves measuring and recording individual size, reproductive activity, damage, and other indices. We know little about the phenology of the species such as number of leaves, population size, and its sensitivity to various factors. Level 1 monitoring might clarify our understanding of this species. Such factors as elevation, aspect, drainage condition, canopy closure, and degree of slope might make important contributions to the size and phenology of the plants.

Level 2 monitoring focuses on assessment of the population as a whole and includes an estimate of the number of individuals in a population and the approximate areal extent of the population. Environmental parameters and other factors, such as flood and fire effects, infestations of forest pathogens, herbivore grazing and insect damage, disease, trampling, and several other impacts are recorded under both Level 1 and Level 2 monitoring.

Five localities were selected for Level 1 monitoring: Bill Williams Mountain, James Canyon, Maple Spring, Upper West Fork of Oak Creek, and West Clear Creek. Each site will be visited once every 5 years. We have completed baseline data on all five sites for Level 1 monitoring, which provides information on the vigor, size classes, and phenology for each population.

Level 2 monitoring, which provides a commitment to visit the localities on a regular basis, was begun after the completion of the *Cimicifuga arizonica* conservation assessment and strategy. Sixteen localities, including four localities on Level 1 monitoring, were selected for Level 2 monitoring. Four to five localities will be visited annually and each locality will be visited every 5 years. This will enable us to detect the effects from some of the factors listed above on the *Cimicifuga* population and habitat.

areas near perennial or intermittent streams in the canyon between 1606–2515 m elevation. The moist ecotonal habitat ranges from forest cover and riparian habitat including springs areas to splash zones of waterfalls. Rich, fertile soils high in humus are typical. The surrounding vegetation is generally mixed conifer with an understory of deciduous shrubs and trees that is often very dense and shady (Phillips et al. 1982, Warren 1991). Important overstory associates of *C. arizonica* include *Acer grandidentatum* Nutt. ex Torr. & Gray (big-toothed maple), *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. (white fir), *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir), *Alnus oblongifolia* Torr. (Arizona alder), and *Cornus stolonifera* Michx. (red osier dogwood). *Cimicifuga arizonica* is often the dominant understory species when present.

It is believed that shade from deciduous trees, cold air drainage, high relative humidity, and snow pack are some factors needed for the continued survival of the species (Phillips et al. 1982). Air temperature is somewhat cooler in the canyons than on the canyon rims and cold air drainage occurs at night. The canyons where *Cimicifuga arizonica* occurs run in various directions: canyon direction or aspect does not appear to be a big factor as long as the canyons are deep and narrow enough to provide shade for a large part of the day. Most of the canyons are large and have perennial streams or ephemeral streams with water in the spring during the snow melt season and sometimes during the summer monsoons.

Kaye and Kirkland (1994) hypothesized that hardwood trees, such as maples, play an important role in forest dynamics where *Cimicifuga elata* grows. The deciduous trees, where *Cimicifuga arizonica* grows, also are bare during early spring, permitting sunlight to reach the forest floor, which would stimulate growth of bugbane when moisture is plentiful. The leaves on the deciduous trees in the summer provide shade to maintain the area in a moist condition. Deciduous trees contribute humus and the soil may be less acidic under them than under conifers. Kaye and Kirkland (1994) noted that hardwood trees require additional light and lessened competition so the disturbance factors, such as floods, wind-throw, fire, and infestations of forest pathogens, may be important for the creation of new sites and colonization by hardwoods and *Cimicifuga elata*. One or more of these factors may be very important in creating sites favorable to Arizona bugbane.

## Results

The population of **West Clear Creek** was not healthy when it was first visited by B. Phillips in 1984. Most of the plants were vegetative only; about 2 percent had fruit. The leaves and stems were turning brown and then black and in many cases the total aboveground plants appeared dead. Although this population was surveyed in September, the die-back did not appear to be natural senescence due to lateness of the season, since other species were quite healthy. During Level 1 monitoring in August 1991 (Figure 1), the plants still appeared unhealthy. The data showed that 23 percent of the plants were diseased.

As mentioned above, *Cimicifuga arizonica* was first collected on **Bill Williams Mountain** in August of 1883 by H.H. Rusby. The Bill Williams population has larger plants than West Clear Creek, as shown by the number of leaves (Figure 2). Leaf counts for that population range from 1 to 17 leaves. The Bill Williams population occurs at 2546 m in elevation. This location is the highest elevation of the known populations. The first Level 1 monitoring was conducted in 1990 and the second visit was September 9, 1995. All plants recorded in 1990 were still present and healthy in 1995, and five new plants were recorded. The population appears to be healthy and stable.

Direct effects resulting from livestock grazing or trampling were noted at the **Maple Spring** population in 1982, but they were minor and did not appear to adversely affect this population (Goodwin 1982 in USDA Forest Service 1978–1994). Signs of disease have been observed also in the Maple Spring population, as indicated by wilted leaves and/or fungal infections. Level 1 monitoring from 1992 indicated that 60 percent of the plants showed symptoms. The cause(s) and effect(s) of the disease are unknown at this time.

Signs of cattle grazing on the flower heads were noticed in 1993 in the **West Fork of Oak Creek** population. Level 1 monitoring was done in the same summer, but in a different site. This population is the northwesternmost within the Oak Creek population area. The plants appeared healthy and vigorous.

**James Canyon** is the newest population, found in 1994 by a spotted owl crew. We conducted Level 1 monitoring in 1994. Since we have only observed this site one time, we don't know the long-term history for this population; however, it appears to be healthy. This location had 800+ plants and most were flowering.

Four localities were visited in 1995 for Level 2 monitoring: Lower West Fork, Pumphouse, Fry, and Secret canyons. Plants at all sites were very healthy. Two sites in Lower West Fork had been eradicated in January and February 1993 by the tremendous floods that occurred in the Oak Creek Canyon drainage area. Several other sites had been impacted by the floods. These sites appeared to be recovering and plants are re-establishing. More sites and higher plant numbers were recorded in Pumphouse, Fry, and Secret canyons than when the sites had been visited initially in 1984. Many juvenile plants were noted and adult plants were flowering and fruiting profusely. Little pathology was noted at these sites.

## Discussion

Most of the populations are obviously doing very well. All plants recorded in 1990 in the Bill Williams population were still present and healthy in 1995 and five new plants were recorded. The population appears to be healthy and stable. This population has larger plants than the other sampled populations, as shown by the number of leaves (ranging from 1 to 17 leaves in 1990 and 1–13 leaves in 1995). The four other populations' leaf counts range from 1 to 8 leaves (Figure 1).

The large numbers of juvenile plants in Pump-house, Fry, and Secret canyons is tentatively attributed to the effects of January and February 1993 floods washing away deep litter and exposing mineral soil and perhaps reducing competition, making favorable seedbeds for the species. Several factors could be contributing to the plants appearing unhealthy in West Clear Creek, including climate and trampling/browsing by deer. Signs of disease have been observed also in the Maple Spring population, as indicated by wilted leaves and/or fungal infections. Level 1 monitoring from 1992 indicated that 60 percent of the plants showed symptoms. The cause(s) and effect(s) of the disease are unknown at this time.

Laura E. DeWald, Assistant Professor, Northern Arizona University School of Forestry, has recently initiated research that will contribute to the conservation of Arizona bugbane through improved understanding of the genetic structure of the species. The specific objectives for this research are (1) to quantify the morphological variation among the four wild populations and one captive population of Arizona bugbane, (2) to quantify the genetic diversity within and between the four wild populations and one captive popula-



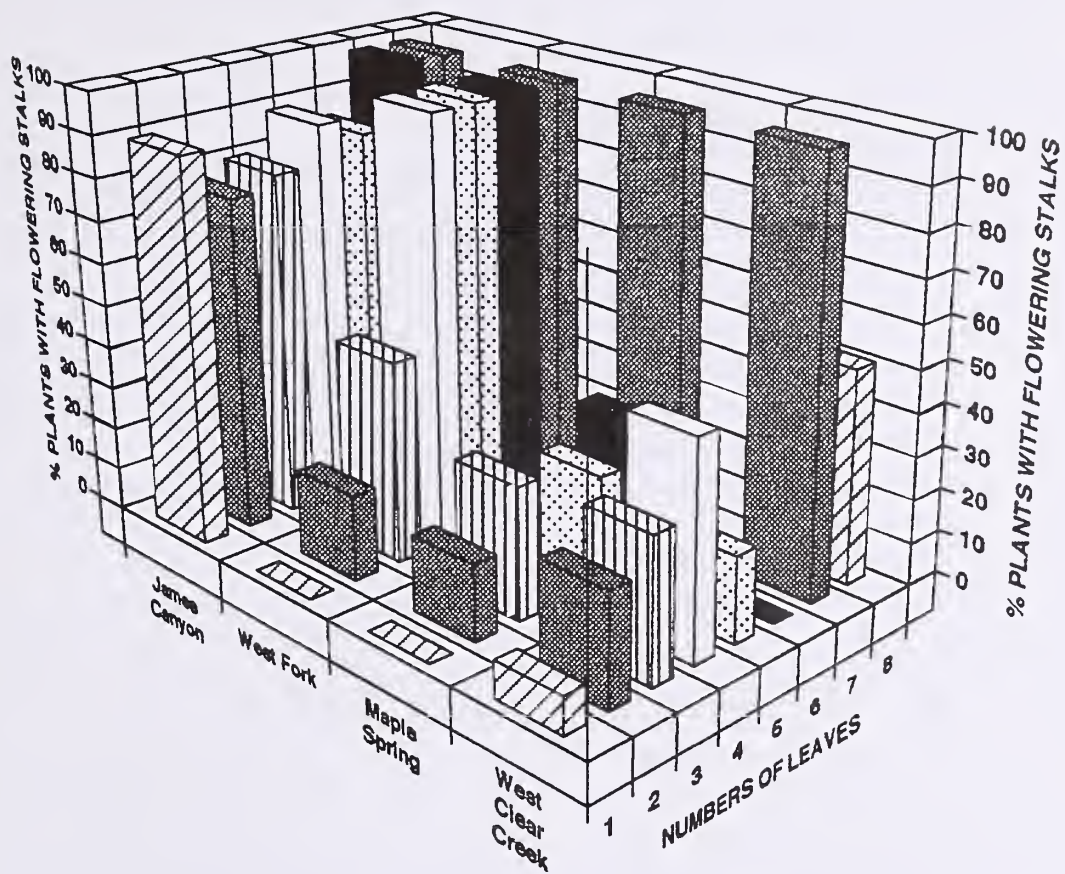


Figure 1. *Cimicifuga arizonica* plots: four sites, phenology 1991–1994.

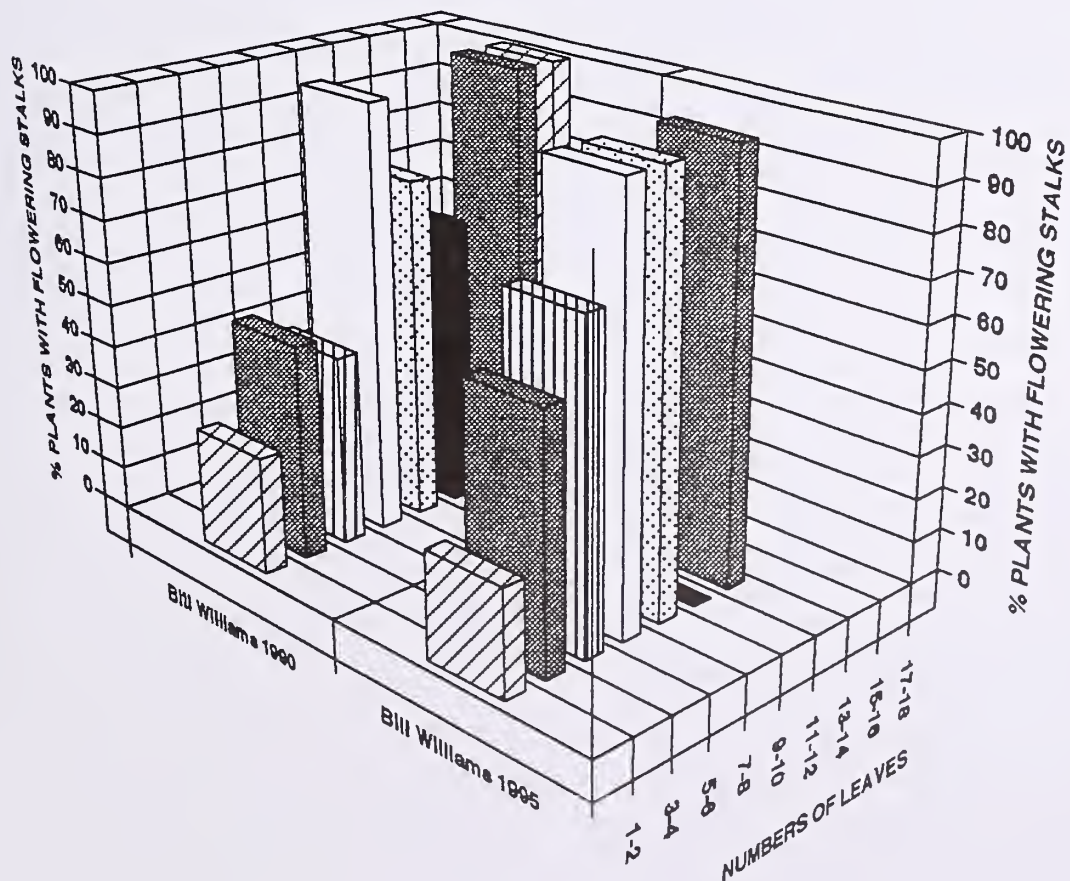


Figure 2. *Cimicifuga arizonica* Bill Williams plot: phenology 1990 and 1995.

tion of Arizona bugbane, and (3) to compare the within and between population levels of genetic diversity of the four wild populations to the captive population of Arizona bugbane.

The knowledge we gain about Arizona bugbane as we continue to implement this conservation assessment and strategy will contribute to the management and conservation of this rare endemic species in Arizona and will indicate the seriousness given to the plant management by the U.S. Forest Service.

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# Consequences of Land Management Practices on Willows and Higher Trophic Levels

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**Abstract:** A broad perspective on the current status of willow populations in Arizona is developed based on a landscape scale view of plant demography in space and a long-term temporal view since the Pleistocene. For germination and establishment, willows need moist mineral soil at higher levels of precipitation than normal for at least 2 years. In Arizona, regeneration may be successful only once or twice in a century even if disturbance creates mineral soils. However, declining disturbance over the landscape has greatly reduced the possibility for willow regeneration. Since the end of the Pleistocene glacial/pluvial period, when conditions in Arizona were probably excellent for willows, gradual drying has resulted in willow species with decreasing population sizes and ranges, with some only in relictual stands. With little or no regeneration and small isolated populations, the biodiversity of insect herbivores and their carnivores supported by willows has inevitably declined, probably by 50 percent of species or more. Simple land management practices could divert the course to local extinction of several willow species in Arizona.

## Introduction

There is a growing realization that to preserve any species it is important that the whole ecosystem should be preserved, and ecosystem preservation rather than species preservation should be our focus. Any terrestrial ecosystem is composed of landscapes with mosaics of plant species, each species supporting a trophic system of herbivores and carnivores. Plant species and community composition change over the landscapes and through time such that the view of a dynamic biotic landscape is essential in understanding the manner in which such landscapes will change in the near and longer-term future for all species in the trophic system of plants, their herbivores, and carnivores.

The focus of this paper is on willow species in Arizona and their associated insect herbivores and carnivores, with emphasis on the dynamics of populations over the landscape in space and time. Associated studies in Alaska, Finland, and Japan have aided the formulation of a general perspective on the dynamics of willows and herbivores in space over the Northern Hemisphere and in time since the last Pleistocene glaciation.

Superimposed on the natural course of vegetation dynamics are the human influences on water use and flow, fire, large mammalian herbivores, and soil disturbance, all relevant to the demographics of willows and their herbivores. There-

fore, to understand plant species and their conservation, landscape dynamics should be viewed as far as possible in a *primaevae* setting first, free of human activity, for this is the landscape in which the species have been evolving and to which they have become adapted. From this perspective we can then develop a view of how humans have modified the natural system and how the modification impacts plants and their herbivores. This step-wise approach is taken in our paper. We examine willow demography in space, then time, and willow growth and willow modular structure relevant to conservation of the willows and higher trophic levels.

## Willow Demography in Space

Willows produce small seeds with short-term viability. Seeds can disperse extensively on a cottony plume of hairs and germinate rapidly on moist mineral soils. Plants establish where fresh alluvial soils have been deposited and when competition from other species is minimal. They are colonizing species, usually among the first to colonize newly available mineral soil (e.g., Viereck et al. 1993).

In northern Arizona, seedlings of willows such as *Salix lasiolepis* Benth. became established only under rare conditions of average or above average winter precipitation followed by above average precipitation in June (Sacchi and Price 1992). Such

conditions occurred in only 2 out of 30 years in the vicinity of Flagstaff. In addition, second-year seedlings were intolerant of drought in June, meaning that higher than average precipitation was necessary for at least 2 consecutive years for survival of cohorts to year 3 when root systems were developed sufficiently to withstand the typical June drought (Sacchi and Price 1992). Under these constraints we may expect successful establishment of willow cohorts in northern Arizona to occur as rare events, perhaps only once or twice in a century.

Not only are those weather conditions suitable for willow establishment rare in northern Arizona, but also the deposition of alluvial soil beds for germination in the absence of competition is infrequent and patchy. Runoff is insufficient along the small intermittent streams on the Colorado Plateau to produce large beds of alluvium suitable for germination and survival.

On a primaeval landscape fire was common and extensive on the Colorado Plateau (Dieterich 1980). The fires common in ponderosa pine forests would have burned no doubt into riparian habitats with willows (Stein et al. 1992), creating extensive expanses of mineral soil probably suitable for establishment of willow seedlings. In addition, uninterrupted runoff from snow melt would have caused more erosion and deposition than at present, creating natural mineral soil beds.

On a landscape managed by humans, the effects of fire have been reduced drastically and runoff is reduced, both with consequences for willow regeneration. On the other hand, disturbance has been increased by construction activity with roadside ditches, borrow pits, cattle tanks, channelization, and city parks, with many disturbances creating conditions that enhance the persistence of surface water, moist soil conditions, and viability of willow seedlings. However, the presence of cattle and probably increased numbers of elk and deer have no doubt increased grazing levels above the natural level. These grazers kill young plants.

The interplay of natural and human activity result in a mosaic of willow patches over a landscape, with regeneration from seed being rare, cohorts of willows aging through time, and an unstable age structure with a preponderance of mature or overmature plants. This causes concern among conservationists for the viability of several species of willow in Arizona: *S. arizonica* Dorn, *S. bebbiana* Sarg., and *S. scouleriana* Barratt. Whether upland species like *S. scouleriana* or wetland species like *S. bebbiana*, the same kinds of forces are

resulting in insufficient disturbance and moisture combined to allow frequent regeneration and the development of populations with a stable age structure in any of the species.

### Willow Demography in Time

At the end of the last Pleistocene glaciation, willow species were among the major colonists on the mineral soil left behind the glaciers. Generally willow species are well represented in the pollen record for woody plants immediately after glaciation (e.g., Anderson et al. 1986, Ritchie 1977, Edwards et al. 1985, Hu et al. 1993). Willows remain dominant colonizers of new alluvium along the glacial rivers in Alaska today (Viereck et al. 1993). Unfortunately, no pollen record has been published for northern Arizona. But it is most likely, since there were small glaciers on the San Francisco Peaks (Sharp 1942, Péwé and Updike 1976), and high precipitation during the glacial/pluvial period, that conditions for willow species were excellent and stands were extensive.

During the interglacial period we are now in, at the southern end of the major willow distribution, we can expect conditions to have become increasingly less favorable for willows in terms of climatic and edaphic factors. Ranges have no doubt contracted over the past 11,000 years until in some cases we have relict populations in Arizona, such as Arizona and Bebb's willow, which were probably members of contiguous populations over much of the area south of the glaciation.

What has occurred through time in Arizona is also more or less displayed in space on a north-south gradient from Alaska to Arizona. In Alaska, willows are still dominant members of landscapes with large glacial river valleys in a perpetual state of change resulting from erosion and deposition of alluvium. In Arizona, the landscape has become much drier and much more static with so little disturbance and soil moisture that some willow species are unable to regenerate.

We may expect the present interglacial to persist for another 11,000 years. We are only about halfway through this interglacial period and yet even now some willow species are not regenerating in this state. With the gradual loss of individuals in willow populations through senescence and death, we may well anticipate extinction of species in Arizona before the next glacial/pluvial period is initiated. While willow species do persist, we can expect to see declining populations of old and senescent plants, with minimal or no regeneration.

## Consequences for Higher Trophic Levels

Changes in willow populations over the landscape in space and time have strong influences on the biodiversity of organisms dependent upon willows. As willow populations age, many herbivore species become less able to persist locally. As willow populations decline in area occupied and number of individuals, they are likely to support a declining number of insect herbivores. These effects no doubt impact the higher trophic level of carnivores in turn. These two considerations of aging populations and reduced numbers and range over the landscape will be discussed in turn.

### *Age Effects on the Fauna*

As stems of willows age, shoot growth per year declines from rapidly growing long juvenile shoots to slower-growing, shorter shoots producing catkins (Craig et al. 1986, Price et al. 1987a, 1987b, Roininen et al. 1993). This pattern is seen in tree species like *S. bebbiana* and shrub species such as *S. lasiolepis* in Arizona. In the shrub species, multiple stems are produced from a common rootstock and clones develop by layering from branches depressed to the ground by heavy snow. Thus, while a tree produces one main stem, the shrubs produce many stems through ramification, which are referred to as ramets. Ramets age in the same kind of way as the stems of trees.

Many insect herbivores on willows are able to utilize only rapidly growing plants and without constant production of young individuals or juvenile growth, such herbivores became uncommon, rare, or locally extinct. Examples include several species of sawflies, both galling and free-feeding, other galling insects, and several aphid species.

As an example of the tight linkage between rapid willow growth and herbivore utilization, the shoot-galling sawfly, *Euura lasiolepis* Smith, on *S. lasiolepis* is one of the best studied (Craig et al. 1986, 1989). Although shorter shoots on older ramets are very common in a clone and long juvenile shoots are scarce, oviposition by sawflies is concentrated on the longest, rarest shoots. It is only on these long shoots that larvae are able to survive. Hence, populations decline as willow clones age and the shoot population becomes shorter (Price et al. 1990).

With so little disturbance on the landscape, young juvenile shoots on some willow species, such as *S. scouleriana*, have become so sparse that sawflies restricted to such shoots have become

very rare and would certainly be considered as endangered species if more was known of them and they were vertebrates. One species of bud-galling sawfly is known at only one locality on *S. scouleriana* in the inner basin of the San Francisco Peaks. Here rock slides and heavy snow cause breakage of mature ramets and basal resprouting produces juvenile shoots. This is the only site in Arizona we know in which natural disturbance is sufficient to maintain vigorously growing ramets and something like a stable age structure of ramets.

As herbivores become rarer on increasingly sparse willow resources, so the effects move up the trophic system to the carnivores. These include many species of parasitic wasps, such that any one herbivore species supports several to many carnivore species. Thus, as willows become rare and endangered, much biodiversity is lost, because the richness of the fauna based on any willow species increases with each trophic level.

Of course, there are probably some herbivores on willows that survive best on older growth and these might be favored by present conditions. However, it is only in stable-aged populations of young to old individuals that the full biodiversity in higher trophic levels can be maintained.

### *Island Biogeographical Effects on the Fauna*

The Theory of Island Biogeography formulated by MacArthur and Wilson (1967) has been applied to plant species and populations as if they were islands on which insect herbivores live. Whether for oceanic islands or plant islands, in general there is a decline in species richness as islands become smaller and more distant from a source of colonists—a mainland (e.g., Price 1984). As a rule of thumb when islands decrease in size to only 10 percent of their former size through habitat fragmentation or contraction of geographic range, then a 50 percent reduction of the species richness on that island will be inevitable (Wilson 1992).

We are unaware of any estimates of the geographical range of willow species in Arizona during or following the Pleistocene, but certainly their biogeographical ranges have declined dramatically since then and have become increasingly isolated from the core populations, which have migrated into cooler, wetter regions. To estimate a 90 percent reduction in the geographic range of some willows, such as *S. arizonica* and *S. bebbiana* in Arizona, with a consequent loss of 50 percent of their specialized insect fauna, would probably be a

gross underestimate. More likely only 1–3 percent of the former range is now occupied and the fauna on such willows are largely extinct or heavily threatened in Arizona. Certainly, there has been significant reduction in biodiversity on most willow species with currently narrow and isolated ranges in Arizona. Given land management practices today, we can predict only continuing decline in willow populations and reduced biodiversity of herbivores and carnivores on these willows.

### Discussion

This attempt to provide a broad perspective of willow species dynamics over the landscape and through time enables development of a rational management plan, because we understand where willow populations have probably been in Arizona and where they are going. Clearly, for several willow species such as *S. arizonica*, *S. bebbiana*, and *S. scouleriana*, and perhaps the majority of willow species in Arizona, populations show unstable age distributions. There are generally far too few individuals in the younger age classes and far too many in the old age classes. Populations are persisting largely because of the longevity of individuals rather than by their replacement. Under these conditions there is inevitably gradual attrition, leading eventually to local extinction before another ice age is initiated.

This ebb and flow of species over the landscape is of course a natural process and local extinction of species is as natural as the origin of new species. Modern landscape management no doubt accelerates the process in Arizona by reducing natural disturbance. But management for conservation of the willow species appears to be a simple matter conceptually, usually involving restoration of water drainages, exclusion of cattle, creation of moist mineral soil patches for germination of seed and establishment of seedlings, and perhaps the judicious use of controlled burns.

Only one willow species in Arizona seems to have benefited somewhat from human disturbance, through construction. That is *S. lasiolepis*, which grows in wet sites around 2,300 m a.s.l. on the Colorado Plateau. Here road construction especially has opened up new habitat in the form of roadside ditches and borrow pits. However, this activity has less than compensated for destruction of riparian habitat by building and channelization and populations are dominated by old or senescent ramets.

Other willows in northern Arizona grow at higher elevations away from major construction, but are nonetheless impacted by reduced water flows, increased grazing pressure, and reduced fire.

Understanding the likely natural course of plant distribution over the landscape through tens of thousands of years appears to clarify planning and decisions on conservation of plant species and the biodiversity they support. Apparently, willows are in inevitable decline in Arizona for natural reasons, probably accelerated by land management practices. Such decline is likely to result in local extinction of some willow species before the next glaciation. Thus there is a simple conservation decision to intervene or not. If intervention is advocated, then sooner is better than later, because with larger populations genetic heterogeneity is likely to be higher and more of the biodiversity in upper trophic levels will be conserved.

### Acknowledgment

For research on willows, their herbivores and carnivores, we are grateful for financial assistance from Northern Arizona University and the National Science Foundation (BSR-9020317 and DEB-9318188).

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# Interagency Conservation Biology Program for Arizona Willow (*Salix arizonica* Dorn)

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**Abstract:** Arizona willow (*Salix arizonica* Dorn) was proposed for listing as an endangered species with critical habitat by the U.S. Fish and Wildlife Service in November of 1992. At that time, it was known only from the vicinity of Mount Baldy in east-central Arizona; no one was aware that the species occurred in Utah. Subsequent to the proposed rule, a 1913 collection from what was then named Sevier National Forest in Utah was located at the Forest Service National Collection housed at the Rocky Mountain Herbarium, Laramie, Wyoming. The final determination for listing the species was placed on hold pending the results of field work in Utah during the 1994 field season. Survey work initiated by the U.S. Forest Service in June of 1994 resulted in re-location of Arizona willow in southern Utah on the Dixie and Fishlake National Forests. Newly discovered populations in Utah far exceeded the number of total plants from Arizona, and significantly expanded the known range of Arizona willow. The U.S. Fish and Wildlife Service and U.S. Forest Service agreed to work on the development of a conservation plan for the species that would provide for implementation of short- and long-term protective measures to reduce threats to the species and its habitat. The resultant Arizona Willow Conservation Agreement and Strategy identified specific conservation measures for the species and formed the basis for withdrawal of the listing proposal in April 1995. It also functions as a collaboratively developed and supported recovery plan for Arizona willow.

## Introduction

As the Endangered Species Act (ESA) continues to suffer from the unwarranted notion that it is the "pit bull of environmental laws," the federal agencies charged with its implementation and regulatory oversight are increasingly seeking opportunities to demonstrate the efficacy and workability of the law by pursuing innovative means to protect rare and endangered species, while still meeting the spirit and intent of the Act. In 1994, federal agencies within the departments of Interior, Agriculture, Commerce, Transportation, and Defense entered into an important Memorandum of Understanding (MOU) for the implementation of the ESA (U.S. National Agencies 1994a). This MOU established a general framework for the cooperation and participation of its cooperators to achieve a common goal of conserving species listed as threatened or endangered under the ESA and to manage the ecosystems upon which these species depend. A second MOU, entered into by the U.S. Forest Service (FS) and the departments of Interior and Commerce, also in 1994, established the general framework for cooperation in the conservation of species that are trending toward federal listing under the ESA (U.S. National Agen-

cies 1994b). It was under this new environment of greater cooperation and innovation that the development of the interagency Arizona Willow Conservation Agreement and Strategy took its successful form (Arizona Willow Interagency Technical Team 1995).

## Background

Arizona willow (*Salix arizonica*) was described by Dorn in 1975 from specimens collected from the White Mountains in east-central Arizona by Granfelt in 1969, who recognized them as a distinct form (Phillips et al. 1982). At that time, the species was known only along high-elevation streams and wet meadows in the vicinity of Mount Baldy, Apache County, Arizona, primarily on the Apache-Sitgreaves National Forests and Fort Apache Indian Reservation. Extensive surveys conducted by Phillips et al. (1982), Galeano-Popp (1988), Granfelt (1989a, 1989b), Subirge (Apache-Sitgreaves National Forests, Springerville, Arizona), and others located the species in 15 drainages, often as single individuals widely scattered along streams.

Arizona willow exhibits several growth forms, including rounded shrub, prostrate mat, and large



hedge or thicket. Plants may occasionally reach a height of 3 m or be as short as several centimeters, but more typically are less than 0.75 m (Galeano-Popp 1988). The mature leaves are broadly elliptic to ovate, with rounded or cordate bases, and are 0.5–3 cm in length. The short petiolate leaf is gland-tipped, with finely serrate margins (7–21 teeth or glands per cm). The midrib of the leaf tends to remain pubescent, while the rest of the upper surface of the mature leaf may be slick and shiny, and is usually glabrous. The lower leaf surface is non-glaucous. Pistillate catkins are densely flowered, 1–4.5 cm long, with glabrous ovaries. The inflorescence has brown, black, or bicolor floral bracts 1–2.5 mm long, with wavy hairs and acute tips (Dorn 1975). Present-year branches are yellow-green, red-brown, or brownish in color and are pilose. The branches from previous growth are usually bright red, which helps distinguish this species from other willow taxa in the area (Figure 1).

### Regulatory History

Arizona willow's status as a very localized endemic prompted the inclusion of the species in category 1 of the December 15, 1980, Federal Register notice of plants under review for listing as endangered or threatened species (USDI 1980, 45 FR 82480). Category 1 includes those taxa for which the U.S. Fish and Wildlife Service (FWS) has sufficient information on biological vulnerability and threats to support the proposal to list under the ESA. This designation for Arizona willow was based on small populations and threats to riparian habitats by livestock (Fletcher 1978). In 1983, the willow was reclassified (USDI 1983, 48 FR 53640) to category 3C, which includes those taxa that have proven to be more abundant or widespread than previously thought and for which substantial threats do not exist. The inclusion of Arizona willow in category 3C was based on an assessment by Phillips et al. (1982) showing that, although the willow was a narrow endemic, it was locally common, with all known populations apparently healthy and reproducing. Category 3C species may be re-evaluated for possible inclusion in category 1 or 2 if further research or changes in habitat indicate significant decline in the taxa.

Arizona willow was placed in category 2 (USDI 1985, 50 FR 39526) due to further questions concerning vulnerability and threats to the small populations. Category 2 are those taxa for which there is evidence of vulnerability but for which

there are not enough data to support listing proposals. Studies by Galeano-Popp (1988) and Granfelt (1989a) presented additional information on vulnerability and threats faced by this species and supported moving the species again to category 1 (USDI 1990, 55 FR 6184). On November 20, 1992, FWS issued a proposed rule (USDI 1992, 57 FR 54747) to list Arizona willow as an endangered species with critical habitat based on historic and current threats to the species. Threats identified in the proposed rule include livestock and wildlife impacts, water impoundments and diversions, roads, recreational use, development and maintenance of ski resort facilities, disease, alteration of natural hydrologic regimes, and changes in the riparian community species composition and structure, including invasion of nonnative vegetation, especially Kentucky bluegrass (*Poa pratensis*), brought about by historic and current livestock use.

In June of 1993, FWS was notified of a previously misidentified herbarium specimen of Arizona willow collected in 1913 from what was then named Sevier National Forest in southern Utah. Surveys initiated by FS in the summer of 1994 resulted in the rediscovery of Arizona willow in Utah on the Dixie and Fishlake National Forests, Cedar Breaks National Monument, and adjacent private land (Figure 2). The extent of some individual populations and the stature of Arizona willow plants in Utah far exceeded all known populations in Arizona. On May 31, 1994, the Southwest Center for Biological Diversity filed a Summons and Complaint based on FWS's failure to meet statutory deadlines for publication of a final listing determination for Arizona willow. The FWS, in negotiation with the plaintiffs, reached an agreement to take final action (list or withdraw the proposed rule) by April 30, 1995, allowing time for additional field surveys.

### Management Involvement and Formation of the Technical Team

The discovery of significant Arizona willow populations in Utah presented new biological information that required a reassessment of range-wide threats to the species, and also provided an opportunity to pursue new management options such as described in the recently developed inter-agency MOUs for conservation of species trending towards federal listing and for the implementation of the ESA. On September 6, 1994, the regional foresters of the Southwestern and Intermountain



Figure 1. Arizona willow (*Salix arizonica*) morphological characteristics. From G. Argus, Canadian Museum of Nature, Ottawa, Ontario, Canada, with permission. The scale bars shown are as follows: upper left 1 mm; upper right 1 mm; center left 1 cm; bottom center 1 mm; bottom right 1 mm.

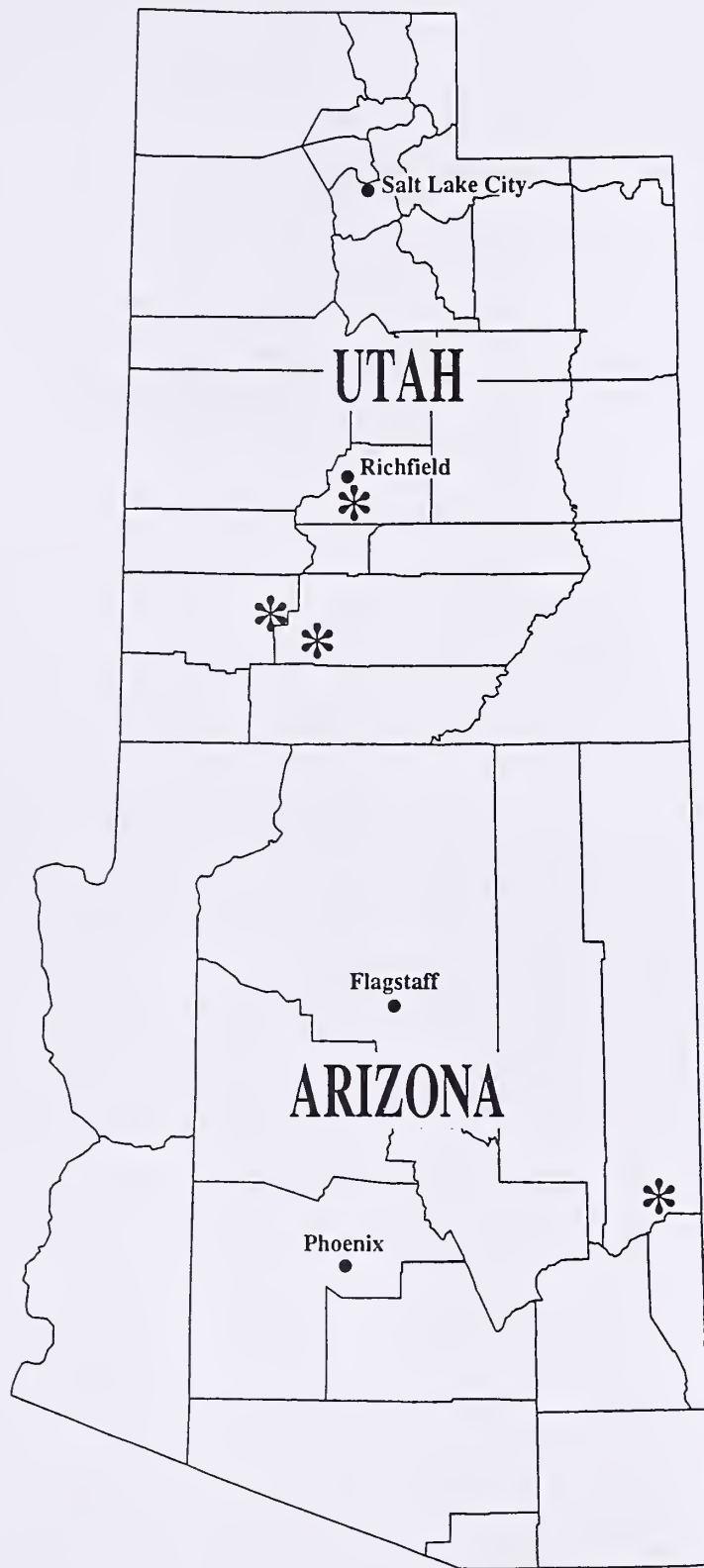


Figure 2. The known distribution of Arizona willow (*Salix arizonica*) following the completion of the 1994 field surveys.

Regions of the Forest Service, and the regional director of the FWS Southwest Region, made a joint decision to develop a conservation agreement and strategy for Arizona willow on federal lands to ensure the long-term conservation of the species throughout its range within its natural ecosystems. This would be accomplished through the implementation of actions to immediately reduce site-specific threats, provide long-term commitments to protect and improve habitats, and carry out proactive conservation actions. The Arizona willow interagency technical team was formed to develop and implement the Arizona Willow Conservation Agreement and Strategy. Concurrent with the development of the federal agency conservation agreement for federal lands, the White Mountain Apache Tribe developed its own Arizona willow management plan for the Fort Apache Indian Reservation, which is consistent with, and complementary to, the strategies and intent set forth in the Arizona willow conservation documents.

### Development of the Conservation Document

The Arizona Willow Conservation Agreement and Strategy was developed by the FWS, FS, and National Park Service (NPS) in cooperation with the Arizona Game and Fish Department, Utah Division of Wildlife Resources, and White Mountain Apache Tribe. Many non-agency experts provided the interagency technical team with additional scientific data and support in the development of this document.

There are three key components to the Arizona willow conservation document: the assessment, the strategy, and the agreement.

1. Conservation Assessment—This presents the existing data available for Arizona willow throughout its range. The biology of the species, including its description, distribution, and habitat requirements, are discussed in this portion of the document. Land uses, habitat modification, and impacts from past and current threats are evaluated. Current protection mechanisms available to Arizona willow are reviewed.

2. Conservation Strategy—This outlines a framework for management actions that provide for the immediate and long-term conservation of the species within its ecosystem throughout its range. This portion of the document details specific actions and timeframes that the management agencies agree to implement. These actions are to

reduce threats, improve habitat conditions, initiate monitoring studies, and support research projects on the biology and ecology of the species to determine appropriate management practices for the long-term conservation of the species.

3. Conservation Agreement—This is the formal written portion of the document agreed to by FWS, FS, and NPS. It documents the specific actions and responsibilities for which each party agrees to be held accountable. The agreement is a signed commitment by agency executives and line officers to implement their respective portions of the conservation document. The FWS may enter into similar agreements with state agencies, tribes, or the private sector to achieve conservation of species through voluntary cooperation.

### What Does the Conservation Document Do?

The agreement documents specific actions and responsibilities to be undertaken by each party to achieve recovery of the species. It provides for immediate protection to Arizona willow plants, establishes a program for long-term conservation and recovery, and applies an ecosystem approach to the management of high-altitude riparian habitats. Fifteen other rare plant and animal species will be the direct beneficiaries of this conservation effort.

Some specific conservation actions for Arizona willow include construction of protective fences, exclosures, and cages, resting pastures from grazing, and application of FS Standards and Guidelines for management of riparian areas. The Agreement includes an annual schedule of actions and budgeting to ensure that conservation activities are implemented in a timely manner.

The agreement sets up an interagency technical review team that oversees monitoring of Arizona willow populations, reviews annual progress and implementation, and makes recommendations for management. The agreement is adaptive in that it allows for changes and/or modification to the document based on the results of monitoring, new research data, or management considerations.

### Advantages of Conservation Agreements

The main advantage in developing conservation documents is to provide signed commitments and agency strategies to implement programs for the protection and conservation of rare species. It is not one agency telling another what to do. These

collaboratively developed documents allow agencies to work together to achieve common goals, assemble interdisciplinary teams of specialists, and provide recommendations to land managers. They ensure adherence to all legal requirements and provide a basis for, and commitment to, the new direction.

A conservation agreement can also function as a collaboratively developed and supported recovery plan for a species. Recovery plans are usually developed by FWS for species that have been formally listed as threatened or endangered under the ESA. The Arizona Willow Conservation Agreement and Strategy is different in that it is a jointly developed document, and has the up-front support and commitment of all involved parties.

Although Arizona willow is still considered rare and potentially vulnerable, the new distribution data, in combination with the management commitments in the Arizona willow conservation documents, reduce the relative magnitude and severity of threats. The FWS has determined that Arizona willow does not warrant listing under the ESA and has placed this plant in category 3C. The notice of the withdrawal of the proposed listing rule was published April 28, 1995 (USDI 1995, 60 FR 20951). This is the first instance where FWS has withdrawn a proposed rule based on the development of a conservation agreement.

Ultimately, this process and approach for the conservation and management of rare species will save time and taxpayer dollars compared to listing under the ESA. It eliminates the need for listing, delisting, critical habitat designation, section 7 consultation, and development of recovery plans, while still providing for the conservation and long-term protection of the species.

### National Support for the Arizona Willow Conservation Agreement and Strategy

This document is the first agreement to meet the intent and to implement the national MOUs developed in 1994. It has received support from the highest department and agency levels in Washington, D.C., such that a media event was scheduled for a signing ceremony that was held in Phoenix, Arizona, on May 19, 1995. A Declaration of Support was signed by representatives of the Secretary of Interior (representing FWS and NPS) and chief of the Forest Service offices. We believe the Arizona Willow Conservation Agreement and Strategy represents an important step, not only in the conservation of the Arizona willow, but as a

renewed focus and commitment to the ESA, without further eroding the integrity of this landmark statute.

### Acknowledgments

We appreciate the contributions and dedication of the many individuals involved with the conservation of Arizona willow and the development of the Arizona Willow Conservation Agreement and Strategy. This includes many agency and non-agency participants. We would like to give special thanks to the Intermountain Research Station, Rocky Mountain Forest and Range Experiment Station, Brigham Young University, Arizona Game and Fish Department, Utah Division of Wildlife Resources, The Arboretum at Flagstaff, Robert Dorn, Carl-Eric Granfelt, George Argus, and the professional and support staffs of FS and FWS. During the development of this document, a tragic accident claimed the lives of two of our team members, Gary L. Davis and David W. Payne. The Arizona Willow Conservation Agreement and Strategy is dedicated to their memory.

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# The Verde Valley Sonoran Desertscrub: An Ecosystem at Risk

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**Abstract:** Fragmentation and degradation of the isolated Sonoran Desertscrub ecosystem in the Verde Valley is occurring due to human population explosion and the resultant increased impacts on national forest lands. In 1994 and 1995, potential habitat for *Purshia subintegra* (Kearney) Henrickson (Arizona cliffrose) and four other rare plants was surveyed. Combined with general animal surveys and an elucidation of geology and soils, the results indicate the importance of expanding the existing Verde Valley Botanical Area into a broader ecosystem-based unit.

## Introduction

*Purshia subintegra* (Kearney) Henrickson (Arizona cliffrose) was listed as endangered by the U.S. Fish and Wildlife Service on May 29, 1984 (U.S. Fish and Wildlife Service 1984). Arizona cliffrose, a member of the Rose Family (Rosaceae), is a rare Arizona edaphic endemic, restricted to nutrient deficient calcareous soils (Anderson 1986, Anderson 1993). It is known from four disjunct populations on the northern edge of the Sonoran Desert (U.S. Fish and Wildlife Service 1994). For 30 years the species was only known from the type locality near Burro Creek, Mohave County. In September 1968, a second population was discovered near Bylas, Graham County (Pinkava et al. 1970). In 1984 and 1985, two additional population areas were discovered near Cottonwood, Yavapai County, and near Horseshoe Lake, Maricopa and Yavapai counties.

The recovery plan for *Purshia subintegra* was recently finalized (U.S. Fish and Wildlife Service 1994). This plan listed several downlisting criteria for the species. (1) Scientific data indicate that each of the recovery units sustains a population that is viable or that is on a significant upward trend towards viability that is maintained for at least 15 years. (2) Unfragmented and high-quality habitat sufficient to ensure long-term survival and recovery is protected within each recovery unit. (3) Regulatory mechanisms or written land management commitments that provide for adequate long-term protection of Arizona cliffrose and its habitat are being implemented and will continue to be implemented after downlisting. (4) The Service determines that Arizona cliffrose is no longer an endangered species, as defined by the Endangered Species Act.

## The Arizona Cliffrose Population of the Verde Valley

The Verde Valley Cottonwood population (and the other populations of Arizona cliffrose) are in the *Larrea tridentata*-*Canotia holacantha* (creosote bush-crucifixion thorn) association of the Arizona Upland Subdivision of the Sonoran Desertscrub (Brown 1982), because crucifixion thorn is a dominant at each site. The disjunct ranges of several plant species parallel the disjunct pattern of Arizona cliffrose (Anderson 1986) and four rare Arizona endemic plants occur in the same habitat as Arizona cliffrose (U.S. Fish and Wildlife Service 1994).

The Verde Valley encompasses the middle part of the Verde River Basin. It is bounded by the Mogollon Rim on the northeast and the Black Hills on the southwest and is approximately 32 km at its widest. During the Tertiary a series of lava dams blocked the natural river drainage and a series of lakes and playas formed resulting in deposits known as the Verde Formation, which varies in thickness from a few meters to over 915 m.

At the upper end of the Verde Basin, the Verde Formation is a Pliocene limestone with interbedded clastic and tuffaceous sediments (Nations et al. 1981). The greatest densities of *Purshia subintegra* are found on open flat ridgetops or other level areas and along shallow, first-order drainages. It occurs on all but the steepest slopes. It is found rooted in either white calcareous soils derived from a limestone member of the Verde Formation, red soils from a calcareous sandy member of the Verde Formation, or a mixture of both (Schaak and Morefield 1985, Denham and Fobes 1992). *P. subintegra* was not found on the Verde Formation at the southern end of the basin

(Anderson 1986), which is stratigraphically lower and contains Miocene evaporite deposits (Nations et al. 1981).

The Cottonwood Arizona cliffrose population is the largest, healthiest population, and it occurs at the highest elevation. The Coconino Forest Plan (U.S. Forest Service 1987) designated part of this population as the 472 ha Verde Valley Botanical Area. This was done for the protection of the unique plant community, including Arizona cliffrose. This plan states (p. 194) that the management emphasis of the botanical area is to:

maintain, as nearly as possible, existing conditions and natural processes for public enjoyment, demonstration, and study. Interpretive and educational demonstration opportunities are emphasized and enhanced through selective facility development. Natural events are not rehabilitated. Off-road driving is prohibited.

### The Human Population of the Verde Valley

The earliest evidence for humans in the Verde Valley is during the Archaic period (8000 B.C.–A. D. 1) (Beard 1990). Population expansion occurred between A. D. 1125 and 1300 and great pueblos were eventually constructed: Montezuma Castle near present-day Camp Verde, Tuzigoot near present-day Cottonwood, and others. Between A. D. 1300 and 1400 consolidation of the pueblos took place into approximately 40 major sites throughout the valley, surrounded by smaller communities and agricultural areas. These dwellings were abandoned by approximately A. D. 1425 and the Northeastern Yavapai, who were nomadic hunter-gatherers, came to occupy the Verde Valley. The first Anglo settlement in the Middle Verde Valley was established in 1865. Construction of check dams and irrigation ditches, the removal of beaver, and an increased flow of water because of decreased vegetation due to livestock impacts on uplands transformed the Verde River from a wide, slow-moving waterway with plentiful marshes and ponds to a narrower fast-flowing stream.

The mining era, a period of approximately 70 years, began in the 1870s in the Black Hills (Twenter and Metzger 1963). Advances in technology made mining more economically feasible over time; however, impacts to the Verde Valley ecosystem greatly increased. Woodcutting for mining timbers, building materials, and fuelwood denuded hillsides of substantial trees in the vicinity of Jerome, Clarkdale, and Cottonwood. Smelters at

Clarkdale and Clemenceau (present-day Cottonwood) released toxic fumes into the Verde Valley that were responsible for serious impacts to agricultural and native vegetation (Beard 1990).

The human population of the Verde Valley continues to grow (Table 1), resulting in increased impacts to the Verde Valley and the unique Verde Formation ecosystem. Habitat loss due to urbanization, road construction and realignments, recreational activities such as off-road vehicle use, horseback riding, hiking and running, cattle grazing, control of insect pests, and limitation in pollinators are some of the management issues and concerns for the Cottonwood population (U.S. Fish and Wildlife Service 1994). Surveys were initiated by the Coconino and Prescott national forests to gain a more comprehensive understanding of the distribution of *Purshia subintegra* and other rare plant and animal species in the Verde Valley in order to be able to better address current impacts, potential land exchange requests, and future management concerns.

### Methods

Surveys were conducted by Lutz (1994a,b, 1995) and Popowski (1994) from May through September 1994 and April 1995 for *Purshia subintegra*, *Eriogonum ericifolium* Torr. & Gray var. *ericifolium* (Heather-leaf wild buckwheat), *Eriogonum ripleyi* J.T. Howell (Ripley's wild buckwheat), *Eriogonum* sp. (undetermined species) and *Salvia dorrii* (Kellogg) Abrahams ssp. *mearnsii* (Britton) L. Benson (Verde Valley sage or Mearns sage) in areas of potential habitat for Arizona cliffrose. Potential habitat for Arizona cliffrose can also support the occurrence of these other four species of interest. Additional surveys conducted by contractors (Baker and Wright 1995) and other Forest Service personnel (Hanneman 1995) added to our knowledge of the distribution of these taxa.

Table 1. Historical and projected population of the Verde River Basin (1963–2040). Data for 1963 from Twenter and Metzger (1963). Data for 1990, 2000, and 2040 from Arizona Department of Water Resources (1995).

	1963	1990	2000	2040
Verde River Basin		102,740	125,141	234,433
Camp Verde	1500	6243	8415	18,579
Clarkdale	1000	2144	2812	5940
Cornville		2089	2924	6834
Cottonwood	3000	7037	9576	21,461
Lake Montezuma		1841	2342	4688



Maps from the Forest Service Terrestrial Ecosystem Survey (TES) (U.S. Forest Service, n.d.) and Surficial Geology of the Northern Verde Valley, Yavapai County, Arizona (House and Pearthree 1993) were utilized to locate potential search areas. First the TES maps were placed over maps showing the current population of *Purshia subintegra*. Arizona cliffrose occurs primarily on two soil types that are characterized by Tertiary lakebed deposits of calcium carbonate, with one steeper than the other. Plants also grow in a small area mapped as a different soil type; however, this is a low drainage area and the Arizona cliffrose occurs primarily on drainage cuts with limestone ledges in this mapping unit.

Aerial photographs were examined prior to going in the field to narrow the search areas. Transects approximately 30 m apart were walked. Binoculars were used to observe the vegetation from a distance in some areas, then plants were checked at closer range if warranted. Wildlife records were made from ocular sightings and sign (such as tracks, droppings, digging, burrowing, stick nests, feathers, pellets, and skulls).

Forest Service geologists and soil scientists added some interpretations to the field observations of the geology and soils made on May 18 and September 28, 1994 (Mathews 1995).

## Results

Arizona cliffrose was not located beyond the limits of the population known previously. However, many new locations of the other four species of interest were discovered and mapped (Figure 1). In addition, clones of *Agave delamateri* Hodgson & Slauson (Tonto Basin agave) (W. Hodgson, personal communication) were discovered by Baker and Wright (1995) in the vicinity of Montezuma Castle National Monument at the southeastern end of the Verde Valley.

Refinement was made of the characterization of the soils where *Purshia subintegra* grows in the Verde Valley (Lutz 1995, Mathews 1995). *Purshia subintegra* appears to grow at the northern end of the Verde Formation where there is a mix of red clastic and carbonate sediments. Based on their composition, together with the geographic setting, most of the clastics were probably derived from the redbeds of the Schnebly Hill Formation, transported fluvially into the lowlands of the Verde Valley, and eventually deposited in thin layers in a lacustrine environment. In much of the area where Arizona cliffrose occurs, the carbonate and clastic

components of the parent material have been somewhat reworked and mixed in the soil. The carbonate component would tend to create an alkaline environment, while the clastics would provide for better drainage, as well as contributing trace elements such as iron and manganese (Mathews 1995). Arizona cliffrose seems to prefer basins and other protected areas with a dominant south, southwest, or west aspect in this highest elevation population area. The plants generally (with exceptions) occur at elevations below 1128 m. Any occurrences at higher elevations would probably be as isolated patches in exceptionally protected areas (Mathews 1995).

*Eriogonum ericifolium* var. *ericifolium*, *Eriogonum ripleyi* and *Salvia dorrii* ssp. *mearnsii* prefer a very white carbonate soil with sparse vegetation (Lutz 1995). In the Middle Verde area, Lutz noted a preference by these plants for a particular geologic layer, although the plants are not limited to this layer. Mearns sage has the greatest range and tolerance for habitat diversity of these three species, growing also on red Supai Sandstone soils in areas with denser shrub cover. We hypothesized that this very white carbonate layer might be the diatomite layer described by Nations et al. (1981) as occurring in the vicinity of the Middle Verde Road, so we collected soils for analysis, which has not been completed to date.

Mammal species typical of this desert environment were observed by sightings or sign: *Canis latrans mearnsi* (coyote), *Urocyon cinereoargenteus scottii* (gray fox), *Lepus californicus deserticola* (black-tailed jack rabbit), *Sylvilagus audubonii arizonae* (desert cottontail), *Eutamias dorsalis* (cliff chipmunk), *Citellus variegatus* (rock squirrel), *Antilocarpa americana* (antelope), *Neotoma* spp. (woodrats), several species of Heteromyidae and Muridae, and many species of birds (Popowski 1994).

## Discussion

The recent surveys in the Verde Valley enable us to have a more complete understanding of the distribution and range of the endangered listed *Purshia subintegra* and several Forest Service sensitive plants of the Verde Valley Sonoran Desert-scrub. In addition, the geology, soils, and animal components of this unusual ecosystem have been correlated with the plants' distributions.

Synthesis of this information is starting to reveal new ideas regarding this ecosystem. The carbonate/clastic composition of the sediments

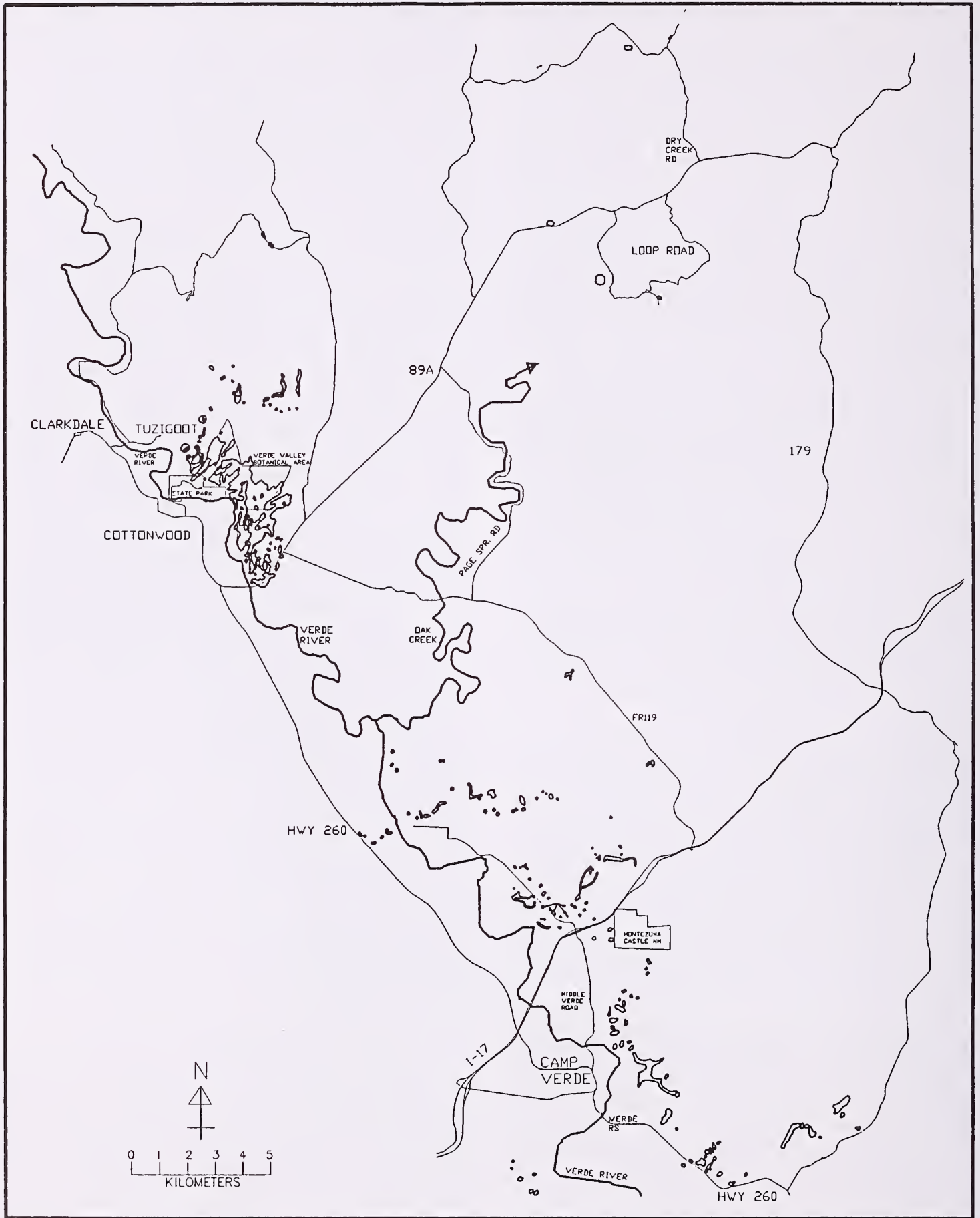


Figure 1. Sensitive plants of the Verde Valley, Arizona.

where Arizona cliffrose grows, along with the microclimate resulting from the geomorphological characteristics of the area, may both be significant factors (Mathews 1995). If so, much of the southern part of the Verde Valley, which is underlain by evaporite deposits, as well as the broad tablelands and rolling hills that are present throughout the Verde Valley wherever the lacustrine deposits have not been highly eroded or dissected by drainages, would not be suitable habitat for Arizona cliffrose. Areas underlain by more massive limestone deposits, which formed in the deepest parts of the lake, would also be excluded if this is true. However, the southeastern part of the Verde Valley that contains the other Forest Service sensitive species is an integral part of the Verde Valley Sonoran Desertscrub ecosystem.

Using the habitat criteria above, *Purshia subintegra* should be growing further north on the east side of the Verde River than currently known. Historically, emissions from the smelters in the vicinity of Cottonwood were credited with considerable destruction of vegetation within the Verde Valley (Beard 1990). Although the map (Figure 24 in Beard 1990) has question marks for the extent of the smoke damage east of the Verde River near Cottonwood, these smelter emissions could have affected Arizona cliffrose distribution by killing adult plants. Heavy metals deposited in the soils could also be limiting seedling establishment (Mathews 1995).

As shown in Table 1, the human population of the Verde Valley continues to grow. Cottonwood and Camp Verde, which were estimated at 3000 and 1500 inhabitants in 1963 (Twenter and Metzger 1963), had 7037 and 6243 inhabitants in the 1990 census and are projected to have 9576 and 8415, respectively, by the year 2000 (Arizona Department of Water Resources 1995). This increase in people in the Verde Valley is resulting in increased pressure on the Verde Valley Sonoran Desertscrub ecosystem of the public, as well as private, lands.

The Ehrlich-Holdren Three Factor Model (Impact = Population x Affluence x Technology, or  $I = P \times A \times T$ ) can be used to assess the environmental impact of a population in a given area (Miller 1994). As any of these factors increases, the impact increases. If any factor is zero, there is no impact. The number of people in the Verde Valley today has a greater impact than the same number during the Archaic Period or in the early 1900s, because consumption per capita or affluence (A) is

greater today and the environmental destructiveness of the technologies used to provide and use resources (T) is greater.

The human population of the Verde Valley will probably continue to increase as projected. However, it is possible to ameliorate the impacts of this population growth through foresight and progressive planning to lessen the environmental destructiveness of this increase. The Forest Service is working with state and local planners to develop more comprehensive and ecosystem-based plans for the Verde Valley. Part of this planning effort will be directed toward making the Verde Valley Botanical Area more encompassing of the true distribution of the Sonoran Desertscrub community and establishing management to lessen the human impacts on this fragile ecosystem.

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# Recommendations for the Preservation of Rare Plants and Unique Habitats Within the Chino Valley Ranger District, Central Arizona

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**Abstract:** Five Forest Service sensitive plant taxa have been documented over 3 years of botanical surveys within Yavapai and Coconino counties, central Arizona, in the Chino Valley Ranger District. These are *Erigeron saxatilis*, *Eriogonum ripleyi*, *Hedeoma diffusum*, *Salvia dorrii* ssp. *mearnsii*, and *Talinum validulum*. Another sensitive species, *Ivesia multifoliolata*, occurs within a mile of the Ranger District boundary. Populations of several additional rarely collected plant taxa were also recorded. Unique habitats occurring within the Ranger District included narrow canyons dominated by large ferns, pygmy woodlands of *Cupressus arizonicus*, and groves of *Quercus gambelii* x *Q. turbinella*. The greatest impact on these and other habitats appeared to be accelerated pluvial runoff owing to more than a century of heavy livestock grazing. General management for rare plant species should begin with the restoration of upland grass and forb populations.

## Introduction

Botanical field surveys have been conducted for the past 3 years within the Chino Valley Ranger District (CVRD) of the Prescott National Forest, Arizona (Baker and Wright 1993, 1994, 1995). Their focus has been the documentation of rare plant populations, unique habitats, and evidence of threats to the stability of these populations and habitats.

Assuming that decreases in overall diversity of both species and habitats correlate with the absence of populations of sensitive species (Holsinger and Gottlieb 1991, West 1993, Westman 1990), it is argued that the least expensive and most effective method for fighting extinction is through multispecies management, focusing on the preservation of habitat types (Scott et al. 1987).

Aerial photographs were first scrutinized for potentially unusual sites, and the surveys then proceeded according to uniqueness of habitat types. Fertile specimens were collected of each species for the preparation of at least one herbarium sheet. For unusual populations, at least one duplicate was collected as a gift for determination by the taxonomic specialist. Locality information was recorded in a redundant fashion using township and range as well as the UTM system. Important aspects of the plant that would not be preserved on a herbarium sheet were also recorded, and photographs were often taken as well. Data were also recorded for unique habitats, unusually healthy habitats, unusually unhealthy habitats, and factors that may have been affecting the health of these habitats.

## Sensitive Taxa and Unique Habitats

Forest Service sensitive taxa documented during our surveys include *Erigeron saxatilis* Nesom (cliff fleabane), *Eriogonum ripleyi* J.T. Howell (Ripley wild-buckwheat), *Hedeoma diffusum* Greene (Flagstaff pennyroyal), *Ivesia multifoliolata* = *Potentilla multifoliolata* (Torrey) Keck (Arizona cinquefoil), *Salvia dorrii* (Kellogg) Abrahams ssp. *mearnsii* (Britton) L. Benson (Mearns sage), and *Talinum validulum* Greene (Tusayan flame-flower). Approximately five to ten thousand individuals of *Erigeron saxatilis* occurred within Tule Canyon of the Sycamore Canyon Wilderness Area. A single dense population of *Eriogonum ripleyi* occurred near the Verde River at the site of the dormant United States Copper Mine northwest of Perkinsville. Individuals were especially abundant along roads and on mine tailings. Populations of *Hedeoma diffusum* occurred most often on shaded slopes in shallow soil derived from Coconino Sandstone or along cracks in the bedrock. They occurred less frequently on outcroppings of Kaibab Limestone. Individuals of *Ivesia multifoliolata* occurred sporadically along the bottom of Tule Canyon, generally along crevices in sandstone and among river cobbles. Larger populations were associated with basalt cobbles in shallow washes feeding the canyons. Several dense populations of *Salvia dorrii* ssp. *mearnsii* occurred on pink soil derived largely from a combination of sandstone and limestone within and near the United States Mine site. The largest population, north of the Gray Hills, contained roughly 50,000 individuals. Populations of *Talinum validulum* were occasional

on limestone and basalt soils of recent origin, especially near the edges of mesas. Roughly 80,000 individuals occurred within the Juniper Mesa Wilderness Area, ca. 700 individuals within the Woodchute Wilderness Area, and at least 100,000 near the western boundary of Prescott National Forest, west of Camp Wood.

A unique community of a hybrid oak occurred at ca. 1830 m elevation on the northeast slopes of Woodchute Mountain, just northwest of Jerome. The oak appears to be intermediate between *Quercus gambelii* Nutt. (Gambel oak) and *Q. turbinella* Greene (scrub oak). These small groves (ca. 100 m wide) of monopodial trees had arching trunks that formed a closed canopy about 4 m tall. The understory was open and diversity was low.

Within the Sycamore Canyon Wilderness Area above Cedar Creek, at an elevation of ca. 1820 m, the shallow soils of Coconino Sandstone ridgelines supported an unusual plant association of pygmy *Cupressus arizonica* Greene (Arizona Cypress) and *Arctostaphylos pungens* Kunth (point-leaf manzanita). The individuals of Arizona cypress exhibited bizarre forms owing to their twisted trunks and patchy compact foliage.

Narrow north-running sandstone canyons within Tule Canyon provided moist retreats for the large-leaved ferns *Cystopteris reevesiana* Lellinger, *Dryopteris filix-mas* (L.) Schott, and *Polystichum lonchitis* (L.) Roth, and mesic herbs of usually higher elevations, such as *Osmorhiza depauperata* Phil., *Maianthemum racemosum* (L.) Link, *Senecio wootonii* Greene, and *Valeriana arizonica* A. Gray. The rarely collected fern, *Phanerophlebia auriculata* Underwood, occurred in one of these canyons.

### Conditions of the Range and Suggested Management

The CVRD has been overgrazed for more than a century, as is characteristic of most western rangeland (Ellison 1960). Most ranchers, U.S. Forest Service personnel, biologists, and other members of the community interviewed have agreed that past use has led to a decrease in overall species diversity. Although change, at least in part, is often attributed to recent drying trends (Bahre 1991, Van Devender 1990), there is considerable evidence within the CVRD that most, if not all, of the loss in both biomass and species diversity within the last century is attributable to land use.

Evidence of overuse by browsers and/or grazers occurred throughout the survey areas. Palatable shrubs, such as *Purshia stansburiana* (Torrey)

Henrickson (cliff-rose), were often clipped to near ground level. In many areas grass density was near zero and grasses occurred only as refugees protected by boulders, cactus, or inaccessible benches. Fence lines often manifested potential for habitat improvement, where vegetation cover was dramatically different from one side of the fence to the other. In several areas deep channel erosion and a paucity of perennial riparian vegetation indicated severe flooding. Very little "A" soil horizon remained within the CVRD and, in many areas, only the "C" horizon remained except where the soil was protected by woody perennials.

The volume of water runoff is inversely correlated with upland plant densities, especially those of fibrous-rooted and generally palatable perennial grasses. The damage to soil and vegetation in these areas should not be treated too lightly since areas similar in elevation, topography, latitude, and amount and distribution of precipitation in other parts of the world have been desertified beyond recovery (Bryson and Baerreis 1967, Wheeler 1960).

Generalizations concerning range potential and the possible methods necessary to achieve a desired potential can be unproductive when applied to a range composed of diverse habitats. Sites varied greatly with respect to soil composition, soil depth, topography, elevation, management history, and species composition. In almost all communities within the CVRD, proper management could dramatically increase biomass and species diversity, and reduce the density of plants poisonous to livestock (James et al. 1992).

McNaughton et al. (1982) suggest that three fundamental processes affect the structure of grasslands: carbon assimilation and allocation, nitrogen assimilation and allocation, and rainfall-evapotranspiration. In terms of management, these processes may best be approached in terms of shade, available soil nitrogen, and soil moisture. Dodd and Lauenroth (1979) reported that through application of supplemental water and nitrogen to blue grama grassland, the aboveground annual net primary production was increased by factors of 1.3 to 2.7 with nitrogen and from 2.0 to 4.6 with water, while the addition of both water and nitrogen increased productivity by factors between 5.0 and 13.0.

**It is usually cost effective in the long term to experiment with several management tactics.** For example, short-duration grazing following the introduction of supplemental feed might create soil conditions conducive to the re-establishment

of native grasses (see Goodloe 1969, Savory 1978). Savory's design includes the construction of several pastures so that some are allowed to rest. This would be especially valuable with respect to the survey area, considering the high degree of site variation and hence the differing periods of rest needed to maintain a sustained yield. Pastures would be best defined along natural boundaries, such as mesa edges and drainages, although local ranchers claim they can avoid additional fencing by proper herding techniques. Most problems with short-duration grazing seem to come from extending the grazing duration for too long or grazing on soils that are not sufficiently stabilized.

Grazing is a complex question, since domestic animals compete with native animals. The role of domestic animals has probably been overrated by interested parties as there is ample evidence that the most important consumers occur below the soil surface and the predominant aboveground consumers are the arthropods (Scott et al. 1979).

The principal external source of nitrogen to midcontinental grasslands is from the atmosphere. The reintroduction of nitrogen back into the soil by cattle dung and urine is insignificant compared to the nitrogen loss from the presence of cattle (Woodmansee 1979). In this regard, cattle grazing results in a long-term deficit with respect to nitrogen, aboveground biomass, and net primary productivity. Perhaps steps can be taken to replenish some of the nitrogen consumed by cattle. Since nitrogen loss from feces and urine is minimized by a healthy soil fauna and humus content (Woodmansee 1979), maintaining a healthy grassland is more energy effective than continually attempting to restore it.

In areas of high *Juniperus osteosperma* (Torrey) Little (Utah Juniper) density, the CVRD has conducted hand slashing of selected trees in an attempt to enhance grass and forb cover. The slash is spread evenly over the soil, which reduces areas of dense shade and adds partial shade to barren areas. The shade of the slash seems to let in sufficient light for grass and forb seedling germination while enhancing average soil moisture by reducing surface evaporation. The success rate of juniper woodland management probably depends on the degree of soil development. The most suitable time, both economically and ecologically, to manipulate juniper woodland is probably when aboveground biomass is great enough to carry a fire. At this time, either controlled fire or slash treatments can be used to open the woodland. Local trials should determine which method is

best under specific conditions and under specified management protocol. Slash treatments by the U.S. Forest Service southeast of Ashfork and by cattle rancher Dennis Moroni east of Camp Wood have shown almost immediate improvement in terms of plant species diversity and herbaceous biomass. This method is far superior to herbicide application, juniper pushing, soil ripping, and brush crushing, all of which have inherently undesirable effects and/or have provided uneconomical results.

**Although controlled burning reflects a natural phenomenon, it can act as a two-edged sword.** Applied during certain times of the year or in certain areas, fire can have damaging effects, such as the enhancement of both economically and aesthetically undesirable grasses (Hull and Pechanec 1947, Robertson and Kennedy 1954). On the other hand, in areas invaded by weedy shrubs, such as *Gutierrezia sarothrae* (Pursh) Britt. & Rusby (Campbell and Bomberger 1934, Ellison 1960, Humphrey 1958), fire has been useful in enhancing palatable grasses (Reynold and Bohning 1956).

Although little is known of the specific role of fire in the region, at least four of the historically most important grasses of northern Arizona, *Bouteloua gracilis* (Kunth) Lag. ex Steud. (blue grama), *Pascopyrum smithii* (Rydb.) A. Löve (western wheat grass), *Sporobolus cryptandrus* (Torrey) A. Gray (sand dropseed), and *Elymus elymoides* (Raf.) Swezey (squirrel-tail) (Vose and White 1991), are enhanced by burning. In Chino Valley, old agricultural fields occupied largely by *Kochia scoparia* (L.) Schrad. (summer-cypress) and *Salsola tragus* L. (Russian-thistle) slowly convert to the beginnings of a mixed prairie dominated by sand dropseed and western wheatgrass (see also Costello 1944). After sand dropseed becomes dominant, however, new growth dramatically decreases, presumably because of the accumulation of dead, gray, matted leaves. This syndrome is common in many grass species (Beard 1953, McNaughton et al. 1982, Hadley and Kieckhefer 1963). After burning our Chino Valley plot in August, summer rains activated new growth from the perennial crowns of the sand dropseed, and the plots soon became lush and green, while adjacent, untreated areas remained gray. Burning removes the older, shading leaves, which allows light to penetrate to subtending shoots. Both burning and mowing have the added benefit of increasing soil temperature, an important factor early in the growing season (Rice and Parenti 1978). Similar observations with western wheat grass have been made by Forest Service

range conservationist Jay Ebby (personal communication 1994).

At a number of sites within the CVRD, especially where a barren soil surface is prevalent, soil lichens and algae seem to play an important role in soil stability. Abundant evidence has been presented from various arid and semiarid rangelands that support these observations (Clair et al. 1993, Eldridge 1993, Harper and Marble 1988). Harper and Marble (1988) report that blue-green algae, lichens, and mosses increase soil water retention, increase nitrogen availability, and improve the microenvironment for the establishment and growth of vascular plant seedlings. Harper and Pendleton (1993) show that cyanobacteria and cyanolichens enhance growth and essential element uptake by associated herbs. These organisms and their underlying substrate are extremely delicate, however; they are easily damaged by mammals (Clair et al. 1993) and they recover very slowly from disturbance (Belnap 1993). Domestic livestock could be kept away from these areas until sturdier grass and forb species have completely stabilized the soil.

**Reclamation should focus on the reestablishment of native palatable grasses, sedges, rushes, and forbs,** since these often correlate with the general health of communities, have economic value, and have received the heaviest grazing pressure. Since germ plasm for many palatable species has been extirpated by resource depletion and seed predation, reseeding is necessary to reestablish climax species within an economically feasible time frame. Without exception, reseeding should be done using native species. The introduction of non-natives only confuses the processes of evolution that have developed over millions of years.

In various sites, grasses such as *Bouteloua curtipendula* (Michx.) Torrey (side-oats grama), *B. gracilis*, *Hilaria mutica* (Buckl.) Benth. (tobosa), *Pascopyrum smithii*, *Poa fendleriana* (Steud.) Vasey (mutton grass), *Stipa speciosa* Trin. & Rupr. (desert needlegrass) and *Stipa neomexicana* (Thurb. ex Coult.) Scribn. (New Mexican feathergrass) were remarkably abundant. The plant diversity within many of these areas was notably greater than within areas where grass cover was poor. Seed and mulch from these areas of dense grass cover could be harvested and used to reseed more barren adjacent areas. The mulch would promote higher soil moisture levels (Dysterhuis and Schmutz 1947) and reduce seed predation. This very simple process may also have a positive effect on the cut area (Penfound 1964). Net photosyn-

thesis in western wheat grass, for example, has been shown to increase after shoots were clipped (Dyer et al. 1982). Also, as mentioned earlier, removal of old leaves improves net productivity.

**Species diversity and hence populations of rare species would be enhanced through the designation of permanent grazing-free areas.** These would also be of great value for range research and as sources for germ plasm. Although such exclosures often require many years to show significant changes (see Brady et al. 1989, Gardner 1950), they would not need to be of great size. In fact, Quinn and Robinson (1987) provide evidence that a sum total of small areas contains a greater plant diversity than an equal area divided into larger subunits. These small grazing-free areas could be incorporated into a system of wildlife corridors between larger preserves.

Much of the Limestone Grazing Allotment, southwest of Ashfork, had been resting for 5 years prior to our survey. Although rested sites in general appeared to possess a much greater above-ground biomass and plant diversity than similar sites under recent grazing pressure, they varied greatly among themselves. Some areas within the allotment, for example, supported a grass-forb cover of nearly 100 percent, while others, with apparently similar soils and topography, supported little or no cover. Indeed, I would have hypothesized that the basalt soils of the northeastern portion of the allotment were naturally sterile of non-woody species if it were not for the abundance of grass and forbs along the railroad right-of-way. There were also large tracts of barren sandstone and limestone-derived soils. Through proper management, the overall net primary productivity within most of the grasslands within the CVRD could probably be increased by 10-fold to 100-fold.

The management of riparian communities is largely dependent on the management of the uplands. When runoff from the watershed has been reduced by the enhancement of grasses and other soil stabilizing vegetation, riparian communities recover quickly, at least in terms of vegetation density. The rapid return of riparian vegetation is a response to the prolonged availability of moisture and to the abatement of catastrophic flooding. Following this logic, **it would be more effective in the long run to restore grass and forb cover of upland areas before attempting to restore riparian habitats.** When direct riparian management tactics are enacted, the focus of restoration should be on the reintroduction of soil-stabilizing monocots, such as cat-tails, sedges, rushes, and grasses.



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# Monitoring of *Penstemon clutei* A. Nels. on Tornado Salvage

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**Abstract:** *Penstemon clutei* is a sensitive plant found on the Coconino National Forest. The plant is a Region Three Forest Service Sensitive species and is recognized as a category 2 Species by the U.S. Fish and Wildlife Service. The plant was the subject of a monitoring project as a mitigating measure for a timber salvage sale within its known range.

## Introduction

*Penstemon clutei* A. Nels. is an endemic that is known to occur only in the volcanic field associated with Sunset Crater. It is a federally listed category 2 and Region 3 Forest Service Sensitive species. It is a short-lived perennial that is estimated to live 5–10 years (Phillips et al. 1992). The species can be distinguished from other species of *Penstemon* found in the area by the sharply toothed leaves that are amplexicaul on the lower portions of the plant, by the bright pink flowers when in bloom, and by the size—up to 3 feet tall. The more common *Penstemon barbatus* (Cav.) Roth, which is found in the area, has entire, non-amplexicaul leaves and red flowers.

The plant was first described in 1927 as a subspecies of *Penstemon pseudospectabilis* M.E. Jones by A. Nelson, but was given a distinct taxonomic separation by Keck in 1937 based on the narrow ecological niche that *Penstemon clutei* occupies. The plant prefers flat or gently sloping sites with open ponderosa pine and a layer of cinders 5–10 cm thick, with a layer of silty soil of similar thickness below. This soil structure has an important function in water retention after rains. The underlying soil retains water for a longer period of time than the cinder layer above after summer moisture is received. This allows the plant to retain moisture in the area of the root system during periods when no moisture is received (Phillips et al. 1992). Areas of deep cinders without the soil layer, areas on the slopes of cinder cones where soil is unstable, and areas of dense vegetative cover by other vegetation such as blue grama (*Bouteloua gracilis* (H.B.K.) Lag.) do not provide the required microhabitat for this plant.

*Penstemon clutei* can tolerate at least some level of disturbance and has been observed in the past to respond positively to various disturbance factors. A wildfire in November 1973, known as the Burnt Fire, burned a large area of the known habitat of *Penstemon clutei*. Goodwin states that "*Penstemon clutei* is one of the pioneering species" in

the area of this wildfire (1979). He also described the population within the burn as being "the largest population" and the individual plants as being "very vigorous" and "reproduction abundant." Some of the results from the fire included opening up the canopy and reducing litter and competing vegetation. There have been some experiments with prescribed burning in the past, but the plants apparently did not respond as well to the disturbance. *Penstemon clutei* can also respond to human-caused disturbances such as logging. In 1992, Phillips et al. observed that the highest concentration of reproducing adults and seedlings were growing around stumps or near the edges of decaying logs.

## Need for Monitoring Project

On October 26, 1992, a major winter storm system passed through the Flagstaff area. A tornado associated with this storm struck an area northeast of Flagstaff near O'Leary Peak. It was the only known occurrence of a tornado in the Flagstaff area. The tornado damaged stands of ponderosa pine habitat on Coconino National Forest and on adjacent Park Service property within Sunset Crater National Monument, uprooting many large ponderosa pine trees. The damage occurred in pine stands on the forest southeast and west of Sunset Crater National Monument, as well as in the monument itself. This area is within the habitat of *Penstemon clutei*. District personnel proposed a salvage sale of the downed and severely damaged material within the National Forest portion of the path of destruction. This sale was known as "Tornado Salvage."

The potential impacts to *Penstemon clutei* were evaluated while planning for the sale. Although past observations indicated that *Penstemon clutei* responds to disturbance, concerns over the level of disturbance from heavy equipment and possible destruction of the seed bank from churning of the soil were addressed. Past observations by district personnel and others have indicated that under certain conditions vehicles that leave the estab-

lished roadways in the area can become stuck and can cause substantial soil disturbance, potentially causing heavy churning of the soil layers previously mentioned as a necessary habitat component for *Penstemon clutei*. These conditions appeared to occur most often in the summer months. These observations and the concern over the protection of the habitat of *Penstemon clutei* led to the development of the primary mitigating measure. This mitigation was the restriction of sale operations, including the operation of heavy machinery to the winter months when the soil was frozen, therefore reducing the potential for soil damage. Several other mitigating measures were developed during the planning of Tornado Salvage to minimize the impacts of the salvage operation on *Penstemon clutei*. These included on-site visits during periods of active harvest to evaluate equipment disturbance, monitoring, and restriction of the equipment to designated skid trails within occupied habitat. Another mitigating measure was to scatter the remaining slash over the area. In some instances, slash from timber sales is pushed into piles using a tractor. This can be a factor in soil disturbance. As the tractor moves over the area pushing the slash, the top layer of soil is sometimes disturbed.

Concern over disturbance of the soil structure and potential damage to the seed bed were factors considered in the monitoring project described below. These were more long-term concerns than actual loss of individual plants. Another objective was to confirm the validity of the mitigation measures, because in recent years the volcanic area that is the habitat for *Penstemon clutei* has been designated for a variety of uses, including recreation and fuelwood gathering.

### Monitoring Methods

The salvage sale was separated into two units geographically as a consequence of the storm path, which was southwest to northeast. One unit was southeast of Sunset Crater National Monument and the other was west of Sunset Crater National Monument near O'Leary Peak. Monitoring the project consisted of three parts: plant searches, photopoints, and regular visits by the district sale administrator or a harvest inspector.

Plant searches were conducted on both units in November 1992. No transects or other specific sampling techniques were used, primarily because distribution of *Penstemon clutei* was unknown within the area. The uneven distribution due to the specific microsite requirements made it difficult to use a specific sampling technique. However, by

using local landmarks all of the area was searched and very few, if any, of the plants would have been missed. The plants were dormant, but still easily recognizable. The serrate leaves and pinkish coloration of the foliage made the plants easy to identify. There were also some dried seed stalks from the previous growing season. This search was conducted by walking around over the unit and counting plants as they were found. The plants were separated into two categories, adults and seedlings, based on plant size and presence or absence of a seed stalk. Plants were considered to be seedlings if no evidence of a seed stalk was present and if the plant was small. Relatively large plants with or without seed stalk were considered adult plants. The purpose of the monitoring project was to gain a general understanding of the impacts of salvage sale activities on *Penstemon clutei* habitat. Funding constraints prevented the development of a statistically valid sampling system. The plant search was repeated on the total area of the unit near O'Leary Peak after completion of sale activities in August 1994. A partial search was done on the unit southeast of the Monument. A complete search was not done, because after the initial search it was determined that most of the unit was only marginal habitat for *Penstemon clutei* and most of the plants found in 1992 were outside of the area of proposed activity.

Fourteen photopoints were also established in November 1992. These points were designed to cover a variety of situations that might occur. For example, one photopoint was placed in the roadway where hauling would occur to monitor the effect of activities associated with roads, such as traffic use and road blading. There were also some points that would be affected by harvest activities, such as skidding and decking of logs. Some points were established in areas where no disturbance was anticipated. All photopoints were marked using metal tags attached to nearby trees. Compass bearings and distances to the selected plant or plant groups were recorded. Directions to the photopoints were noted and anticipated outcomes to the plants were recorded as damaged, destroyed, or avoided. Sketches were made of the surrounding areas and nearby landmarks. For example, if there was a nearby rock or log, this was included in the sketch. At least one photo was taken of the selected plants. The photos and the accompanying information were combined on a field form designed for the project that was kept on file for comparison after the sale was completed. Two wire rings were used to help define

the plants in the photos. These rings, constructed from heavy wire and wrapped with brightly colored flagging, were placed around the selected plant during field visits when the photo was taken. Also included in the photo was a sign including photopoint number and date. This sign was constructed of heavy paper and attached to a standard size clipboard. The photopoints were revisited on at least four occasions between November 1992 and August 1994: two pre-harvest visits and two visits after harvest was completed. On each occasion the condition of the plant was recorded and a photo was taken.

During the sale, the area was visited regularly by the district sale administrator or the harvest inspector. The biological technician that was responsible for monitoring *Penstemon clutei* was also designated as the harvest inspector. During visits to check the status of *Penstemon clutei*, the biological technician could also check the area for soil disturbance and other factors of disturbance associated with sale activities that might affect the plants or habitat. Any activity that needed attention could be reported to the sale administrator. This made field visits more efficient because the visits could serve two functions.

## Results

Photopoint information recorded for the project shows that most of the predictions made on the fates of individual plants were correct. The following examples are some predictions that were verified during the monitoring project:

- The photopoint of a plant that was in the road was destroyed by blading activity that occurred during the sale and it had not recovered on the August 1994 visit.
- At four points, it was anticipated that the plants would be avoided by sale operations when the points were established in November 1992 and these plants survived and were present in August 1994.
- At four points, it was anticipated that the plants would be damaged or destroyed by salvage operations and these plants were destroyed. Two were destroyed by road blading activity. Two others near harvested logs were destroyed by skidding activity.
- The control reference point, located outside of the cutting unit boundary where no activities occurred, remained generally the same throughout the monitoring project. While

plants at this point were not actually counted because they were outside of the activity area, no substantial change in the plant number was noted.

- At three points, plants died of causes other than sale activity. The cause of death of two of these plants was unknown; the plants appeared dried and shriveled on one or more visits and were dead on the visit in August 1994. At the third point a plant had been eaten by an unknown herbivore, probably a rabbit. This photopoint could not be relocated on the final visit, but it is assumed that the severe clipping eventually caused it to die.

## Plant Survey Results

During the August 1994, visit an increase in population was noticeable by visual inspection. Therefore, a plant search was done of the entire unit. The salvage sale unit near O'Leary Peak showed an increase in *Penstemon clutei* plants from 831 plants in November 1992 to 2099 plants in August 1994. No distinction was made during this count between seedlings and adults. The count confirmed the observed increase in population.

A complete plant search was done on the unit southeast of Sunset Crater National Monument in 1992, but was not repeated in August 1994. This unit was marginal habitat, mostly covered by deep cindered soils. In November 1992, there were 110 plants recorded on or near the only photopoint within the unit, and most of these were seedlings. During the August 1994 visit, 85 adult plants and 200 seedlings were recorded in the same area. Disturbance was visible on the site, but the *Penstemon clutei* population still increased. At this photopoint, there were some drag marks left in the soil and on an April 1994 visit, this plant was recorded as destroyed. However, an adult-size plant was present at the photopoint location in August 1994, growing in the same location. Sale activity in the area occurred during the winter months, so it is possible that while the top of the plant was torn away, the plant was able to grow back from the roots.

## Discussion

Observations during the initial surveys determined that the area near O'Leary Peak was better *Penstemon clutei* habitat than the unit southeast of the monument, primarily due to a difference in soil structure. The soil near O'Leary Peak seems to contain the beneficial layering of cinders and soil

as previously described. The other unit southeast of Sunset Crater is mostly deep cindered soils. While both areas remained in the monitoring plan, most of the monitoring efforts were concentrated on the area near O'Leary Peak. Most examples of the situations desired for the photopoint monitoring were found in the O'Leary area and the area also contained a significantly larger population of *Penstemon clutei*.

The storm that caused the damage was a very unusual occurrence for the Flagstaff area. One of its noticeable effects was the reduction of tree canopy within the area of damage. Initially it appeared that all of the trees in the area had been uprooted. However, as months passed and tree foliage began to dry, it became apparent that some smaller trees remained alive, which resulted in a ponderosa pine stand of smaller trees with less canopy closure. This could be a situation similar to that found by Goodwin (1979) in areas affected by Burnt Fire, where he noticed an increase in both numbers and vigor of *Penstemon clutei* plants and a decrease in canopy. The storm caused substantial ground disturbance by uprooting trees and other vegetation. The exact effect of such disturbance on *Penstemon clutei* is unknown; however, no adverse effects on the plants were observed. Disturbances from logging and fire have been observed by others, but no soil disturbance of the magnitude caused by the storm has been observed before.

The increase in population of *Penstemon clutei* is at least partially attributed to the timber harvest activities. The sale activities did destroy or damage some individual plants, but there was a substantial post-harvest increase in the population of *Penstemon clutei*. Certain activities such as the blading of roads destroyed individuals, but other activities provided some slight disturbances which may have benefited the *Penstemon clutei* population. The skidding of logs and other activities on the project could have helped disperse the seeds of *Penstemon clutei* to parts of the area unoccupied by the species before the activities. By planning most of the activities during winter months, no deep disturbance of the beneficial soil layering resulted. Through the reduction in competing vegetation,

distribution of the seed, and only slight disturbance to the top of the soil, the sale-related activities contributed to the increase in population of *Penstemon clutei*.

## Conclusion

The monitoring of *Penstemon clutei* was an example of a successful monitoring project. The objective was to monitor the habitat of *Penstemon clutei* for adverse factors during the operation of a salvage sale within the known habitat. The monitoring plan was simple to design and revisits were easy to accomplish. The project accomplished the desired results and did not require a lot of technical expertise or special equipment. A more complicated model could have been constructed and may have provided better information on population trends and related subjects, but the monitoring plan using the photopoints, plant searches, and visits to the project area provided the desired information on the impacts on *Penstemon clutei* habitat.

Projects within the range of *Penstemon clutei* can be completed if certain precautions are observed. By restricting activities to the colder months of the year, the soil layering that is an important component of the habitat of *Penstemon clutei* can be preserved. Visits to the area to monitor activities provided additional protection to *Penstemon clutei*. Disturbance was restricted to an acceptable level and the habitat was preserved.

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# Monitoring a Population of Grama Grass Cactus

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**Abstract:** During May of 1994, while conducting a special status plant clearance to meet NEPA requirements for a proposed trials bike race, a large population of grama grass or paper-spined cactus was discovered near San Ysidro, New Mexico. The population consisted of 121 individuals that were identified and planetable mapped. Other information compiled during the establishment of baseline monitoring studies included plant heights, top diameters (both excluding spines), the occurrence (frequency) and number of flower buds, and general view photos of the habitat area.

## Introduction

Populations of grama grass or paper-spined cactus, *Toumeyia papyracantha* (Engelm.) Britt. & Rose (synonym *Pediocactus papyracantha*) (Fletcher et al. 1984), near San Ysidro, New Mexico are threatened by collectors, domestic livestock grazing and trampling, and motor bikes (Knight 1983, Fletcher and Moir 1993, Peddie 1993).

During April and May of 1994, in order to meet NEPA requirements, the Bureau of Land Management was in the process of legally clearing an area for an environmental analysis of a trials bike race when a large population of grama grass cactus was discovered in the area.

This small, well-camouflaged cactus was first collected by Augustus Fendler in May, 1847 (Sivinski and Lightfoot 1994). It is presently a category 2 candidate for federal listing.

Moderate to heavy livestock grazing has occurred in the area for over 200 years. Coronado wintered near Bernalillo, NM in 1541 with a commissary of sheep, goats, and horses (approximately 20 miles east of the site). From 1776, when Spanish settlers began grazing the area, ecological phyto-edaphic communities have evolved under continuous heavy grazing. At the present time approximately 36 cows graze the area (3,947 acres) for 9 months (October–June). Grama grass cactus grows within grass stubble and near shrubs and half shrubs. This affords it some protection from grazing. Cactus nurse plants in the area include broom snakeweed, black grama, blue grama, alkali sacaton, and broom snakeweed. Grama grass cactus tends to grow no taller than the surrounding protective vegetation.

The grama grass cactus is a fairly widespread species, occurring in most of central New Mexico, east-central Arizona, and the vicinity of Dell City, Hudspeth County, Texas (Sivinski and Lightfoot

1994). Threats to *T. papyracantha* include trampling and removal of sheltering grass cover by livestock, urbanization, highway and road construction, collection, predators, disease, and off-road vehicles.

A simple mapping/monitoring technique is needed for grama grass cactus where populations can be easily relocated in succeeding years after the baseline studies are established.

## Description of the Site

The geology consists of rocks from the Petrified Forest Member of the Chinle Formation (Triassic). The formation is a reddish orange and brownish maroon shale with subordinate reddish sandstone and minor green shale, brown clastic limestone, and small pebble conglomerate (Woodward and Ruetschilling 1976). Thickness is about 1000 feet. The Tierra Amarilla Anticline, an important geologic landmark, is located approximately three-quarters of a mile northeast of the site.

The mean elevation of the site is 5,640 feet, aspect is south, and percent slope ranges from 0 to 30 percent. The average annual precipitation is 10–12 inches and the average annual temperature is 52–54°F, with a frost-free period of 120–140 days (April–September).

The soil mapping unit is rock outcrop–Zia Complex; included in this unit are small areas of the Skyvillage series near the rock outcrop. The Skyvillage series soils are classified as loamy, mixed (calcareous), mesic, Lithic, Ustic Torriorthents (USDA 1987).

The Skyvillage soils have 10–18 percent clay with a moist bulk density of 1.35–1.55 g/cc. Permeability is moderately rapid (2.0–6.0 in/hr) and available water capacity is very low (0.11–0.16 in/in). Soil reaction is 7.4–8.4 pH and salinity is less than 2 mhos/cm. The shrink-swell potential is low. There is low potential for frost action and the

hydrologic soil group is D. Effective rooting depth is 6–20 inches. Runoff is medium, and the hazard of water erosion is moderate (USDA 1987). Rainfall runs quickly off the area's rock outcrops, through the site, and eventually downstream into the Rio Salado.

A typical soil pedon (USDA 1987) follows:

Horizon	Description
A	0–2 inches; pale yellow (2.5Y 7/4) fine sandy loam or sandy loam, light olive brown (2.5Y 5/4) moist; weak fine granular structure; soft, very friable, slightly sticky and slightly plastic; few fine roots; slightly effervescent; moderately alkaline; abrupt smooth boundary. Percent clay 10–15.
Cl	2–11 inches; light gray (2.5Y 7/2) fine sandy loam, light olive brown (2.5Y 5/4) moist; massive; slightly hard, friable, slightly sticky and slightly plastic; few fine and very fine roots; 10 percent pebbles; slightly effervescent; mildly alkaline.
R	12–16 inches sandstone (depth to sandstone ranges from 6–20 inches).

The vegetation ranges from desert grassland to juniper savanna in the area. The plant community consists of widely scattered one-seed juniper with an understory dominated by black grama, side oats grama, and blue grama. Grasses occurring in lesser amounts include galleta grass, Indian rice grass, bottlebrush squirreltail, New Mexico feathergrass, New Mexico muhly, and Fendler three-awn. Broom snakeweed is the dominant shrubby species.

From an oblique or vertical view the grama grass cactus appears grass-like. The spines are the most distinguishing characteristic of this species, as described by Benson (1962):

Spines dense, obscuring the surface of the stem; central spines whitish or pale brown, changing to gray, *flexible*, 1 or sometimes 2–4 per aureole, the upper ones then smaller, curving upward, the mass of centrals overarched the apex of the stem, the longer ones usually 3/4 to 1/4 inches (1.9–3.1 cm) long, up to 1/20 inch (1.2 or 1.3 cm) broad basally, subulate, strongly flattened, involute and the midrib evident on the central side, it and the margins puberulent, the cross section very narrow; radial spines ashy white or pale gray, *flexible*, 6–8 per aureole, spreading parallel to the surface of the stem, straight, the longer ones about 1/8 inch (3 mm)

long, up to nearly 1/48 inch (0.5 mm) broad, flat, very thin.

Wildlife that may damage the cactus in the area are the cottontail rabbit, black-tailed jack-rabbit, spotted ground squirrel, kangaroo rat, silky pocketmouse, plains pocketmouse, western harvest mouse, deer mouse, hite-footed deer mouse, northern grasshopper mouse, and white-throated wood rat.

## Methods

Grama grass cactus is a clonal species that occurs in small groups dispersed unevenly throughout suitable habitat. Clonal groupings can range from a few scattered plants to nearly 200 individuals in a 1–2 acre area. Accurate counts of individuals within a population of this extremely cryptic plant are almost impossible to obtain and are infrequently attempted. Since this plant is so difficult to see (especially juveniles), reliable density estimates for large areas are almost nonexistent (Sivinski and Lightfoot 1994). The planetable-based recording system is among the oldest forms of surveying technique. A modern planetable outfit consists of an adjustable tripod, planetable board (a mobile field drafting board), alidade (equipped with stadia wires and magnifying optic lens and/or laser), drawing instruments, stadia rod, and measuring tape (300 ft).

The planetable system is based on direct observation and measurement, and maps and records are made (to appropriate scale) while in the field. Recording topographic features and other structural attributes while they are in full view has many applications (Brinker and Wolf 1977). Civil and mining engineering, forestry, archaeology, geology, agriculture, small tract surveying, and military operations still use planetable technology. Though photogrammetry (aircraft or satellite) is currently the method used for large-scale mapping, it remains impossible to extract everything mapworthy from remote imagery. Ground truthing still requires exactly that—direct observation and recording on the ground.

With the board and tripod assembled and the drawing mylar fastened, the planetable was located on a small mound (without any plants) that provided direct observation of the known *Toumeyia* population cluster. A survey pin was inserted into the ground, directly under the center of the planetable, establishing a base reference point from which measurements and recordation would be made. A previous survey had identified



the general concentration location and the planetable was located in proximity to, but well outside of, the population cluster.

A careful examination of the area was conducted and a pin flag was set next to each identified plant. It became apparent as the flagging progressed that the bulk of the population occupied the bottom and both hillsides of a small shallow basin in roughly an elliptical pattern. It was possible to establish four corner points outside of the population with the plant locations flagged. Each corner was pin flagged and recorded on the planetable. A tape was attached to the base station survey pin and the distance to each individual plant was measured and recorded on the map. As the location measurement was taken, the pin flag was replaced with a nail located approximately 5 cm north of each plant. Plant attributes (height, top diameter, configuration, budding status) were recorded at the same time. The nails identify all of the recorded plants; the assumption is that in future examinations those plants unmarked by nails will be new individuals in the population.

### Results and Discussion

Within the study site (approximately 31.3 square meters), 121 individuals were recorded. Another 10 individuals were found outside the site further down the hillside.

Ten of the population were found in clusters of two, three, and five, respectively. There were four sets of twin stem plants. The mean height was 2.36 cm and the mean top diameter was 1.61 cm. The number of individuals with flowering buds was 33, ranging from one to five with a mean of 1.5. There were 51 flowering buds in the population that may produce seed. Forty-two percent of the population flowered during the second week in May, 1994.

Nurse plants were mainly blue grama and broom snakeweed. Some individuals were in open areas surrounded by bare soil or rocks with the rocks acting as protection. Two exposed plants had been run over by a trials bike, but had not died. These were transplanted approximately 3 meters away from the bike trail.

Age classes were not calculated, but based on diameters and heights, seedlings were presumably 0.2–0.4 cm in diameter and 0.1–1.5 cm in height, whereas large adults were 2.6–5.0 cm in diameter and 3.1–7.5 cm in height. The large adults were presumed to be 5 years old or older. Flowering begins to occur on plants that are 0.7–1.5 cm in size.

Table 1. The population was divided into 5 size classes based on top diameters and 4 classes based on height.

Size Class	No. of Individuals	Percent Comp.
Based on Top Diameters:		
0.2–0.4 cm	18	15
0.5–1.0 cm	23	19
1.1–1.9 cm	35	29
2.0–2.5 cm	33	27
2.6–5.0 cm	12	10
Based on Height:		
0.1–1.5 cm	20	16
1.6–2.4 cm	42	35
2.5–3.0 cm	45	37
3.1–7.5 cm	14	12

### Conclusions

The population of grama grass cactus should be monitored annually during May (flowering) and re-mapped every 5 years (minimum). The area is now restricted for trial bikes and other off-road use, but livestock continues to graze the area. The ecological site condition is mid-seral and the range trend is stable or static to slightly upward. The grama grass cactus is a species that requires more open sites, where grasses are the dominant cover and it is reasonable to assume that the species population is less now than before the advent of livestock.

The planetable mapping procedures and measurements take only one 6-hour day to complete and can be done efficiently with two people. This method should be considered where populations are fairly dense and not too scattered or variable.

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# Inventory and Monitoring of Giant Four-wing Saltbush, Little Sahara Recreation, Area, Juab County, Utah

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**Abstract:** We have been studying the giant four-wing saltbush (*Atriplex canescens* var. *gigantea*), a Great Basin endemic, on BLM lands since 1992. Shrub distribution has been delineated on four scanned USGS quadrangles. In 1994, 20 transects were established within the dunes to inventory population parameters such as shrub density, age, male/female ratios, growth, vigor, damage, and mortality. Shrub communities on active as well as stable dunes were studied. In general, plants appear vigorous and healthy, but no seedlings were observed during the inventory. Shrub damage from all-terrain vehicles is light (22%) and mortality is low (9%) within the communities sampled. Permanent monitoring plots were established during 1995 in areas of high visitor use to track population trends. Threats to the species and population viability will be assessed annually. Species conservation and habitat management strategies will be developed.

## Introduction

The giant four-wing saltbush (*Atriplex canescens* (Pursh) Nutt. var. *gigantea* Welsh & Stutz), a federally proposed category 2 species, is a relict Great Basin endemic that appears to be restricted to the Jericho Sand Dunes near Lynndyl, Juab County, Utah (Welsh et al. 1993). The Jericho Dunes (Little Sahara Recreation Area), which provide habitat for the shrub, are administered by the Richfield BLM District as part of the House Range Resource Area.

This rare shrub occurs most often on the leeward side of active dunes (Stutz et al. 1975, Welsh et al. 1993) and less frequently in the relatively stabilized interdunal valleys (swales) with other sand-tolerant species (Hreha and Meyer 1993). Several dead and damaged shrubs were observed in areas of high visitor use at White Sands Campground, Jericho Picnic Area and Sand Mountain (Hreha and Meyer 1993). Damage to and mortality of *A. canescens* var. *gigantea* within these sand dune plant communities may be attributable to all terrain vehicles (ATVs) and firewood collection (Peterson and Muhlestein 1973, Stutz 1979, Hreha and Meyer 1993). Disease pathogens may contribute to shrub mortality.

Further field research was required to provide additional information to assess the population viability of the species in order to protect this species from threats associated with recreational activities. A conservation strategy will be developed for the plant using data gathered from inventorying population parameters and monitoring plots to annually track population trends and threats to the species.

## Project Objectives

The main objective of the project is to inventory population parameters of *A. canescens* var. *gigantea*, such as mean shrub height and diameter, density, percent cover, male/female ratio, reproductive age classes, vigor, damage, and mortality. The data will be used to assess the population's viability and to aid in the selection of areas where monitoring plots need to be located. Future objectives include setting up monitoring plots to assess population trends and threats to the species and developing a conservation strategy for the species.

## Methods

### *Population Inventory*

Twenty transects (7.5 m width x 150 m length) were established (Figure 1) during October, 1994, to collect population data in areas of high visitor use at White Sands Campground, Jericho picnic-campground area, Sand Mountain, and northwest of Tanner Creek. Transects were located within *A. canescens* var. *gigantea* (shrub) stands and were positioned to sample as much variation within these plant communities as possible. Transect placement was limited by accessibility to the dunes; most transects were located along the periphery of the dune field in areas accessible by four-wheel drive vehicles. We had intended to locate some transects within the interior of the dune field, but the terrain limits access except with ATVs, which were unavailable until late in the 1994 field season. We were able to study shrub communities within the interior of the dunes during the spring of 1995 with the aid of BLM personnel stationed at Little Sahara.

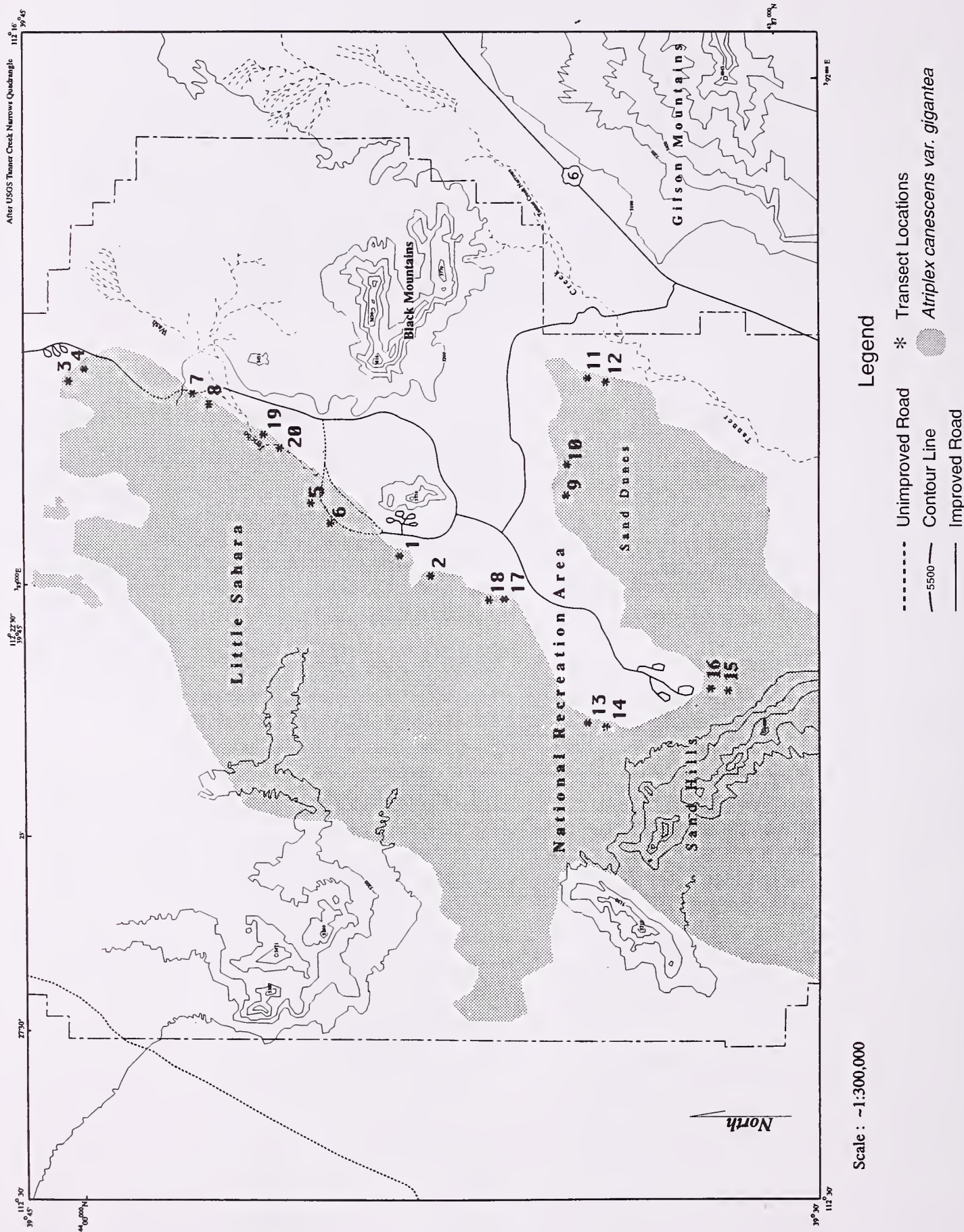


Figure 1. Study site map with transect locations (prepared by Barbara L. Soha, Conservation Volunteer, Red Butte Garden and Arboretum, University of Utah, Salt Lake City).

Transect starting points and end points were marked with surveyor's stakes. A 150 m tape was used to measure transect length. Transects were divided into three 50 m segments and each segment was characterized as either active dune or stabilized swale. Segment characterization was subjective and difficult in some cases. In general, areas with sloping, moving sand and low plant densities were characterized as active and relatively flat areas with dense plant cover were characterized as stable.

Sixty 50 m segments were tallied along the 20 transects. A compass was used to position each transect along a selected compass heading (north = 0 degrees, east = 90 degrees, south = 180 degrees, west = 270 degrees). The tape was laid out along the compass heading for the entire length of the transect.

The site number, date, location, township and range coordinates, and associated plant species were recorded at each transect site. Plant nomenclature follows that of Welsh et al. (1993). Transect orientation (aspect) was recorded by using a compass. Depth of each swale (dune height) was estimated for each transect by measuring the distance from bottom to top of the swale (dune) with a measuring tape and using an angle finder for slope degree.

All giant four-wing saltbush shrubs that occurred within the transect boundaries were sampled. Shrub height and diameter were measured with a meter stick. Male and female individuals were tallied and their ratio was calculated. Reproductive age class (seedling, juvenile, mature, senescent) and vigor classes (excellent, good, fair, poor) were recorded for each shrub encountered along the transect.

Shrub damage (amount and type) and mortality (probable cause) were assessed at each transect. Shrub damage was characterized as the number of broken branches per shrub and the number of prostrate shrubs. Shrub mortality consisted of percent of dead branches (<25%, >25%, >50%, >75%) per shrub and number of dead shrubs.

Shrub densities were calculated by dividing the number of shrubs per unit area of each transect. Percent shrub cover was calculated by dividing the total area occupied by shrubs by the total area of each transect.

Photographs at the starting point or end point of each transect were taken to illustrate methodology, to show relative size and appearance of the shrubs in their habitat, and to document threats.

### *Population Viability Analysis*

Population viability was determined from the data gathered in the population inventory at the 20 transect sites. Data sets on shrub growth (height and diameter), male/female sex ratio, juvenile/reproductively mature shrub ratio, vigor, damage, and mortality were analyzed and used to draw viability conclusions.

### *Monitoring Plots*

Monitoring plots (100 × 100 m) were set up during the 1995 field season at four heavily used sites within the Little Sahara Recreation Area (White Sands campground, Jericho picnic area, Sand Mountain, and Tanner Creek) to monitor shrub growth, reproduction, and vigor, as well as to assess shrub damage and mortality trends. Efforts were taken to select monitoring plots with similar elevation, orientation, interdunal valley (swale) depth or dune height, and plant community characteristics.

Plot corners were marked with tall survey stakes and located within the crowns of the shrubs to avoid detection and potential disturbance by recreationalists (Bagley 1988). Plot locations were recorded, photographed, and referenced to some stable facility (i.e., restrooms or roads within the area). Compass bearings from the reference point and distances to the northeastern corner of the plots were calculated. Plot coordinates were recorded using a Trimble navigation GPS unit.

All giant four-wing saltbushes were tagged and numbered so that individual shrub growth, damage, and mortality could be monitored. The same information collected in the population inventory was recorded for each shrub within the plot. Population trends and threats to the species are assessed annually.

The dune was characterized as active or stable under each giant saltbush within the plot. Dune activity/stability was correlated with shrub growth, vigor, damage, mortality, and threats. In addition, sand height was marked on the corner stakes of each plot so that sand movement (rise or fall in height) could be monitored. Results of a 3-year monitoring study (1995–97) will be submitted to the Utah State BLM Office in Salt Lake City, Utah.

### *Data Analysis*

Means were calculated for all population parameters studied. Vigor, damage, and mortality were correlated with active and stable dune areas

along the transects. Conclusions were drawn as to the growth, vigor, viability, and threats to the species.

## Results and Discussion

### *Transect Location and Orientation*

For analysis purposes, the Little Sahara study area was divided into four recreational sites: White Sands, Jericho, Sand Mountain, and Tanner Creek. Results are presented by transect within each recreational site.

Table 1 lists the location, orientation, dune stability, slope, and swale depth (dune height) of each transect. Twelve transects have a north-south orientation and eight transects have an east-west orientation. In most cases, we entered the dune-field from the eastern edge and walked north, west, or south along the transect line.

The dominant orientation of the dunes within the dune field is from southwest to northeast with predominant wind direction from the southwest (Sack 1981). Plant communities inhabiting the somewhat protected interdunal valleys of the dune field are well developed compared to the sparse vegetation occurring on the windswept active dunes that surround them. In general, plant communities have a northwesterly to southeasterly orientation similar to the longitudinal axis of dune crests within the dune field. However, at dune intersections, the general dune orientation pattern from northwest to southeast is disrupted, resulting in plant communities that sometimes have east-west orientations.

According to Stutz et al. (1975) and Welsh et al. (1993), giant four-wing saltbushes prefer the leeward side of active dunes. Hreha and Meyer (1993) also found many giant saltbushes concentrated at the leeward bases of active dunes near the edges of swales.

### *Dune Activity/Stability*

The number of active versus stable dune segments sampled was similar along the 20 transects. Of the 60 dune segments sampled, 26 were characterized as active and 34 were characterized as stable (Table 1). Sack (1981) characterized 64 percent of the dune field as active dunes and 36 percent as stable or semi-stabilized dunes.

### *Slope (Degrees) and Swale Depth (Dune Height)*

Slope steepness was divided into two widely distributed categories. In general, stable swales (transects 2, 10, 11, 12, 16, 17, 18 and 20) had rela-

tively flat slopes (0–5 degrees) while active dunes (transects 1, 3, 4, 5, 6, 7, 8, 9, 13, 14, 15 and 19) had steep (30–35 degrees) slopes (Table 1). Swale depth ranged from 0 to 36.5 m, with a mean swale depth of 8.4 m. Sack (1981) reported active dune heights (or swale depths) of 2–61 m.

### *Associated Plant Species*

Table 2 lists the plant species that occur with *A. canescens* var. *gigantea*. The two most abundant plant species occurring along 18 transects each were cheatgrass (*Bromus tectorum* L.) and Russian thistle (*Salsola pestifer* A. Nels.). These two weedy species seem to have ubiquitous distributions on both active and stabilized dunes.

Active dunes support Indian rice grass (*Stipa hymenoides* R. & S.), the legume *Psoraleidum lanceolatum* (Pursh) Rydb., sand verbena (*Abronia fragrans* Nutt. ex Hook.), dock (*Rumex venosus* Pursh), and pale evening primrose (*Oenothera pallida* Lindl.). Indian rice grass was present on 13 transects and the legume was present on 11 transects. Sand sunflower (*Helianthus anomalous* Blake) was present on eight transects. Sand verbena (occurring on three transects), dock (occurring on three transects), and pale evening primrose (occurring on four transects) abundances were low (Table 2). All of the above-mentioned plant species that grow on active dunes help to stabilize them. *Psoraleidum lanceolatum*, *Abronia fragrans*, and *Rumex venosus* all have long, thick rhizomes that are adapted to sand dune habitats.

Utah Juniper (*Juniperus osteosperma* (Torr.) Little), big sagebrush (*Artemisia tridentata* Nutt.), greasewood (*Sarcobatus vermiculatus* (Hook.) Torr. in Emory) and rubber rabbitbrush (*Chrysothamnus nauseosus* (Pallas) Britt.) are most often associated with stabilized dunes and swales. Rabbitbrush was observed on half (10) of the transects (Table 2). It appears to occur at the edge of swales along leeward dune margins as well as out in the center of swales. The other three species associated with stabilized dune areas were low in abundance (Table 2). Also see Appendix—Flora and Fauna of the Little Sahara Recreation Area (p. 38) in Hreha and Meyer (1993).

### *Shrub Density and Percent Cover*

Tables 3–5 list the results of the population inventory by transect. A total of 178 plants (162 live /16 dead) were tallied in the 20 transects (Table 3). Shrub densities were 0–33 plants along the transects, which were 367 square meters (3.67 hectares) each. Mean shrub density was 8.9 shrubs per tran-

Table 1. Transect location, orientation, dune stability, slope, and swale depth (dune height).

Site No.	Location	Transect Orientation		Dune* Stability	Slope (degrees)	Swale Depth (feet)
3	South White Sands Campground	205	N-S	A-A-S	35	27.5
4	South White Sands Campground	200	N-S	S-S-S	30	37.5
7	Southwest White Sands Campground	260	E-W	A-A-A	35	35
8	Southwest White Sands Campground	160	N-S	A-A-A	30	15
19	Northeast Jericho Campground	40	N-S	A-A-A	30	17.5
20	Northeast Jericho Campground	270	E-W	S-S-S	0	----
5	Northwest Jericho Campground	210	N-S	A-A-A	35	17.5
6	Northwest Jericho Campground	280	E-W	S-S-S	35	27.5
1	Jericho Picnic Area	15	N-S	S-A-A	30	35
2	Jericho Picnic Area	250	E-W	A-A-A	5	----
17	North Sand Mountain Campground	20	N-S	S-S-S	5	----
18	North Sand Mountain Campground	270	E-W	S-S-S	5	----
13	West Sand Mountain Campground	270	E-W	S-A-A	35	80
14	West Sand Mountain Campground	340	N-S	S-A-A	30	30
15	South Sand Mountain Campground	270	E-W	A-A-S	35	120
16	South Sand Mountain Campground	360	N-S	S-S-A	0	----
9	Northwest Tanner Creek	200	N-S	S-S-S	30	20
10	Northwest Tanner Creek	140	N-S	S-S-S	0	----
11	Northwest Tanner Creek	240	E-W	S-S-S	0	----
12	Northwest Tanner Creek	350	N-S	S-S-S	0	----
Totals		12	N-S	26 A	8 (0-5)	0-120 ft
		8	E-W	34 S	12(30-35)	(0-36.5 m)

\*A = Active, S = Stable (Segments 1-2-3).

sect. Densities were similar on active (2.68 plants/50 m segment) and stable (3.13 plants/50 m segment) dunes. Giant saltbush cover on average was about 10 percent of the transect area.

*Male to Female Shrub Ratios and Juvenile to Mature Shrub Ratios*

The male to female shrub ratio (Table 3) was 79:83 (49%/51%) or 1:1 as reported by Stutz et al. (1975). Although we observed male and female shrubs growing adjacent to one another on a few of the transects, shrub distribution according to sex does not appear to be random. Males were observed more often on active dune sites while females were observed more often on stable sites in the swales. Males may be better adapted to the more unstable habitats along dune margins, while females may be somewhat restricted to the more

stable sites in the swales. In general, energy expended by male plants for reproductive output appears less than that expended by females, which produce an abundance of large seeds (Stutz et al. 1975).

The juvenile to mature shrub ratio was 20:142 shrubs or 12%/88% (Table 3). We observed 20 plants during the survey, which we assumed were juveniles. There was no way to determine if the plants tallied as juveniles were really young individuals, or rather shoots arising from nearby mature shrubs, without excavating the roots of each juvenile plant. When roots from juvenile shrubs growing on dune margins were excavated, they appeared to be connected (clonal in origin) to roots of a larger mature shrub growing adjacent to them. However, we did not excavate roots of juvenile shrubs that were growing in the swales.

Table 2. Associated species list.

Species	Transects Present	Total
<b>Trees</b>		
<i>Juniperus osteosperma</i>	11, 12	2
<b>Shrubs</b>		
<i>Artemisia tridentata</i>	10, 11, 12, 17	4
<i>Chrysothamnus nauseosus</i>	1, 3, 4, 6, 7, 9, 10, 11, 14, 20	10
<i>Gutierrezia sarothrae</i>	12	1
<i>Leptodactylon pungens</i>	12	1
<i>Eriogonum corymbosum</i>	1, 13	2
<i>Sarcobatus vermiculatus</i>	17, 18	2
<b>Forbs</b>		
<i>Abronia fragrans</i>	10, 13, 16	3
<i>Helianthus anomalus</i>	1, 2, 5, 7, 8, 13, 14, 19	8
<i>Linum perenne</i>	4, 6	2
<i>Oenothera pallida</i>	1, 5, 6, 10	4
<i>Psoraleidum lanceolatum</i>	1, 3, 5, 6, 7, 8, 9, 13, 15, 16, 19	11
<i>Salsola pestifer</i>	all except 10 & 12	18
<i>Rumex venosus</i>	2, 17, 18	3
<b>Grasses</b>		
<i>Bromus tectorum</i>	all except 2 & 8	18
<i>Stipa hymenoides</i>	1, 2, 3, 4, 5, 6, 7, 9, 13, 14, 15, 19, 20	13

Table 3. Male/female and juvenile/mature shrub ratios.

Site No.	No. Plants per Transect	Male: Female Ratio	Juvenile: Mature Ratio
3	5	1/4	0/5
4	8	2/5	0/7
7	7	5/2	0/7
8	2	1/1	0/2
19	5	1/4	0/5
20	0	—	—
5	10	5/5	0/10
6	25	14/11	5/20
1	20	8/11	2/17
2	4	3/1	0/4
17	7	2/4	0/6
18	0	—	—
13	4	2/2	0/4
14	4	3/0	2/1
15	10	7/3	1/9
16	6	1/5	0/6
9	33	15/10	6/19
10	12	3/5	0/8
11	4	1/3	0/4
12	12	5/7	4/8
Totals	178	79/83	20/142

We did not observe any shrub seedlings during our inventory. The fact that no seedlings were observed is a cause for concern. If all the plants that were observed are mature and no seedlings are germinating, it might mean that the species is in trouble. However, in this type of environment, particularly on sand dunes, vegetative reproduction is highly adaptable to the site and has been documented as the preferred means of reproduction (Abrahamson 1980, Pye and Tsoar 1990, Urbanska 1990). Plant species adapted to sand dune habitats use a combination of reproductive strategies in order to ensure success. These species colonize sites with seeds (sexual reproduction) and quickly become established with various means (rhizomes) of vegetative reproduction (Urbanska 1990).

Weathered seeds, collected in the fall (1993) from beneath shrubs, germinate readily in soil mix in the greenhouse. It is not understood why seedlings were not observed in the field. Perhaps germination inhibitors were not leached from the seeds in the field (see Hreha and Meyer 1993 for further discussion).

Field germination in the giant four-wing salt-bush may be triggered by certain climatic factors (Stutz et al. 1975), especially temperature and moisture (Larcher 1980). Perhaps warm temperatures during mild winters inhibit field germination in spring, or seeds may germinate only after adequate precipitation is received in the dunes during the cooler months of autumn. We observed field germination during the fall in many of the sand-tolerant species associated with *A. canescens* var. *gigantea*. In general, plants time their germination to coincide with seasonal storm patterns that usually deliver adequate moisture for germination (Larcher 1980). However, Stutz et al. (1975) also reported that reduced germination in this species is probably due to genetic differences.

#### Shrub Growth and Vigor

Mean shrub height was 1.4 m and diameter was 3.0 m (Table 4). Shrub diameter was observed to be approximately twice the height. The large average size of the shrubs encountered in the inventory may indicate that most of the shrubs that were sampled are mature individuals.

No attempt was made to count the number of branches or stems per plant. However, shrubs were observed to have about 6–15 stems per plant.

Vigor was high: 41 (25%) shrubs had excellent vigor, 66 (41%) shrubs had good vigor, 41 (25%) shrubs had fair vigor, and only 14 (9%) shrubs had



Table 4. Shrub height, diameter, and vigor.

Site No.	Height (inches)	Diameter (inches)	Vigor Classes			
			Exc.	Good	Fair	Poor
3	67.6	160.6	2	2	1	-
4	73.4	113.1	-	2	3	2
7	53.3	171.6	3	3	1	-
8	54.0	206.5	-	1	-	1
19	61.2	238.6	5	-	-	-
20	----	----	-	-	-	-
5	60.2	225.9	4	3	1	2
6	62.7	114.2	3	17	3	2
1	53.8	99.8	7	8	3	1
2	58.3	109.5	3	1	-	-
17	40.8	147.2	-	1	4	1
18	----	----	-	-	-	-
13	54.3	116.3	2	-	2	-
14	50.3	63.3	-	2	1	-
15	40.6	109.2	3	2	2	3
16	49.0	129.8	3	1	-	2
9	52.1	107.0	2	10	13	-
10	59.6	125.5	1	3	4	-
11	46.8	111.5	2	2	-	1
12	39.3	50.1	1	8	3	-
Totals	1.4 m* (4.5 ft)*	3.0 m* (10.0 ft)*	41 25	66 41	41 25	14 (plants) 9 (%)

\*Mean, n = 20.

poor vigor (Table 4). Plant vigor was recorded as poor if plants had small leaves, chlorotic leaves, or sparse foliage. In general, most plants seemed healthy and vigorous. We observed that plants drop about half of their leaves at the end of the growing season.

#### *Shrub Damage and Mortality*

Shrub damage was light, with 22 percent (35 shrubs) of the population affected (Table 5). Of the 35 damaged shrubs, 17 shrubs had broken branches. Shrubs with broken branches had an average of 4.4 broken branches per plant. Four of the 17 shrubs with broken branches had been run over by ATVs (tire tracks were observed in the sand next to these plants).

The causes of other broken branches were not evident; however, many thick branches seem to break when they are not supported by sand. In the stabilized swales, female shrubs appear to grow

and produce seed for many years, but as these plants get larger and bulkier, the heavier branches are prone to break under their own weight.

We observed 18 prostrate shrubs with collapsed branches radiating from their centers. Sixty percent of these prostrate shrubs were females and 40 percent were males. Maybe, in years of high seed production, female shrubs are more prone to breakage than males. However, broken branches may result most often from large plant size and senescence rather than from additional weight from seed production as observed in females.

Another 35 shrubs (22% of the population) had dead branches. Of the 35 shrubs with dead branches, 10 shrubs had less than 25 percent dead branches, 11 shrubs had more than 25 percent dead branches, 6 shrubs had more than 50 percent dead branches, and 8 shrubs had more than 75 percent dead branches.

Table 5. Shrub damage and mortality assessment.

Site No.	No. of Plants	Damage*	Dead Branches	Dead Shrubs
3	5	1	1	0
4	8	1	3	1
7	7	0	3	0
8	2	1	1	0
19	5	0	0	0
20	0	0	0	0
5	10	3	2	0
6	25	1	2	0
1	20	4	7	1
2	4	1	1	0
17	7	6	5	1
18	0	0	0	0
13	4	0	0	0
14	4	1	0	1
15	10	6	3	0
16	6	4	1	0
9	33	5	3	8
10	12	1	3	4
11	4	0	0	0
12	12	0	0	0
Totals	178	35	35	16
(%)		22	22	9

\*Number of shrubs with either broken or prostrate branches.

The lower branches around the periphery of the giant four-wing saltbush shrubs seem to die out before the upper branches in the center of the shrub. This die-off of lower branches may be due to the lack of light. After the branches die, they turn grayish black from weathering.

Mortality (Table 5) was low at 9 percent (16 dead plants). Many shrubs appear to have been dead for quite some time and the cause of their death could not be determined. Dead shrubs had an average height of 0.9 m and diameter of 3.5 m. This supports normal mortality patterns: larger senescent shrubs are more likely to die than smaller (younger), more vigorous shrubs.

*Plant Vigor, Damage and Mortality on Active/Stable Dunes*

Shrub vigor (Table 6) is higher on active dunes compared to stable dunes (see Hreha and Meyer 1993, Figure 25, p. 31). Active dunes probably provide better sites for growth compared to stable swales. Active dunes might possibly protect shrubs from damage by supporting and covering tender and succulent shoots (Stutz et al. 1975). Sand cover might also enhance moisture retention.

On the other hand, shrubs growing on active dunes (Table 6) seem to be a target for damage by recreationists compared to those growing in swales or stable dunes (see Hreha and Meyer 1993, Figures 20 and 21, p. 24). Barren dunes with few shrubs (clear of vehicular obstacles) seem to be the most popular areas for ATVs, especially in the Sand Mountain area.

When the dunes move across an area and shrubs are exposed, they are prone to collapse (see Hreha and Meyer 1993, Figure 24, p. 26). The weight of the large unsupported branches causes them to fall in a prostrate position radiating from the center of the shrub. As these old and heavy branches weather, they very often break off at the center of the plant at about the point from which stems emerge from the dune surface. Plants with prostrate branches are more prevalent on stable rather than active dune areas (Table 7). Perhaps active dunes help support the weight of branches and prevent breakage.

Shrubs with dead branches seem to be distributed similarly on both active (16 plants) and stable (19 plants) dunes (Table 7). Dead branches, especially at the base of these shrubs, are probably a normal part of the growth cycle of the giant four-wing saltbush.

Dead shrubs are almost entirely restricted to stable dune areas (94%; Table 7). This may be the result of better protection of their branches by sand cover on active dunes. Shrubs growing on stable sites would also be more prone to desiccation by hot drying winds.

*Centers of Density, Damage, and Mortality within the Recreation Area*

The arm of the sand dunes northwest of Tanner Creek has the highest shrub density (15.3 shrubs per transect) of the four recreation sites studied. The Jericho picnic-campground site has an intermediate shrub density (10.6 plants per transect). Shrub densities at the White Sands (5.5 shrubs per transect) and Sand Mountain (5.1 shrubs per transect) sites are similar (Table 8).

Sand Mountain had the highest number (17 or 50%) of damaged shrubs of all the areas studied, while the Jericho site had nine, Tanner Creek had six, and White Sands had three damaged shrubs (Table 8). Sand Mountain is the center of visitor activity within the Little Sahara Recreation Area and shrub damage would be expected to be highest where visitors spend most of their time.

The Jericho site had the highest number (12) of shrubs with dead branches. White Sands (8) and

Table 6. Shrub vigor on active and stable dunes.

	Active Dunes			Stable Dunes		
	Number of Individuals		Percentage of Plants in Active Dunes w/Condition	Number of Individuals		Percentage of Plants in Stable Dunes W/Condition
Vigor	28	Excellent	43	13	Excellent	13
	20	Good	30	46	Good	48
	10	Fair	15	31	Fair	33
	8	Poor	12	6	Poor	6

Table 7. Plant damage and mortality on active and stable dunes.

	Active Dunes		Stable Dunes	
	Number of Individuals	Percentage of Plants in All Dunes w/Condition	Number of Individuals	Percentage of Plants in All Dunes w/Condition
Damaged shrubs				
Broken branches	13	76	4	24
Prostrate branches	4	22	14	78
Mortality				
Dead branches	16	46	19	54
Dead shrubs	1	6	15	94

Sand Mountain (8) had similar numbers of shrubs with dead branches. Tanner Creek had the lowest (6) number of shrubs with dead branches (Table 8).

Tanner Creek had the highest mortality (12 shrubs or 75%). Shrub mortalities at the Sand Mountain (two shrubs), White Sands (one shrub), and Jericho (one shrub) sites were low (Table 8).

The causes of dead branches at the Jericho area and mortality in the Tanner Creek vicinity were not determinable. Monitoring plots should be set up in both of these areas, as well as Sand Mountain, to assess shrub damage and mortality trends.

#### *Threats*

The plants seem healthy and their growth was observed to be vigorous, but 22 percent are being damaged by recreational activities within the dunes. This concerns us, because as the human population rises, more pressure will be put on this area for recreation. As a consequence, the plant population will be even more at risk and could become endangered without proper management.

Pye and Tsoar (1990) list several direct threats to sand dune vegetation and habitats from recrea-

tionists. These include picking and trampling of vegetation, increased risk of fire, physical erosion of sand by passage of feet, wind funneling along trails, and vehicle tracks leading to blowouts. Motorized vehicles also pose other threats, such as direct crushing of aerial shoots and roots of plants. Tires can compact sand and increase water repellence, which leads to erosion by surface wash. Erosion of dune surfaces results in reduced infiltration and increased drought damage to vegetation (Pye and Tsoar 1990). These types of threats should be monitored annually.

We observed several rodent burrows, a few mammal burrows, a few deer tracks and droppings, and coyote feces in the shrub's habitat. Animal use of the species' habitat appears not to be threatening the population at this time. Some evidence of grazing was observed on these shrubs. Next year we would like to record the number of grazed plants in our monitoring plots.

#### *Population Viability*

Population viability assessment (PVA) is relatively new to the discipline of conservation biology (Shaffer 1981, Gilpin and Soule 1986, Menges

Table 8. Comparison of shrub density, damage, and mortality by recreational site at Little Sahara Recreation Area, Juab County, Utah.

Area	Density <sup>1</sup>	Damage <sup>2</sup>	Mortality <sup>3</sup>
White Sands	5.5	3	8:1
Jericho	10.6	9	12:1
Sand Mountain	5.1	17	8:2
Tanner Creek	15.3	6	6:12

<sup>1</sup>Mean number of plants per transect, n = 20.

<sup>2</sup>Number of plants with broken or prostrate branches.

<sup>3</sup>Number of plants with dead branches:number of dead plants.

1986, 1990, 1991, Boyce 1992). Most of this research has been conducted on animal populations (Menges 1991, Boyce 1992). However, Menges (1986, 1990, 1991) has applied these research techniques to assess the viability of plant species threatened with extinction.

Detailed long-term autecological information is necessary to formulate estimates of minimum viable population numbers and to assess a particular population's viability (Menges 1991). A species' population parameters (demographics), ecology, environment including natural disturbances, and genetic characteristics are often analyzed when trying to determine viability. Future threats to the species are also considered. Some researchers (Boyce 1992) feel that it is impossible to determine population viability because of all the ecological factors and stochastic events involved. Basically, researchers provide their best educated guess concerning the future survival of these endangered species. When adequate population data is available, the process of conducting viability assessments is much easier.

Population viability for endangered plant species is usually determined by comparing recruitment rates with mortality rates (Menges 1990). The ideal situation would be for recruitment rates to be higher than mortality rates in these species. Growth, vigor, damage, and threats to the species are also studied and analyzed.

### Conclusions

In general, the plants that occupy the sand dunes seem healthy and vigorous, but if visitor use of the site increases and damage (22%) to the shrubs continues, their survival may be jeopardized. Threats resulting from visitor use should be monitored on an annual basis.

Shrub mortality was low at 9 percent this year. Recruitment may be nonexistent and shrub seedlings were not observed. The giant four-wing salt-

bush may be in danger of extinction if it is solely dependent on vegetative reproduction. Every effort should be taken to comb the monitoring plots for seedlings this spring. If climatic patterns inhibit germination, or if other stochastic events should affect the population, damage to individual mature shrubs may decrease population numbers to a point critical for survival.

### Acknowledgments

This research was funded by a challenge cost-share grant from the Utah State Office of the Bureau of Land Management in Salt Lake City, Utah to Red Butte Garden and Arboretum (RBG&A). I appreciate their continued support of this project.

I would also like to thank Therese Meyer, Conservation Horticulturist (RBG&A), who has experimented with seed germination and propagation of this species, and Barbara Soha, Conservation Volunteer (RBG&A), for preparing Figure 1.

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# Distribution and Biology of *Trifolium longipes* subsp. *neurophyllum* (Greene)

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**Abstract:** *Trifolium longipes* subsp. *neurophyllum* (Mogollon clover) is designated category 2 by the U.S. Fish and Wildlife Service. This report is a précis of a status assessment that was made to draw together all known facts about its biology, distribution, and population stability. Mogollon clover is morphologically distinct, having large heads and elongated trifoliate leaves. It is found between 6500 and 9000 ft in permanently, or seasonally, damp meadows and near seasonal or perennial streams in eastern Arizona and New Mexico. The first collection was made in 1903. Confusion between subspecies *pygmaeum* and subspecies *neurophyllum* has occurred. A collection was made of subspecies *reflexum* among plants of subsp. *neurophyllum* in Arizona. Prior to this observation subspecies *reflexum* has been reported only north of the range of subspecies *neurophyllum*. Although it may be locally abundant, it has been observed relatively rarely. It is clear that moist meadows and undisturbed stream banks are in decline and therefore its habitat is threatened.

## Introduction

*Trifolium longipes* (Nutt.) subsp. *neurophyllum* (Greene) Gillett has been proposed for listing as an endangered or threatened species and has been designated in category 2 (C2) by the Fish and Wildlife Service (Federal Register 1993). In Arizona it is also designated category 2 (C2), a taxon for which the Fish and Wildlife Service has insufficient information to support a proposed rule to add the species to the threatened and endangered species list (Rutman 1992). In New Mexico it is on "List 2," which is reserved for those species considered to be rare and/or subject to long-term use or cumulative land-use impacts and are thus vulnerable to events that could eventually threaten it with extinction or extirpation (Sivinski and Lightfoot 1994). It is listed as Forest Service Sensitive, Region 3 (Palmer 1991). For these reasons a status assessment was made and this report outlines the results of this work. It is hoped that this information will be useful to those people working with sensitive plants in the Southwest and to those interested in the *Trifolium* genus. Possibly the unanswered questions about this plant's biology, genetics, and habitat requirements will stimulate further research efforts.

## History and Biology of the Species

In the family Fabaceae (Leguminosae), the genus *Trifolium* comprises approximately 240 species that are divided into 16 sections. Species of *Trifolium* are distributed throughout Europe, western Asia, North Africa, and western North Amer-

ica. Approximately 85 species are native to the United States; about 20 are endemic to South America. The plants are annual, perennial, wild, and cultivated. Many cultivated species with origins outside the Americas are now naturalized in the USA (Taylor 1980). *Trifolium longipes* subsp. *neurophyllum* is in the *Lupinaster* section.

The first collection of *Trifolium longipes* subsp. *neurophyllum* was made from the middle fork of the Gila River in the Mogollon Mountains, Socorro County, New Mexico, by O.B. Metcalfe in 1903. The specimens were given to E.L. Greene at the Herbarium at Notre Dame University, Indiana, who recognized them as a distinct species of the *Trifolium longipes* group, and named it *Trifolium neurophyllum* (Greene 1903–1906). Kearney and Peebles (1960) included the species in the taxonomic key of the *Trifoliums* of Arizona, remarking that the large heads were a very distinctive characteristic. Gillett (1966, 1969) reviewed the taxonomy of the species of *Trifolium* L. in the section *Lupinaster* (Adanson) Seringe. He included *T. neurophyllum* in the *T. longipes* complex in his treatment, but regarded *T. neurophyllum* as a separate species. Martin and Hutchins (1980) in the *Flora of New Mexico* also included it as a species in their taxonomic key. Isely (1980) reviewed the *Trifolium* genus and assigned the plant varietal status, *Trifolium longipes* (Nutt.) var. *neurophyllum* (Greene) Isely, apparently in agreement with an unpublished thesis by Martin (1943). In *The Flora of North America* it is given as *Trifolium longipes* (Nutt.) subsp. *neurophyllum* (Greene) Gillett (Gillett 1993).

Common names are Mogollon clover, White Mountains clover, and big clover.

*Trifolium* species are known as true clovers. The technical description of the species is in Gillett (1969) and the Arizona Game and Fish Department has provided a nontechnical description (1994). *Trifolium longipes* subspecies *neurophyllum* is a perennial herb that is generally 14–60 cm tall, although it can grow to a height (length if prostrate) of 80 cm. Leaves are divided into three leaflets that are 10–35 mm long, narrowly lanceolate, sharp pointed, and small toothed. The large flowers are in globose clumps, the clumps on a hairy, leafless, bractless stalk. The calyx is tubular, ends in long teeth, and is distinctively hairy (white villous). The flowers are 10–20 mm long and purple, purple-white, or white. The holotype is shown in Figure 1. The pods are 1–2 seeded (although 3–6 have been reported). It flowers in late July, through August, and into September when field identification is best accomplished. Identification using vegetative features alone can be very difficult.

Other species of *Trifolium* that may be found in the same area are *Trifolium repens* L. (white clover; introduced), *Trifolium pratense* L. (red clover; introduced), *Trifolium wormskjoldii* var. *wormskjoldii* Lehm. (*T. fendleri* Greene; fendler clover; native), *Trifolium wormskjoldii* Lehm. var. *arizonicum* (Greene) McDerm. (*T. arizonicum* Greene; *T. lacerum* Greene; native) and, in areas where the habitat may overlap (e.g. at the conifer/meadow interface), *Trifolium wormskjoldii* var. *longicaule* Woot. & Stand. (*T. pinetorum* Greene; woods clover; native). In Arizona, *Trifolium longipes* var. *pygmaeum* may, in some cases, share the same habitat, although observations of association with the latter have not been confirmed. In several past instances herbarium specimens of *Trifolium longipes* var. *pygmaeum* have been misidentified as *T. neurophyllum*. Another member of the *T. longipes* complex, *Trifolium longipes* var. *reflexum* (A. Nels.) J. Gillett, has been observed to grow amongst *Trifolium longipes* var. *neurophyllum* in Arizona. A specimen was collected in 1991 that was south of the original range (Figure 2). The specimen is deposited at the USDA Forest Service Herbarium, Albuquerque, New Mexico. This subspecies is very similar to subspecies *neurophyllum* but has white flowers and does not have the white villous calyx. *Trifolium rydbergii* (syn = *Trifolium longipes* var. *reflexum* (A. Nels.) J. Gillett) is reported for New Mexico but north of the range for subsp. *neurophyllum* (Gillett 1969, Martin and Hutchins 1980).

*Trifolium* species can be self-pollinating, cross-pollinating, or both. In general, self-pollinated species have small flowers and large flowers are characteristic of cross-pollinated species. From this generalization *T. longipes* subsp. *neurophyllum* could be a largely cross-pollinating species. Bumblebees (*Bombus* spp.) are often pollinators for very large headed species; honey bees (*Apis mellifera*) will be the pollen vector if other pollen sources are limited. Precise vectors for var. *neurophyllum* have yet to be determined. *T. longipes* does not possess the tripping mechanism that is found in other species of clover. Rhizobium (specifically strains of *R. trifolii*) are generally found in symbiotic relationship with *Trifolium* species (Bergensen 1982). However, nodulation of *T. longipes* subsp. *neurophyllum* has not been specifically reported.

A live plant was taken in 1964 from an area at 6900 ft and cultivated in a greenhouse for a year in Canada (B. Cody, personal communication 1995). Chromosome number and morphological characters remained the same as the original plant, suggesting that the original plant had no environmentally sensitive characteristics. No studies on seed production/germination, etc. were reported (B. Cody, personal communication 1995).

### Distribution and Habitat

*Trifolium longipes* subsp. *neurophyllum* is geographically on the periphery of the other *T. longipes* subspecies. The *T. longipes* polyploid complex covers a large portion of the western United States (Gillett 1969). Chromosome races in this complex exist at the diploid ( $2n = 16$ ), tetraploid ( $2n = 32$ ), and hexaploid ( $2n = 48$ ) levels and have a base number of  $x = 8$ . The diploid varieties, of which Mogollon clover is one, cover far less area than that covered by hexaploid and tetraploid populations. *Trifolium longipes* is absent from the Great Basin proper, most likely because of the scarcity of suitable habitat due to progressive desiccation of the region since Pleistocene times. The range of the species is within the White and Mogollon mountains of eastern Arizona and adjacent New Mexico at elevations of 6500–9000 feet. This area is part of the region designated M313 on the ECOMAP (in the National Hierarchical Framework of Ecological Units, McNab and Avers 1994).

As of February, 1995, there were 39 reports of occurrences, i.e., individual sites where the plants grew. Those as small as one individual clump (<1 m<sup>2</sup>) probably represent a discontinuous portion of



Figure 1. Drawing of two holotype specimens of *Trifolium longipes* (Nutt.) subsp. *neurophyllum* (Greene) Gillett. Photocopies for use by the artist (Ronald Redsteer) were courtesy of Dr. B. Hellenthal, Notre Dame-Greene Herbarium, Notre Dame, IN.





the major population when a larger population is located within a hundred meters (of the smaller individual clump or cluster of individuals). There are 19 sites in Arizona and 20 in New Mexico. Populations in the Cox Canyon region of New Mexico that were first located more than 10 years ago have apparently been extirpated. Hydrological changes have occurred in the region of the original observation and the area is much drier (E. De-Bruin, personal communication 1994). There have been few attempts to relocate plant populations at historic locations (>10 years between surveys). Plants found in the vicinity of Willow Creek, Arizona in 1991 were probably a rediscovery of plants from which Clyde Jones had collected in 1960 (UNM Herbarium specimen, Albuquerque, New Mexico), and those located in the Salvador Springs area in New Mexico, also in 1991, are likely to be a relocation of the plants from which M. Talbot collected in 1915 (USDA Forest Service Herbarium, Albuquerque, New Mexico). In Arizona, plants have been relocated near Hannagan Meadows where plants were first identified in 1935 (Bainbridge 1991). Specimens are deposited at the New York Botanical Garden Herbarium and the University of Arizona Herbarium.

In several of the occurrence records, numbers of individual plants have been either counted or estimated (greater than, less than, etc.). The numbers of the plants at different sites are described in Tables 1 and 2. Low and high refers to the values given when an estimate was provided. For example, when a population was described as about 100, the low used was 75 and the high was 125. From these observations the mean number of plants per site is quite high, 69 in Arizona and 434 in New Mexico, but standard deviations are so high as to make the mean biologically, as well as statistically, meaningless. The median value is probably more significant and appears to be 16 plants per occurrence in Arizona and 100–150 in New Mexico. For a plant like Mogollon clover these are small patches, when one considers that a square meter could enclose over 100 plants. In addition, in making estimates during flowering it may be tempting, but erroneous, to consider a flowering head as denoting a plant. In New Mexico, only 12 records contained actual numerical estimates; one observation where plant number was estimated to be in the thousands was made when only two plants were in bloom, so the chances of misidentification were high. This is especially true in view of the fact that plants of subsp. *reflexum* were amongst plants of subsp.

Table 1. Number of plants in individual occurrences in Arizona (AZ) and New Mexico (NM). Where the low and high estimate are the same, the plants were counted. Where estimates were made, they were made in the field, but no counting was carried out.

State	Number of Plants		State	Number of Plants	
	Low	High		Low	High
AZ	2	2	AZ	200	300
AZ	2	2	AZ	100	100
AZ	6	6	AZ	150	200
AZ	8	8	AZ	500	1000
AZ	10	10	NM	24	48
AZ	15	15	NM	175	225
AZ	15	15	NM	450	500
AZ	15	15	NM	225	275
AZ	16	16	NM	1000	1500
AZ	18	18	NM	1	1
AZ	100	100	NM	1500	2500
AZ	100	100	NM	2000	2500
AZ	5	10	NM	75	125
AZ	5	10	NM	15	25
AZ	25	35	NM	15	25
AZ	50	60	NM	55	65
AZ	100	500			

Table 2. Estimated mean and median values of the plants per site in Arizona and New Mexico.

	State	Mean	Std Dev	Median
Low estimate	NM	433	653	100
	AZ	69	114	16
High estimate	NM	611	928	150
	AZ	125	237	16

*neurophyllum* in Arizona. Other than flower color, this creamy white flowered *Trifolium* is superficially quite similar to the subsp. *neurophyllum* and it is important to keep this in mind when surveying for the rare subsp. *neurophyllum*.

Herbarium specimens are deposited at the University of Arizona Herbarium (ARIZ), the Arizona State University Herbarium (ASU), the University of Notre Dame Greene Herbarium (ND-G; see Figure 1), the New York Botanical Garden Herbarium (NY), the University of New Mexico Herbarium (UNM), the New Mexico State University Herbarium (NMSU), the Center for Land and Biological Resources Research Herbarium (DAO), Canada, the U.S. National Herbarium (Smithsonian Institute) (U.S.), the USDA Forest Service Herbarium (Southwest Regional Office).

*Trifolium longipes* subsp. *neurophyllum* occurs primarily in seasonally or permanently damp clearings in ponderosa pine and mixed conifer

forest at elevation 6500–9000 ft. They grow in wet, but not marshy, meadows and on benches beside active streams. Some plants have been located in relatively dry environments (not riparian) but these habitats are likely to be seasonally moist. The plants can grow under pines and other trees as long as the canopy is relatively open (R. Mongold, personal communication 1995). Although the plants can tolerate dry conditions, probably by remaining dormant, they apparently need damp conditions to flourish. Associated plants are *Iris missouriensis* Nutt., *Trifolium fendleri*, *T. repens*, *T. fendleri*, *Achillea*, *Alnus*, and ponderosa pine. *Senecio quaerens* Greene has been observed to be in the more mesic habitats occupied by *T. longipes* subsp. *neurophyllum*.

On a local level the distribution of *Trifolium longipes* subsp. *neurophyllum* appears to have been fragmented by water development and other forms of habitat degradation. The influence of the fragmentation of populations on genetic structure, and the potential for species outcrossing with other *Trifolium* species in the region has not been studied. Genetic analysis and pollination studies could resolve these questions.

Although not quantified, the presence of Mogollon clover has been observed to be positively associated with basalt soils and negatively associated with datil soils (P. Morrison, personal communication 1995), which may be one of the reasons why the species is not found eastward into New Mexico. Basalt soils are derived from quaternary volcanoes and datil soils from tertiary volcanoes.

### Threats to the Species

Elimination of potential habitat is a major threat. This loss of habitat can be due to one or several pressures acting alone, or in concert. Road building and timber harvesting activities may change the hydrology of an area. For example, the population at Cox Canyon may have been lost either by direct disturbance (road development), or indirectly by the observed change in the hydrology of the area. Grazing pressures by livestock can lead to changes in soil properties through compaction and disturbance, including initiating gullies through the meadows. Livestock particularly disturbs stream banks and stream channels. There are several indications that substantial grazing may have a negative interaction with the *T. longipes* var. *neurophyllum* population. There is the tendency for individual plants in areas subject to grazing to grow in clumps of *Iris missouriensis* that

are not palatable to livestock and therefore resist trampling. Fenced areas have been observed to enclose higher numbers of individuals than adjacent, unfenced areas. There are morphological changes in response to grazing pressures. Plants have been observed to respond to grazing pressure by becoming more prostrate (R. Sivinski, personal communication 1995). A positive view of this latter phenomenon is that some adaptations to grazing are possible, but there has been no investigation of whether chemical and phenological changes occur along with the morphological changes. These “unseen changes” may be detrimental to the species vitality or reproductive potential. Livestock and elk directly consume the flowering heads and thus eliminate the potential for seed production. This is a special concern because during dry years the plants may not be able to grow or multiply substantially and they rely on prolificacy in wet years to propagate the species. Statistical analysis of these observations, and thus their significance, is missing and needs to be addressed.

No interspecific interactions have been observed although acute shading by ponderosa pines and other trees may be a limiting factor to the plant’s growth. Encroachment of the conifers onto meadow areas has been observed to be affecting suitable habitat for the clover (personal communication, P. Boucher 1994, P. Morrison 1995). It may be that long-time fire suppression has contributed to the increase in pines in such areas (Boucher, personal communication 1994). It may also be that pine encroachment is an effect, rather than a cause of drying sites.

Native and cultivated species of clover are notoriously difficult to cross artificially (Taylor 1980). Little gene transfer between the sections has been possible. Artificial hybridization with another species or variety has not been attempted with *T. neurophyllum* specifically. Native American and cultivated species of clover have generally not been crossed because of species incompatibility. There may be a potential for effective crossing between the geographically near, extant relative *T. longipes* subsp. *pygmaeum* ( $x = 8$  and  $2n = 16$ ) with *T. longipes* subsp. *neurophyllum*, if the conditions were appropriate and the plants were located near each other. *T. longipes* subsp. *pygmaeum* flowers from June to September and so coincidental flowering could occur. However, according to the current information available, this situation is only likely in a small area in the Apache–Sitgreaves National Forest. Specimens a little uncharacteristic of both species have been reported, but conclusive

evidence of hybridization has not been gathered. Observed differences in the morphology of *T. longipes* subsp. *neurophyllum* could well be explained by the morphological plasticity; for example, a prostrate growth habit and smaller plants due to grazing could restrict normal growth.

Because observations and quantitative surveys earlier than 5 years ago are few, the trend of the plant's population and range is not known for certain. However, there is some evidence of decline in population number and range. Fragmentation of populations is suggested by the discontinuous patchiness of the plants along disturbed streams with ostensibly suitable habitat. There also tends to be more plants in the less disturbed regions.

### Conclusion

*Trifolium longipes* subsp. *neurophyllum* is a distinctive plant, but it can be mistaken for other subspecies of *Trifolium longipes*, especially in the vegetative state. Present evidence supports that this plant is found only infrequently, considering the number of areas surveyed and the number of places in which it has been found. It therefore can be considered rare; however, it can be locally abundant, and given the correct conditions, it is not naturally given to sparse local distribution. It is clear that the number of moist meadow habitats and undisturbed stream banks have declined and without care such areas will continue to contract. Therefore, its habitat is threatened. Small populations stand a good chance of extirpation because of grazing pressure from elk and livestock, especially in dry years.

This subspecies may serve an important function as a biological indicator of the hydrological conditions of a given meadow over time. There is still a lot to be learned about the distribution, ecology, associated *Trifolium* species, and community structure of Mogollon clover.

### Acknowledgments

This status assessment was funded by the National Biological Service, Washington D.C. 20240, under the initiative "Species at Risk, 1994."

Appreciation is also expressed to the following people who kindly provided information: Paul Boucher, Gila National Forest, NM; David R. Brunner, Missouri Botanical Garden, MO; B. Cody, Center for Land and Biological Resources Research, Ontario; Ann Cully, USFW, NM; Ellen DeBruin, New Mexico Natural Heritage Program, Albuquerque, NM; Reginald Fletcher, USDA FS, Albuquerque, NM; JM Gillett, Canadian Museum

of Nature, Ontario; Dr. Barbara Hellenthal, Notre Dame-Greene Herbarium, IN; Dr. P.K. Holmgren, NY Botanical Garden, NY; Dr. Duane Isely, ISU, IA; Dr. Phil Jenkins, U of A, AZ; Karen Lightfoot, Santa Fe, NM; Terry Lillybridge, Wenatchee National Forest, WA; Dean Longrie, USDA FS, OR/WA; Roger Mongold, Bandelier National Monument, NM; Pat Morrison, Gila National Forest, NM; Terry Myers, Apache-Sitgreaves National Forest, AZ; Bruce Palmer, USFWS, AZ; Dr. Pinkava, ASU, AZ; Teresa Prendusi, USDA FS, NM; George F. Russell, Smithsonian Institute, Washington D.C.; Sabra Schwartz, AZ Game and Fish Dept., AZ; Dr. R. Spellenberg, NMSU, NM; Tom Subrige, Apache-Sitgreaves National Forest, AZ; Dr. N. Taylor, University of Kentucky, Lexington, KY; Bob Sivinski, Santa Fe, NM; Dr. Peter Warren, TNC, AZ; Dr. Martin Wojciechowski, U of A, AZ.

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# Endemic Calciphiles of the Middle Arkansas Valley, Colorado

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**Abstract:** The Middle Arkansas River Valley of southern Colorado contains a number of endemic species associated with the Niobrara Formation. Many endemic plants occur throughout this stratum, which is subdivided into layers of Ft. Hayes limestone and Smoky Hills calcareous shales, chalks, and limestones. One species, however, *Oxybaphus rotundifolius*, appears only on a few subunits of the Smoky Hills Member. Elemental cation analysis of the soils using an inductively coupled plasma spectrometer showed geochemical differences between the Ft. Hayes limestone soils, which lacked *O. rotundifolius*, and the Smoky Hills sites where it grows. X-ray diffraction of the Niobrara soils dispelled the assumptions in current botanical literature that gypsum occurs abundantly throughout these soils and that local endemics were obligate gypsophiles. Geological data provide useful autecological information about rare species and opportunities for predicting the occurrence of populations.

## Introduction

The Arkansas River Valley of southern Colorado has long been recognized for its unusual flora. Taxa of particular interest include Chihuahuan element disjuncts and local endemics. In the Middle Arkansas Valley, defined here as the region between Pueblo and Cañon City, many of the endemic taxa appear on local exposures of the Niobrara Formation, a massive upper Cretaceous deposit of calcareous shales, chalks, and limestones. Described in the literature as obligate calciphiles, these endemics have also often been characterized as gypsophiles (e.g., Harrington 1964, Naumann 1990, Weber 1990). Despite extensive bedrock exposures in the Arkansas drainage, which would seem to offer suitable habitat, some of these endemics appear to be quite rare. Conservation concerns, raised by the limited distributions of these plants and locally strong development pressures, have resulted in their placement on a category 2 species list for Colorado (Colorado Natural Heritage Program 1995).

Though recognized for its unusual flora, the Middle Arkansas Valley has received little detailed attention from botanists. It remains poorly collected, ecologically unexamined, and sparsely documented for the distribution of the suspected rare taxa. Currently, however, work is underway to rectify this situation. Our study, outlined here, focused on the specific habitat requirements for some of the endemic calciphiles, with particular attention given to one of the rarest species in the region, *Oxybaphus (Mirabilis) rotundifolius* (Greene) Standley (Nyctaginaceae). [Nomenclature for the taxa discussed in this paper follows Weber and Wittmann (1992).]

The association of rare or endemic plant taxa with unusual substrates, such as those rich in carbonates, ultra-mafic compounds, or minerals like selenium or gypsum, has long been accepted by botanists without necessarily being well understood. It is notably difficult to determine if rare species grow in situ, because unusual substrates provide requisite geochemical conditions, physical conditions, biological conditions, or conditions that exclude competitors. Few detailed studies have examined the influences of stratigraphy and geochemistry on the autecology of rare plants; consequently, the use of geological data for predicting rare plant occurrences has limited precedent. The middle Arkansas Valley provides an opportunity for such investigations due to the wealth of geological information available and the variety of substrate-specific species growing there. Correlative studies of floristics and geology may produce valuable results throughout the Southwest, where detailed geologic survey maps exist for large expanses of botanically unknown territory. With few botanists to survey these expanses, focused site selection could yield valuable findings.

In this study we examined the relationship of endemic calciphilic species to the substrate(s) on which they grow in the Middle Arkansas Valley. Our specific objectives were as follows:

- To determine if certain calciphilic endemics, particularly *Oxybaphus rotundifolius*, were restricted to specific stratigraphic units.
- To determine whether unusual substrate conditions correlate with the distribution of *O. rotundifolius*.
- To determine if detailed geological information could assist in predicting the occurrence of rare plant species.

In addition to extensive fieldwork and use of the geological literature and maps, we analyzed soil samples in the laboratory using X-ray powder diffraction (XRD) to test for occurrence of gypsum, calcite and quartz; pH measurements; and elemental cation speciation using an inductively coupled plasma spectrometer (ICP). Seven extracts were used in the ICP analysis: a general elemental survey with HCl, the ion exchangeable and surface adsorbed fraction, carbonate-bound fraction, Fe-Mn oxide fraction, weakly bound organic fraction, strongly bound organic fraction, and the crystalline hydrous oxide fraction.

### Materials and Methods

Fieldwork and laboratory analyses were conducted May–August of 1995. Floristic surveys of the region covered approximately 1000 km<sup>2</sup> in Pueblo and Fremont counties. For initial test sites, we relocated 6 of the 12 previously known populations of *Oxybaphus rotundifolius* (Naumann 1990). Several undocumented populations were also found during the months of fieldwork and they were included in our habitat analyses. Physical parameters including slope, aspect, surficial characteristics, and stratigraphic position were assessed for each site. We removed the top 2 cm of soil around individual *Oxybaphus* plants (chosen to represent the approximate center of the population), and then sampled several grams of subsurface soil surrounding the plant. These samples were mixed together and homogenized in a plastic bag. Where possible, several samples were taken from a population. Comparative samples were also taken from other calcareous sites of known stratigraphic position that did *not* contain *Oxybaphus* populations. Soil samples were frozen at -29°C until the time of processing and analysis.

We used the technique given in Hendershot et al. (1993) with a Cole–Parmer electronic pH meter to obtain pH measurements. For X-ray diffraction analysis, sample subsets were sieved to remove large bedrock pieces, then analyzed using random orientation powder mounts and a Phillips X-ray diffractometer. [Analysis performed by S. Weaver and C. Seaman of the Colorado College Department of Geology.] For ICP analysis, soils were prepared following the techniques of Tessier et al. (1979) as modified by Bower (1995). Analysis was performed on a Thermo–Jarrell Ash Atomscan 16 inductively coupled plasma spectrometer. [Sample preparation by K. Heckmann and P. Beardsley; analysis by N. Bower, Department of Chemistry,

Colorado College.]

### A Summary of the Geology of the Middle Arkansas Valley

The central position of the Pueblo area as illustrative of the Western Interior Cretaceous Basin of North America provided the original impetus for stratigraphic studies of the Arkansas River bedrock. The Niobrara Formation was formed by episodic deposition in the shallow epicontinental Inland Seaway (Kaufmann 1985). The dynamism of the seaway in response to tectonic, eustatic, and climatic factors makes it a particularly rich area for study by sedimentary geologists. Recent interest in sea level–climate links with the Milankovitch cycles of orbital variation has resulted in the mapping of high-resolution exposed bedrock, stratigraphic details cut by the Arkansas River and its tributaries.

Originally described from the Great Plains, the Niobrara Formation has a complex nomenclatural history (Scott and Cobban 1964, Hattin 1982, Barlow and Kaufman 1985). Its outcropping in the Pueblo–Cañon City region, which consists of a basal Ft. Hayes limestone overlain by the Smoky Hills Members (Figure 1) has been extensively documented. Type localities for both members occur in Kansas, but high-resolution stratigraphy in Colorado has provided local analysis of the formation, which is almost 250 m thick in the Pueblo area. The following details about the formation have been summarized from Barlow and Kauffman (1985), Kauffman (1985), Pollastro and Martinez (1985), Kauffman and Pratt (1985), Scott (1964, 1969a, 1969b), and Scott and Cobban (1964).

The basal unit of the Niobrara Formation is Ft. Hayes limestone, a very resistant gray limestone interbedded with thin calcareous shales. Slightly more than 13 m thick, the unit is easily recognized by its characteristic wide ledges and abundance of large bivalve fossils (*Ostrea congesta*). On gentle slopes, the ledges weather to smaller fragments that obscure the bedding. Above the Ft. Hayes limestone, the Smoky Hills Member contains seven subunits (Figure 1):

- (A) Shale and limestone unit (6.7 m): transitional between the hard Ft. Hayes limestones and the typically fissile Smoky Hills layers. The soft shales interbed with the harder limestones and contain some gypsum near the top and bottom of the unit. Bedding layers average ca. 10 cm in thickness, appearing gray or a weathered yellow-gray.

## STRATIGRAPHY OF NIOBRARA FORMATION: PUEBLO, COLORADO

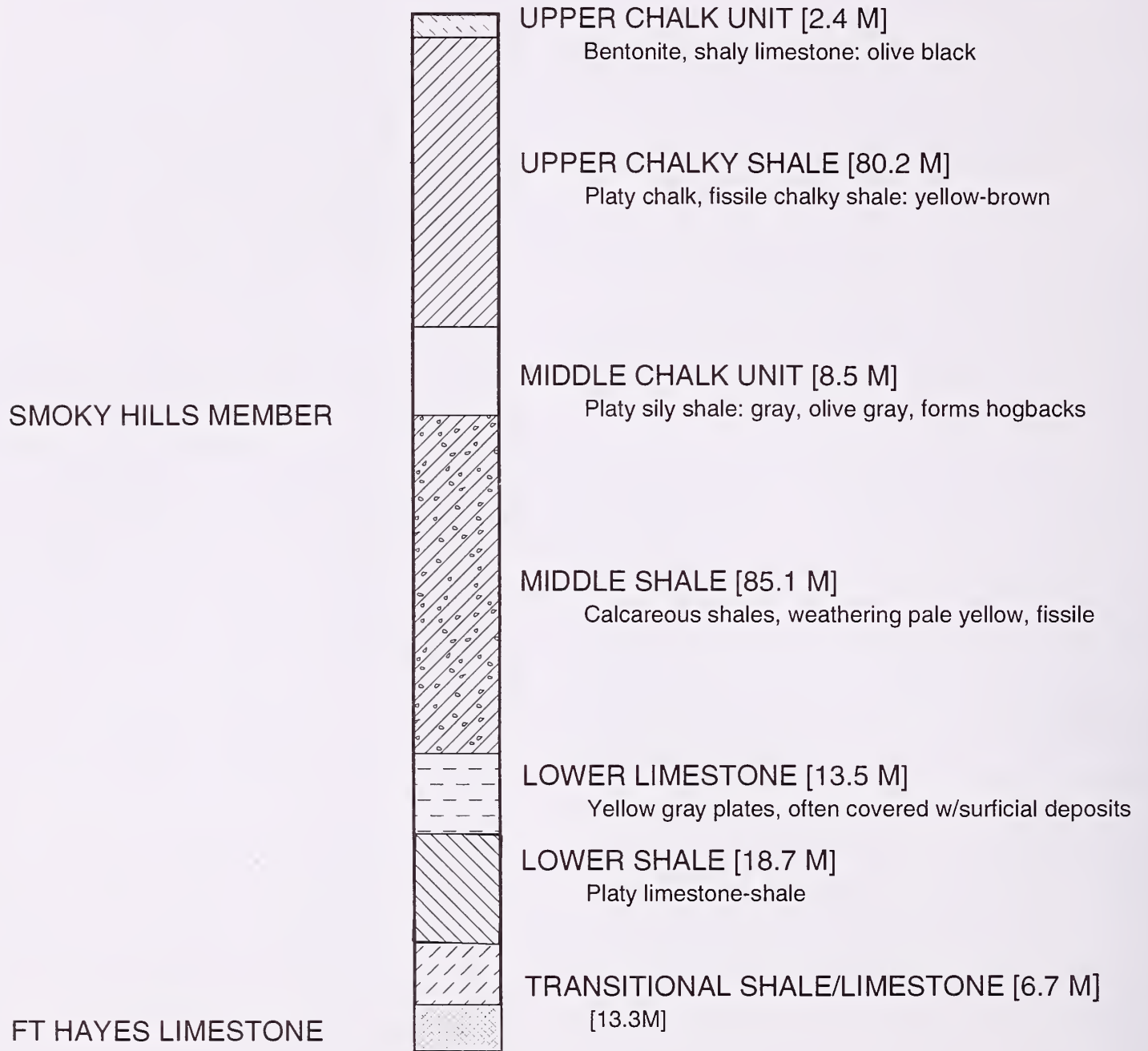


Figure 1. Stratigraphic units of the Niobrara Formation in Pueblo, CO. Adapted from G.R. Scott and W.A. Cobban (1964).



- (B) Lower shale unit (ca. 19 m): slightly resistant calcareous shales forming slopes between the harder layers of the under and overlying units. These shales weather to medium gray with marker units of orange limonite and bentonite. Beds range in thickness from 18 to 62 cm.
- (C) Lower limestone unit (13.5 m): a low ridge-forming unit of argillaceous limestone with shaly limestone interbedded with calcareous shale. Beds weather to olive-brown or buff and range in thickness from 16 to 72 cm.
- (D) Middle shale unit (ca. 85 m): erodes easily, forms a broad valley with few outcrops in the Pueblo area and consists of various calcareous shale types. Colors range from olive-gray to yellow-gray and yellow-brown.
- (E) Middle chalk unit (8.5 m): blocky chalk layers separated by thin layers of chalky shale. It weathers to small, irregular, yellow-gray plates.
- (F) Upper chalky shale (over 80 m): forms a broad valley between the more resistant middle chalk and upper chalk units. Colors range from orange-yellow to olive or yellow-gray. The lower portion contains gypsum or selenite.
- (G) Upper chalk (2.5 m): ledge-forming, olive-black (weathering to orange) chalk. Pierre Shale, a noncalcareous shale of the late Cretaceous Age, caps the Smoky Hills Member.

A summary local geologic column is shown in Figure 2.

### Calciphiles of the Middle Arkansas Valley

Table 1 shows some of the most notable calciphilic species found along the Middle Arkansas. Most occur throughout the area in diverse habitats on various stratigraphic units. A few are uncommon or rare in distribution; the most notable of these is *Oxybaphus rotundifolius*. Our field surveys concur with previous literature reports indicating that this species has a restricted distribution with apparently exact habitat requirements. While a number of other calciphilic species are found with *O. rotundifolius*, no single species can be used as a reliable indicator of its presence. Of the calciphiles, the most often associated species is *Penstemon versicolor* Pennell. Sites populated by *P. versicolor* usually contain *O. rotundifolius*. However, *P. versicolor* can also be found on sites that do not

contain *O. rotundifolius*, and it does not show the same preference for slopes that the latter species exhibits (see below).

Table 1. Calciphilic taxa of the Middle Arkansas River Valley, Colorado.

Widespread	
<i>Frankenia jamesii</i>	SW disjunct
<i>Menodora scabra</i>	SW disjunct
<i>Artemisia bigelowii</i>	Endemic
<i>Echinocereus reichenbachii</i> *	SW disjunct
<i>Melampodium leucanthum</i>	Endemic
<i>Townsendia fendleri</i>	Endemic
<i>Erioneuron pilosum</i>	Endemic
<i>Penstemon auriberbis</i>	Endemic
<i>Eremogone (Arenaria) hookeri</i>	Endemic
<i>Lesquerella fendleri</i>	Endemic
<i>Zinnia grandiflora</i>	SW disjunct
Uncommon	
<i>Bolophyta (Parthenium) tetraeuris</i> *	Endemic
<i>Lesquerella ovalifolia</i>	Endemic
<i>Lesquerella calcicola</i>	Endemic
<i>Nuttallia (Mentzelia) chrysantha</i> *	Endemic
<i>Eriogonum lachnogynum</i>	Endemic
<i>Eriogonum tenellum</i>	Endemic
<i>Hoffmanseggia drepanocarpa</i>	SW disjunct
<i>Oonopsis puebloensis</i> *	Endemic
Limited to Rare	
<i>Oxybaphus (Mirabilis) rotundifolius</i> *	Endemic
<i>Penstemon versicolor</i>	Endemic
<i>Asclepias uncialis</i> *	Distribution uncertain

\*Indicates species of special concern in Colorado.

Other associated calciphilic plant species are quite diverse, but in comparison to *O. rotundifolius*, they appear less specific in their ecological preferences, with no obvious restrictions to stratigraphic subunits of the Niobrara Formation. In addition to *Penstemon versicolor*, the most commonly associated herbaceous species are *Lesquerella calcicola* Rollins, *Lesquerella ovalifolia* Rydberg, *Bolophyta tetraeuris* (Barneby) Weber, and *Stipa hymenoides* Roemer & Shultes. Virtually all sites have low vegetative cover, dominated by cushion plants.

### Physical Characteristics of *Oxybaphus rotundifolius* Sites

This species is found almost entirely on moderately steep (<15°) slopes. Plants can occasionally be found on flat ridgetops, but are much less abundant there than on the slopes below. Slope

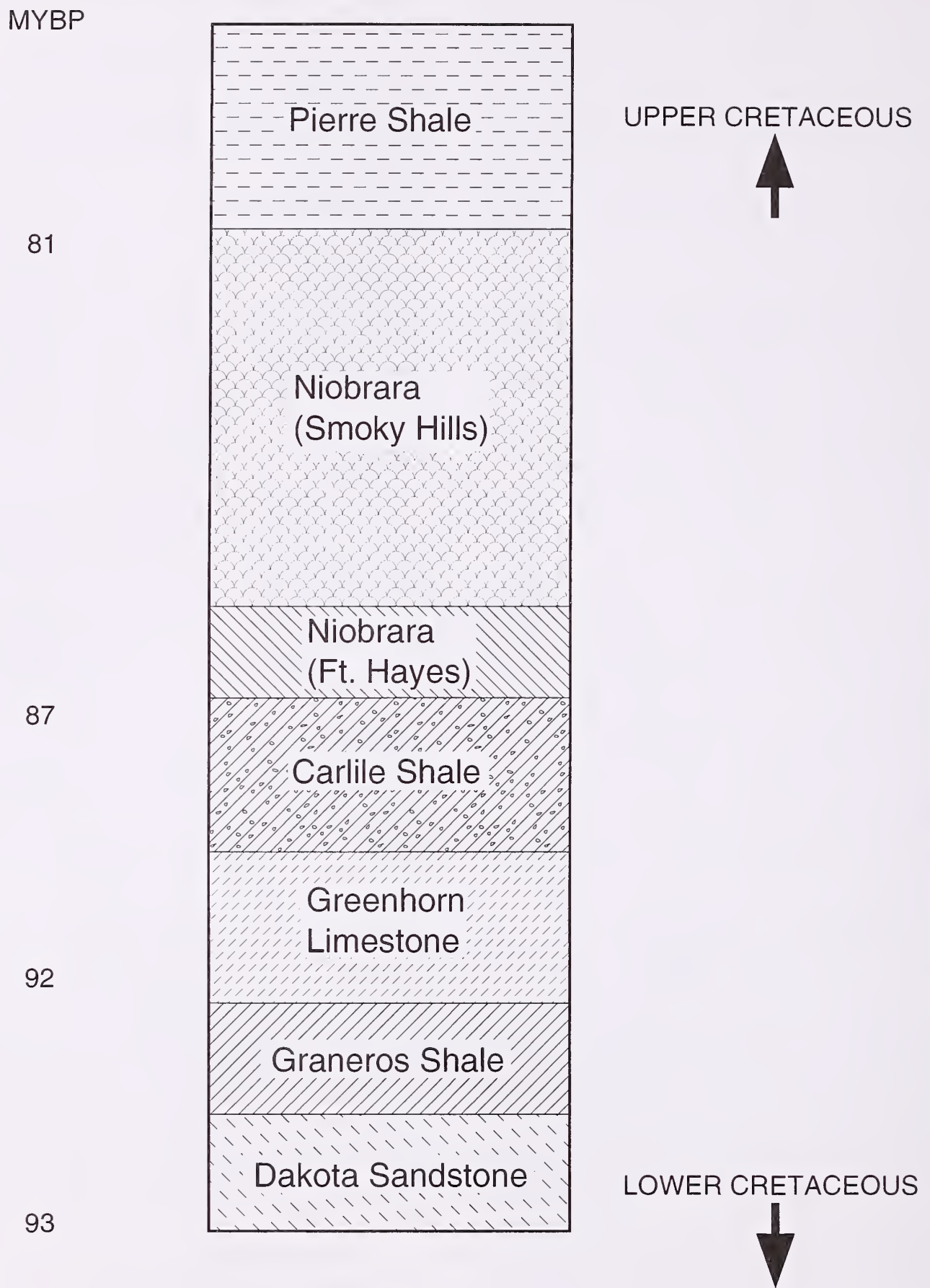


Figure 2. Generalized geological column near Pueblo, CO.

aspect varies considerably, but appears to be most common on south- or southeast-facing exposures. Surficial characteristics are uniform in all sites: a highly weathered bedrock facies consisting of small (less than 4 cm long) platy pieces forming a thin surface layer, with deep fines underneath. Habitats correspond to the middle chalk and upper chalky shale subunits of the Smoky Hills Member, where fissile bedding planes exist. We also found a single small (<30 individuals) population on a Ft. Hayes exposure. This site was anomalous for its highly weathered facies of small rock fragments and its low-angle slope. Ft. Hayes limestone is commonly found as resistant ledges in vertical exposures.

By using detailed geological maps in conjunction with knowledge of habitat preference for slopes, we were able to predict successfully the occurrence of the species in previously unsurveyed areas. Two new populations of *O. rotundifolius* were located using this method.

## Chemical Characteristics of *Oxybaphus rotundifolius* Sites

All sites are moderately to strongly alkaline, with a range of pH levels from 7.3 to 8.6 (Table 2). This range was consistent with values reported in the soil survey for Pueblo County (U.S. Department of Agriculture 1979). We found no significant difference in pH between the Smoky Hills sites with *O. rotundifolius* and the Ft. Hayes limestone sites that lacked it.

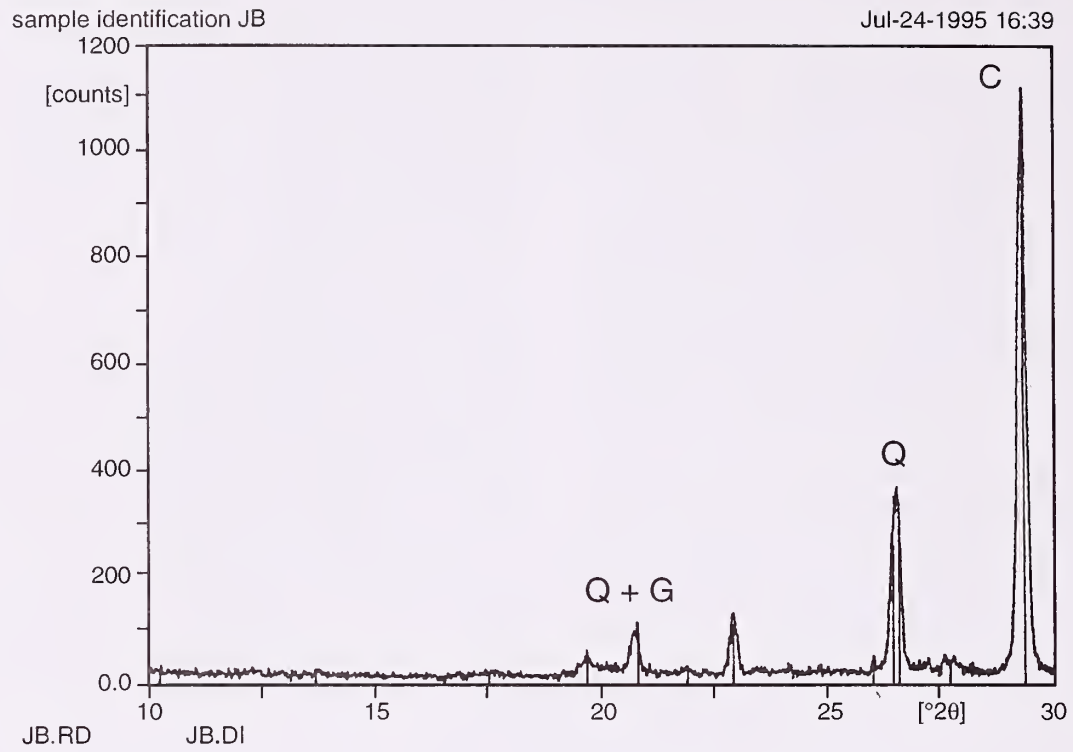
Of 17 soil samples analyzed using X-ray diffraction, all sites, even those lacking *Oxybaphus rotundifolius* populations, showed the strong presence of calcite and quartz (Figure 3). Gypsum appeared in only five samples, one of which was a test sample taken from a known gypsum lens. Of the remaining four samples showing gypsum, three were from sites with *Oxybaphus rotundifolius*. Four other populations of this species did not show any presence of gypsum. Though the botan-

Table 2. Soil pH, Niobrara Formation, Middle Arkansas Valley. Values represent median of two readings for each sample (electronic pH meter).

Origin	Unit	pH*	<i>O. rotundifolius</i>
Pueblo Wildlife Area 1	Smoky Hills	7.79	yes
Pueblo Wildlife Area 2	Smoky Hills	7.40	yes
Pueblo Wildlife Area 3	Smoky Hills	7.69	yes
Pueblo Wildlife Area 4	Smoky Hills	8.30	no
Juniper Brks 1	Smoky Hills	7.98	yes
Juniper Brks 2	Smoky Hills	7.85	yes
Juniper Brks 3	Smoky Hills	7.86	yes
Portland 1	Smoky Hills	7.56	yes
Portland 2	Smoky Hills	7.76	yes
Portland 3	Smoky Hills	7.49	yes
Rte 115/50	Smoky Hills	7.43	yes
Rte 115/50	Smoky Hills	8.21	yes
Rte115/50	Smoky Hills	8.32	yes
Rte 50 contact	Smoky Hills/Ft. H	7.92	yes
Four Mi Creek	Smoky Hills	7.92	yes
Four Mi Creek	Smoky Hills	7.84	yes
Four Mi Creek	Smoky Hills	7.91	yes
Beaver Creek	Smoky Hills	7.80	yes
Rte 115/K Rd	Ft. Hayes	7.81	no
Rte 115/K Rd	Ft. Hayes	7.95	no
Beaver Cr	Ft. Hayes	8.02	no
Pueblo Reservoir 1	Ft. Hayes	8.07	no
Pueblo Reservoir 2	Ft. Hayes	7.84	no
Swallows	Ft. Hayes	8.11	no
Pueblo S.	Ft.Hayes	7.97	no

\*pH values at sites with and without *O. rotundifolius* are not significantly different.

### A. No Gypsum



### B. Gypsum Present

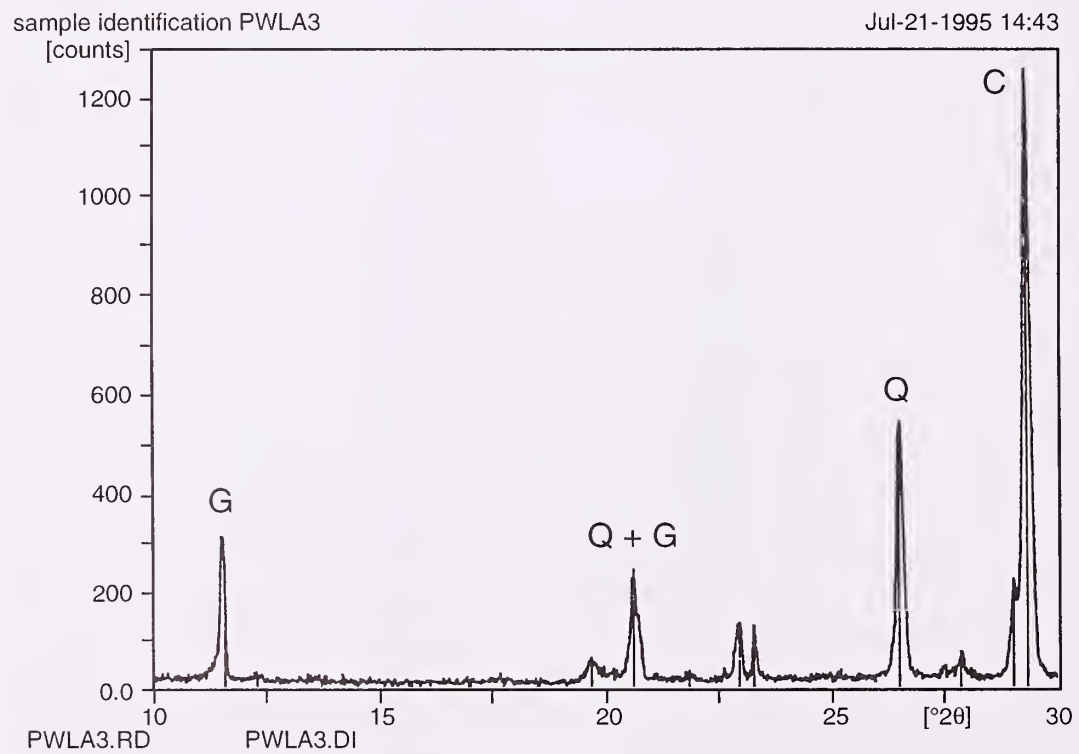


Figure 3. Typical soil profiles in the Niobrara Formation showing presence and absence of gypsum. Twelve of 17 samples were similar to profile A; five were similar to B. G = Gypsum; Q = Quartz; C = Calcite.

ical botanical literature presumes the nearly ubiquitous presence of gypsum in the Middle Arkansas Valley, the geological literature suggests that its occurrence is only sporadic, limited to thin beds in certain strata. Our XRD results contradict the botanical literature, which identified many of the local endemics as gypsophiles. While gypsum may occur in some sites, none of the calciphilic species we surveyed appear to depend on its presence.

For ICP elemental analysis, a survey of soil elements was obtained first using HCl extraction (Table 3). While this method does not indicate elemental availability to plants, it does provide the most general information about overall soil cations. In HCl extraction, we found a strong correlation between the occurrence of *O. rotundifolius* and a low presence of Fe and Mn (Figure 4). Further analysis using different soil fractions (Table 4) focused on the elements Fe, Mn, V, and Cd (the only elements that showed discernible and consistent patterns). Ft. Hayes limestone sites that were lacking *O. rotundifolius* did have chemical differences

Table 3. Elements found in detectable amounts, Smoky Hills Member and Ft. Hayes Limestone (soil concentrations in ppm). Ft. Hayes sites lack *O. rotundifolius*; Smoky Hills sites contain *O. rotundifolius*. Data from eight Ft. Hayes sites and eight Smoky Hills sites. Replicates taken at eight different sites showed minimal intrasite variation. Bold face elements show unusual levels.

Element	Range Ft. Hayes Sites	Range Smoky Hills Sites
B	3-8	2-11
Mg	74-187	67-221
Al	7-62	7-37
Si	27-77	29-96
P	0-1000	0-540
S	4-3600	3-3200
K	10-103	7-37
Ca	68000-80000	60000-80000
Ti	1.6-2.8	0.4-3.0
V	11-39	4.5-56
<b>Mn</b>	<b>26-81</b>	<b>8-24</b>
<b>Fe</b>	<b>35-100</b>	<b>11-67</b>
Co	0.5-1	0-2
Ni	0-1.5	0-1.5
Cu	0-1.5	0-1.5
Zn	1.2-9.5	1.9-11
Sr	141-220	162-221
Y	0-1.5	0-1.5
Cd	0.3-0.6	0.6-2.0
Ba	5-24	12-35
Pb	0-0.5	0-0.5

from Smoky Hills sites with *O. rotundifolius*. They had consistently lower levels of Cd and Mn in the carbonate-bound fraction, lower levels of Cd and V in the Mn-Fe hydroxide-bound fraction, lower levels of V in the weakly bound organic fraction, higher levels of Mn in the strongly bound organic fraction, and lower V, higher Mn, and lower Cd in the crystalline hydroxide-bound fraction. The presence of V and Cd in all the fractions strongly suggests that they are indigenous rather than local pollutants. A consistent pattern of high Cd and low Mn for Smoky Hills soils containing *O. rotundifolius* reoccurs throughout the different soil fractions. These levels appear to be atypical in comparison to levels found in other U.S. soils (McBride 1994).

## Discussion

Elemental analyses *do* show some unusual, or at least different, soil chemistry at *Oxybaphus rotundifolius* sites. In particular, the species correlates with low levels of Mn and high levels of the potential toxins Cd and V. Additional analysis of plant tissues will determine if plants accumulate these elements. While heavy metal or low manganese tolerance may be part of the autecology of *O. rotundifolius*, it would be premature and overly simplistic, however, to conclude that these factors alone explain its restriction to certain sites. Elements that appear high or low in different soil fractions are available to different plants in different ways and under different conditions of moisture and mycorrhizal association. We view these results as preliminary and only potential indicators of habitat requirements. Considerable additional work, including tissue and mycorrhizal surveys, will be needed to determine if these differences are important factors in the distribution of this species. We are cautious about assuming causation with geochemical differences in soils. Sedimentary stratigraphic units will differ chemically because of different environmental conditions in their depositional cycles. For plants, these differences may be only correlates of the physical characteristics and weathering patterns that are more critical components of habitat.

Although the discovery that geochemical differences exist between sites with and without rare species may pose more questions than answers, we suggest that such analyses are still valuable tools in rare plant biology. We found that the geological literature provided us with a detailed understanding of local substrate differences relevant for plant distributions, and that we could use

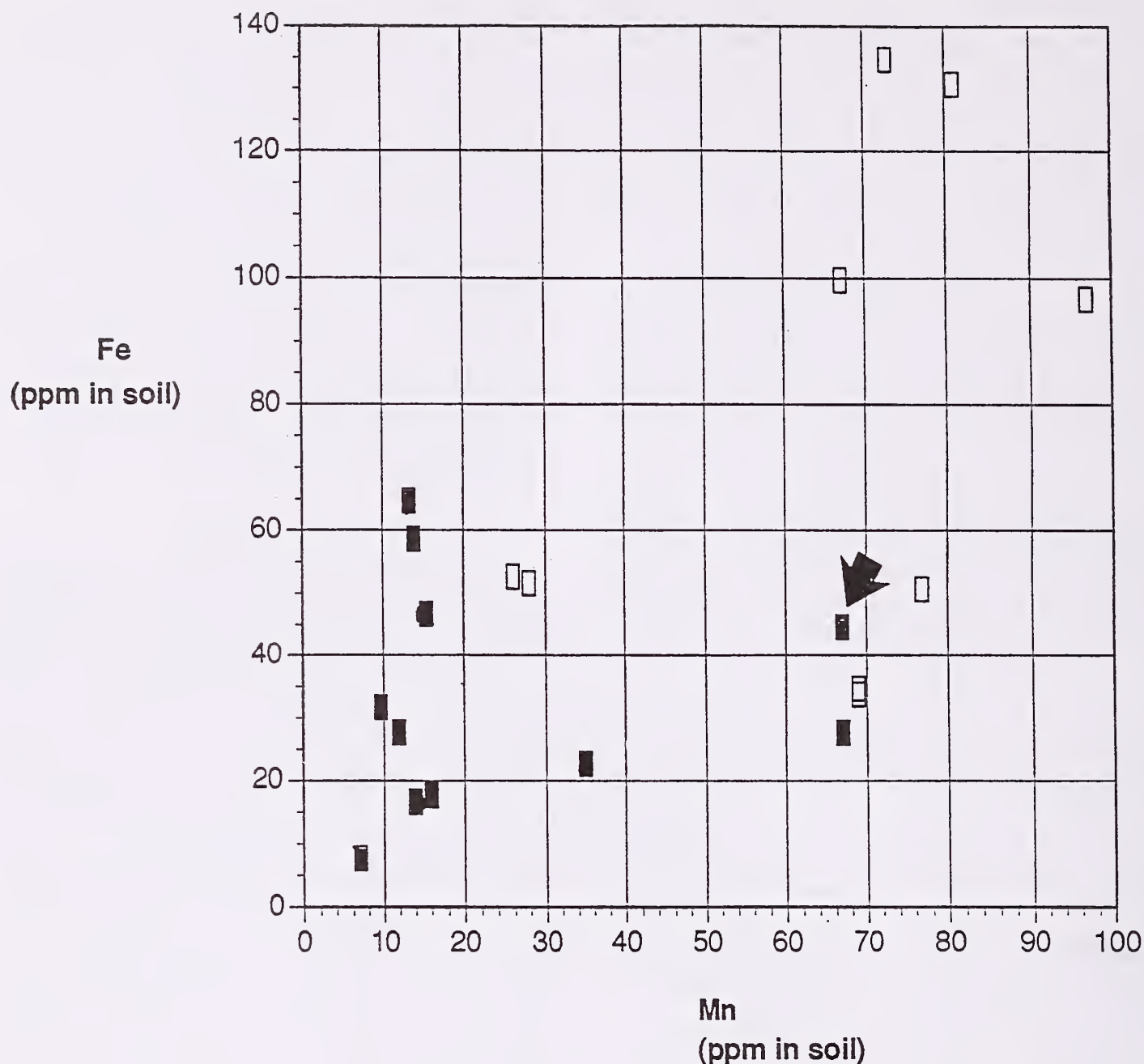


Figure 4. Iron and manganese content of Smoky Hills and Ft. Hayes soils. Filled rectangles are Smoky Hills locations containing *Oxybaphus rotundifolius*; open rectangles are Ft. Hayes locations that lack *O. rotundifolius*. Arrow indicates only known Ft. Hayes location containing this species.

available geological maps to pinpoint locations where new populations would be found. The geological literature provided us with the initial idea that if gypsum was not a ubiquitous component of local soils, then the botanical literature might be incorrect in assuming an obligate gypsophilic nature for local plant species. XRD analysis of soils supported this hypothesis: gypsum certainly occurs in the Middle Arkansas Valley, but we find little evidence that endemic or disjunct species here depend on its presence. Abundant crystalline calcite in the region may well have been

mistaken by botanists for surficial gypsum.

The initial results of cation speciation from ICP analysis pose intriguing questions for further pursuit. Additional elemental pattern analysis, plant tissue samples, and surveys of mycorrhizal associations may help clarify some questions about the relative importance of geochemical, physical, and biological characteristics of *Oxybaphus rotundifolius* habitat. On a practical level, the ICP provides a comparatively easy and inexpensive method of assessing habitat characteristics. This basic knowledge could be used not

Table 4. Comparative elements in Smoky Hills sites (with *Oxybaphus rotundifolius*) and Ft. Hayes limestone sites (lack *O. rotundifolius*). Nd = amounts not detectable by ICP analysis. Numbers indicate ppm in extract concentrations. Soil concentrations are approximately 10x larger ( $\pm$  individual calibrations for different fractionations).

Ext 1: Ion Exchangeable			Ext 2: Bound to Carbonates		
Element	Ft. Hayes	Smoky Hills	Element	Ft. Hayes	Smoky Hills
V	Nd	Nd	V	Nd	Nd
Mn	Nd	Nd	Mn	2.1-7.2	0.9-1.6
Fe	Nd	Nd	Fe	0.22-0.14	0.4-1.2
Cd	Nd	Nd	Cd	Nd	0.16-0.22
<b>Summary:</b> No significant differences.			<b>Summary:</b> <i>Oxybaphus</i> sites higher in Cd; lower in Mn.		
Ext 3: Bound to Mn-Fe Hydroxides			Ext 4: Weakly Bound to Organics		
Element	Ft. Hayes	Smoky Hills	Element	Ft. Hayes	Smoky Hills
V	0.93-1.2	2.5-7.2	V	Nd	0-0.7
Mn	10.6-27.0	2.6-11.4	Mn	0.1-1.1	0.07-0.67
Fe	80-160	114-305	Fe	6.3-23.0	1.0-21.0
Cd	Nd	0.1-0.53	Cd	Nd	Nd
<b>Summary:</b> <i>Oxybaphus</i> sites have higher V, Cd, Fe; low in Mn.			<b>Summary:</b> <i>Oxybaphus</i> sites have higher V? Otherwise, no differences detected.		
Ext 5: Strongly Bound to Organics*			Ext 6: Bound to Crystalline Hydrated Oxides		
Element	Ft. Hayes	Smoky Hills	Element	Ft. Hayes	Smoky Hills
V	Nd	0-1.3	V	0.3-1.1	1.6-5.1
Mn	0.3	0.03-0.1	Mn	1.3-3.6	0.49-5.1
Fe	17	3.4-14	Fe	85-167	105-192
Cd	Nd	Nd	Cd	Nd	0-0.16
<b>Summary:</b> <i>Oxybaphus</i> sites lower in Mn.			<b>Summary:</b> <i>Oxybaphus</i> sites higher in V.		

\*Fewer samples analyzed due to corrosive qualities of extractant-damaging machine.

only to predict a species' occurrence, but also to locate geochemically compatible sites for the re-establishment of species in threatened or endangered habitats.

In conclusion, we suggest that botanical fieldwork in substrate-diverse regions, such as the Southwest, should proceed in conjunction with geological research in both the laboratory and in the literature. Extensive geological data exists which can be extremely useful in assessing habitat, predicting new populations, or simply understanding autecology of our numerous and all too poorly understood rare species. Collaboration with geologists, soil scientists, and chemists can provide new insights for all parties concerned.

## Acknowledgments

Funding for this project was provided by the Howard Hughes Medical Institute Undergraduate Research grant to Colorado College, the Colorado Natural Areas Program, and the Colorado Native Plant Society. We are also grateful for the invaluable field and laboratory assistance of the following groups and individuals: D. Anderson (Directorate of Environmental Compliance, Ft. Carson); P. Beardsley, N. Bower (Dept. of Chemistry, Colorado College); P. Myrow, S. Weaver, and C. Seamann (Dept. of Geology, Colorado College); G. Scott (U.S. Geological Survey); C. Crawford and B. Jennings (Colorado Native Plant Society); M. French (Pueblo Reservoir State Park); Soil Conser-

vation Service in Cañon City, CO; and the Natural Heritage Program of The Nature Conservancy, Colorado.

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# Monitoring Survival, Growth, and Reproduction of *Aletes humilis*

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**Abstract:** Larimer aletes (*Aletes humilis* Coulter & Rose), a globally rare plant, has been monitored for 7 years beginning in 1989 at Phantom Canyon Preserve, Colorado. It is a cushion plant that usually grows in cracks in granite along north-facing cliffs. Information was collected annually on survival and growth of approximately 300 individuals. The first 5 years showed very little change in most variables. Very little mortality was seen over the course of the study. The percentage of individuals that flowered ranged from 50 to 80 percent with most of those producing fruit. Flowering and fruit production data differed greatly in 1994 and 1995 from that collected in 1989 through 1993. While mortality was still low in 1994, only 54 percent of the individuals flowered and very few of those set fruit. A late spring storm may have again interrupted seed set. In 1995, a prolonged cold and wet spring may have interfered with seed set. Data from the first 5 years indicated that these plants were well established and no threats were seen. Data from 1994 and 1995, however, showed the value of long-term monitoring in determining the impacts of unusual climatic events on this plant.

## Introduction

*Aletes humilis* Coulter & Rose (Larimer aletes), a member of the parsley family (Apiaceae), is classified as a C2 candidate for listing under the Endangered Species Act, and is considered sensitive by the U.S. Forest Service Region 2. It grows in cushions 2–10 cm high and about 5–20 cm in diameter (Theobald et al. 1964). *Aletes humilis* produces yellow flowers in early May, but has been found to flower into early July. After flowering the leaves grow up around the flowering stems. Thus, most of the seed falls within the plant.

*Aletes humilis* grows in cracks and crevices on rocky, mostly north-facing granite cliffs along the front range in Colorado (Figure 1). This species has been found at 32 sites in Larimer and Boulder counties in Colorado. It has also been reported, but not verified, from Albany County, Wyoming. Individuals in the populations appear to be vigorous, flowering is abundant, and at least some seed production occurs. The populations are small to moderate, with numbers of individuals ranging from about 50 to several thousand.

*Aletes humilis* mostly grows on sites derived from Silver Plume granite in cracks in massive rock or between boulders, but has also been found in cracks in metamorphic rocks. Sometimes two or more plants grow together within a crack, making determinations of individual plants difficult. It is found largely on sites that do not receive continual direct sunlight, such as north-facing slopes, under trees, or on the north sides of large rocks. *Aletes humilis* grows on level ground, steep slopes, and canyon walls. The soils on which it is found are

usually coarse-textured, being derived from granite parent material.

Elevations of the known locations range from 2100 to 2380 m (6500–7400 ft) (O'Kane 1988). Precipitation data for the locations are lacking; however, annual precipitation is probably on the order of 300 to 500 mm (12–20 in).


## Study Site and Methods

Phantom Canyon Preserve, owned by The Nature Conservancy, is located 30 miles northwest of Fort Collins, in Larimer County, Colorado. This preserve protects 1600 acres of rugged canyon along the North Fork of the Cache la Poudre River. Phantom Canyon remains one of the few low-elevation roadless canyons along the Front Range of Colorado (Figure 2).

Approximately 300 individual *A. humilis* plants of various sizes were tagged and monitored for 7 years (1989–1995) at five sites (Figure 2). At the time, these were the only known sites at the preserve. Subsequently, one additional site has been discovered. Genetic work showed that these sites are distinct populations even though some are less than a kilometer apart (Linhart and Premoli 1993). Demographic parameters of size and reproductive output were measured annually. *Aletes humilis* grows in circular or elliptical cushions. Therefore, plant size was determined by measuring two perpendicular diameters once annually, and the area was calculated for each of the tagged individuals.

Seed production was counted for several years and then estimated for 1994 and 1995. Counts of

# Phantom Canyon Preserve

 Approximate distribution of *Aletes humilis*

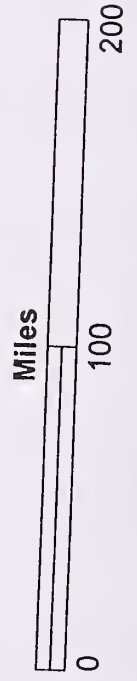
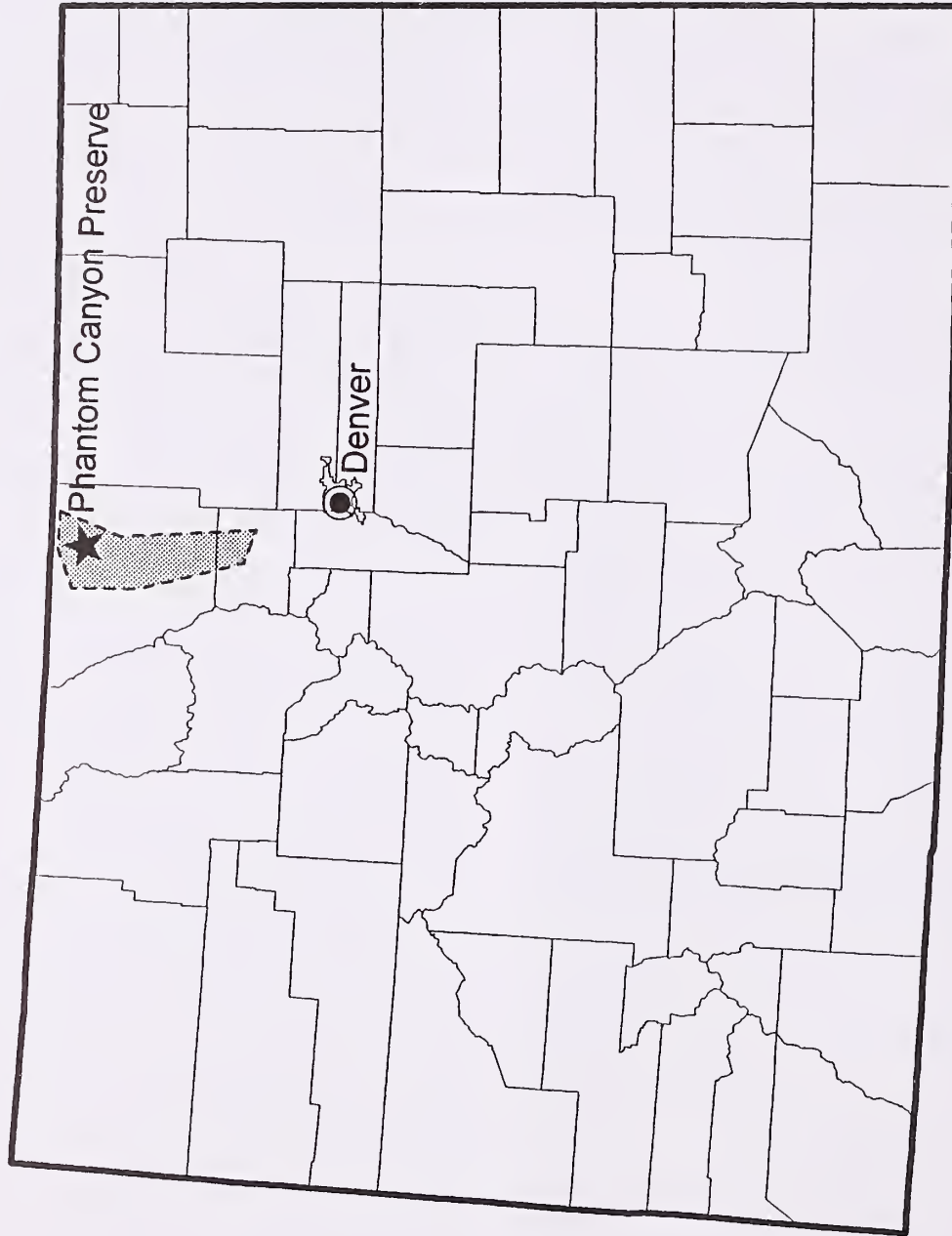


Figure 1. Map of the state of Colorado showing the approximate distribution of *Aletes humilis* within Boulder and Larimer counties.

# Phantom Canyon Preserve

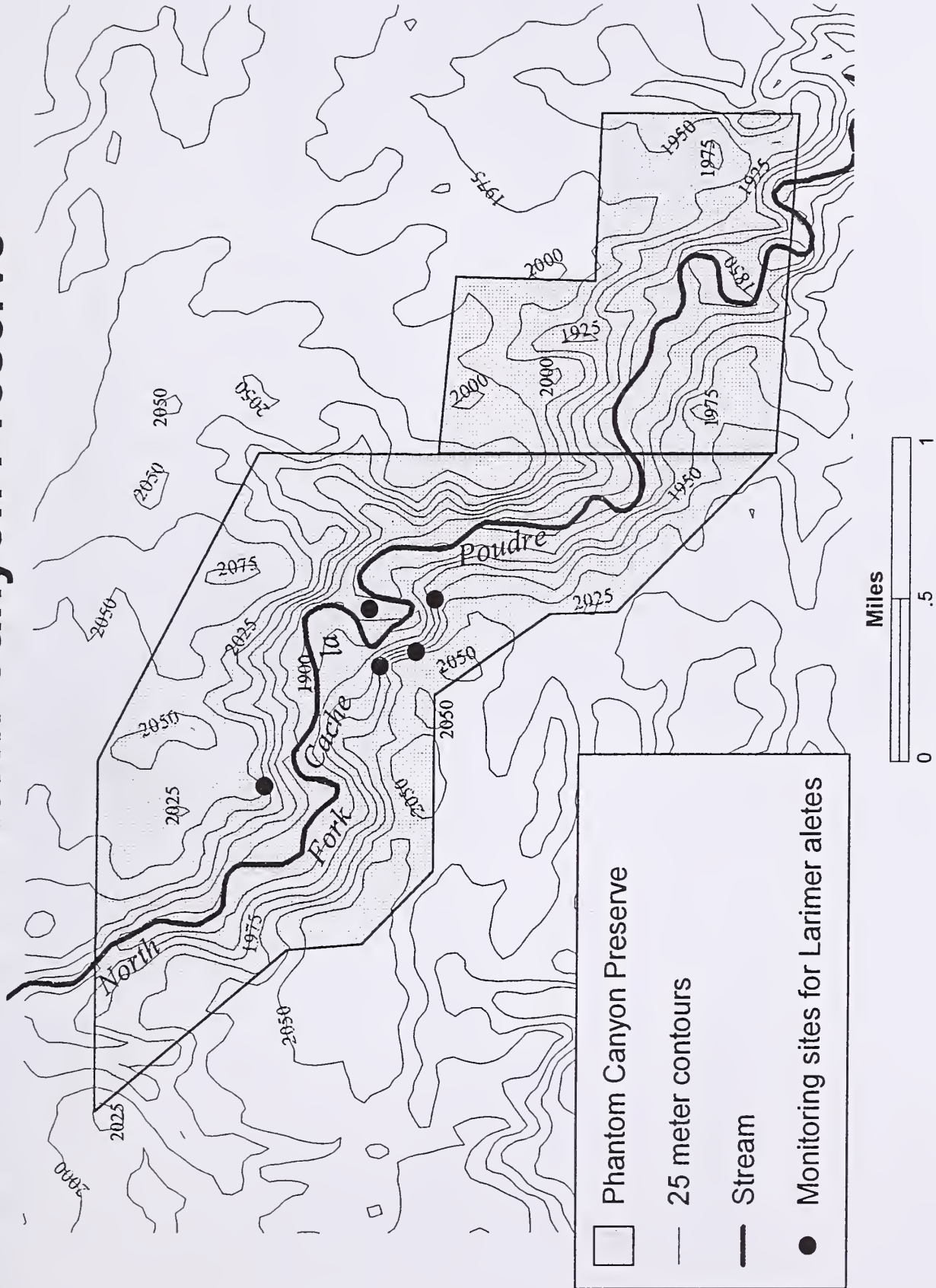


Figure 2. Phantom Canyon Preserve with monitoring sites for *Aletes humilis* marked by dots.

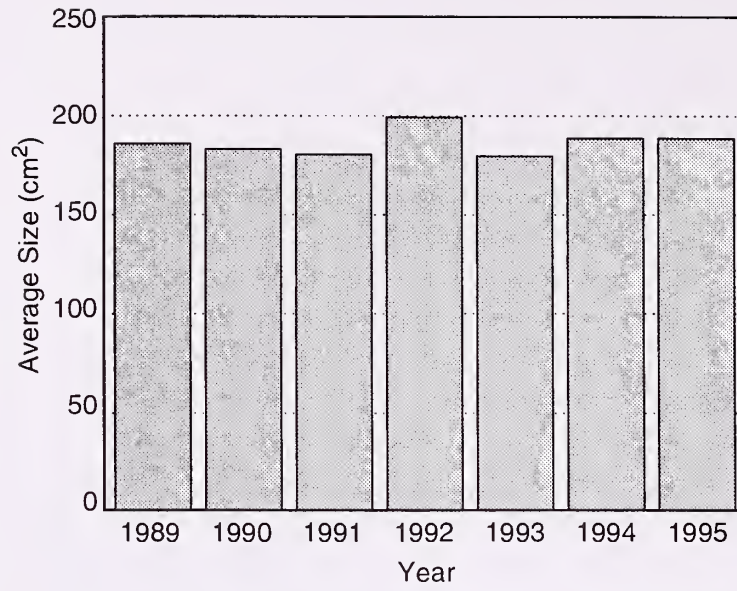


Figure 3. Area of tagged *Aletes humilis* plants.

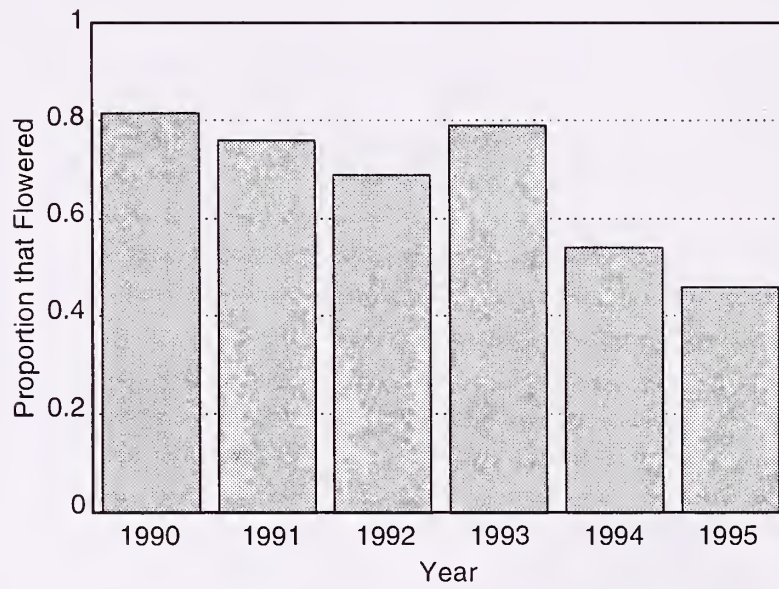


Figure 4. Proportion of tagged *Aletes humilis* individuals that flowered.

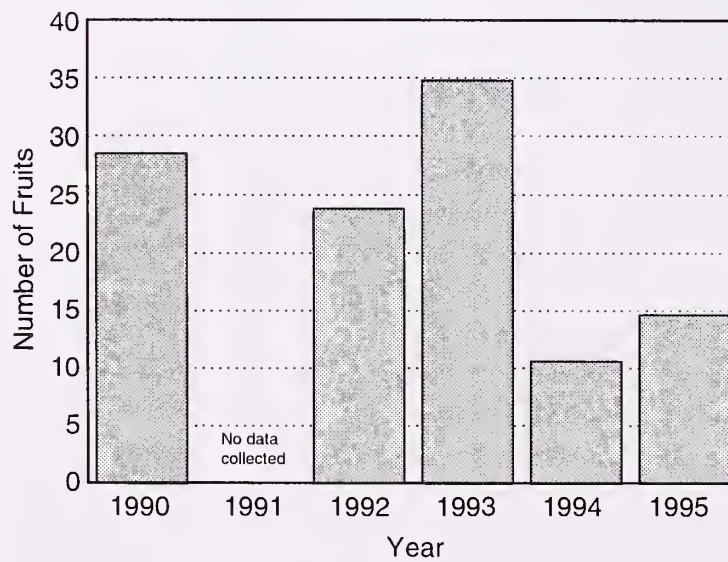


Figure 5. Average number of fruits per tagged *Aletes humilis* plant that flowered; 1994 and 1995 data are estimated numbers of fruits.

seeds took a great deal of time and did not vary much during the first 5 years of the study. Therefore, estimates of seed production were made into the following categories: A = 1–10, B = 11–100, C = 101–1000, and D = greater than 1000.

Counts of all *A. humilis* individuals in the five populations in 1993, 1994, and 1995 yielded estimates of aggregate sizes of the combined populations of 1086–1285 individuals. Thus, about a fourth of the plants at Phantom Canyon Preserve were tagged. In addition, the immediate area around each of the tagged *A. humilis* plants was searched intensively for seedlings.

### Results and Discussion

The first 5 years of the study showed very little change in most demographic variables. Very little mortality was observed over the course of the study. Only 1 percent of our tagged individuals died annually. The average half-life of the tagged plants was calculated to be 60 years. Thus, *A. humilis* appears to be a very long lived plant. In addition, fewer than 10 seedlings were observed in any year during the study.

Over the 7-year study period, the average size of individual plants changed very little, indicating that the tagged individuals had attained their maximum size (Figure 3). Plant growth is probably limited by the small amounts of soil or water that occur within the cracks the plants occupy.

Between 46 and 80 percent of the tagged individuals flowered over the course of the study, showing that the populations are dominated by reproductive individuals (Figure 4). The number of fruits did vary, especially in 1994 and 1995 (Figure 5). A late spring snowstorm in 1994 at Phantom Canyon possibly interfered with flowering and reduced seed set. Unusually wet and cold spring conditions occurred throughout May and June of 1995. During those years most of the tagged plants did not flower or set fruit.

Over the course of the study, approximately

20,000 *A. humilis* fruits were produced, yet we observed less than 100 seedlings. The fruits of *A. humilis* are rather large and tend to fall within the plant. Seedlings were most often found where a mature *A. humilis* had died. The lack of seedling recruitment is likely a shortage of safe sites to germinate and grow and the inability to disperse to open safe sites across steep slopes.

### Conclusion

*Aletes humilis* appears to be a long-lived plant that flowers consistently and sets a modest number of fruits. Very few seedlings are found, however, except in places where older individuals have died. The limiting factor in this plant's survival appears to be a lack of safe sites and the inability to disperse fruits to open safe sites. If most safe sites are currently occupied, recruitment may await the demise of established *A. humilis* individuals. The populations at Phantom Canyon Preserve are well established and even recent low recruitment should not be a problem due to the longevity of the plants.

The substantial decline in fruit production during 1994 and 1995, likely due to unusual spring weather conditions, is probably a transient phenomenon. We expect flowering and seed production to rebound over the next several years. This study demonstrates the value of long-term monitoring in understanding the impacts of unusual climatic events on reproduction.

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# Narrow Endemism in the New Mexico Flora

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**Abstract:** New Mexico is a state with diverse geology, topography, and flora. Approximately 3 percent of the flora consists of endemic species with small geographic ranges. Few of these are paleoendemics and most are recently evolved species. Several families and genera have endemic species; however, the Asteraceae (*Erigeron*), Fabaceae (*Astragalus*), Cactaceae (*Escobaria*), and Scrophulariaceae (*Penstemon*) are most represented in the list of endemic species. Substrate specialization and isolated populations in mountain ranges are important features of plant endemism in New Mexico. The largest numbers of endemics occur in the mountains of the southern and western parts and the sedimentary formations in the northwestern part of the state.

## Introduction

New Mexico has a diverse native flora that combines the species richness of several different regions. The Great Plains, southern Rocky Mountains, Great Basin Desert, Chihuahuan Desert, and northern Sierra Madre flora all converge here. Wootton and Standley (1915) wrote the first manual for the New Mexican flora by combining their extensive personal knowledge of the state flora and studying the collections of previous explorers. This collaboration assembled 2903 plant species for the state and their *Flora of New Mexico* is still a valuable reference for distribution and type locality information. Martin and Hutchins (1980, 1981) produced the most recent *Flora of New Mexico* and identified 3700 plant taxa as occurring or expected to occur in the state. Since that publication, New Mexican botanists have confirmed many of the speculated species, added hundreds of new state records, and discovered several species new to science. The *Working Index of New Mexico Vascular Plant Names* (Roalson and Allred 1995) presently lists 3433 species and 3906 plant taxa. Several hundred of these have been reduced to synonymy (Kartesz 1994), but we anticipate the discovery of several hundred new state records. Approximately 92 percent of the New Mexico flora is native (Rejmánek and Randall 1994).

The study of rarity and endemism in the New Mexico flora began two decades ago with the passage of the Endangered Species Act of 1973 and its subsequent amendment to include protection for threatened and endangered plants. The *Handbook of Rare and Endemic Plants of New Mexico* (NMNPPAC 1984) identified 137 species and was the first of its kind in the western states to illustrate each species and provide distribution maps.

This list has been revised several times in subsequent years. Spellenberg (1993) identifies 153 plants of special concern and provides an excellent discussion on the causes of endemism, rarity, and endangerment in New Mexico. The third edition of the *Inventory of Rare and Endangered Plants of New Mexico* (Sivinski and Lightfoot 1995) lists 210 entities that are endangered or sensitive to the prevailing land uses in New Mexico. The New Mexico Endangered Plant Species Act identifies plants that are rare and endangered in New Mexico, but that may be widespread or common in adjacent states. Therefore, certain elements of the *Inventory* are artifacts of political boundaries and not natural rarities. Surprisingly, a rather small percentage of the New Mexico flora can be considered endemic to the state. We estimate only 3–4 percent of the taxa have a sufficiently restricted distribution to be considered narrow endemic species.

Endemic species are of special concern and are usually studied within the context of the Endangered Species Act. Therefore, the word endemic is often confused with endangered. However, in New Mexico, rare and endemic status is frequently a prerequisite, but rarely an indicator of endangerment. Narrow endemics are also only a subset of the total species we consider rare. Species with small geographic range and narrow habitat specificity comprise only two of the seven forms of rarity (Rabinowitz 1981). Endemic is also used to describe species that are unique to the area within the political boundaries of a particular state. However, if the truth is told, "state endemic species" (except in the large coastal state of California) frequently extend over the borders and into adjacent states. Their significance is often preserved by

their distributional restriction to a particular geologic feature that is shared by the neighboring state.

The word endemic is generally accepted by phytogeographers to mean restricted distributionally to a specific region. In geographic terms, a region can be rather large and have a very high percentage of endemic flora. For instance, there is some overlap of flora between the Chihuahuan and Sonoran deserts, but for the most part their species memberships are highly dissimilar and endemic to their respective regions. Floristic regions are often delimited by the distribution of their dominant endemic flora.

In this study of plant endemism in New Mexico, we will focus on "narrow endemic species," which we somewhat arbitrarily define as species with total range length of less than 100 miles (160 km). The 97 narrow endemics listed in Table 1 are often common in their habitats and not always strictly confined to New Mexico, but are usually endemic to a specific geologic outcrop or range of mountains. We have retained a few obvious substrate specialists with slightly wider distributions (e.g., *Atriplex griffithsii* Standley, *Amsonia tharpii* Woodson), but have generally conformed to the 100-mile limit. We find that this criterion includes almost all the species restricted to unique and distributionally limited habitats. Table 1 also excludes species with serious taxonomic problems, including ambiguous taxa that cannot be relocated at their original collection sites and are known from only a type specimen (*Philadelphus wootonii* Hu, *Rubus aliciae* L.H. Bailey, *Rumex tomentellus* Rechinger, etc.). Our studies of narrow endemics that cross the state borders have been hampered by the lack of a recent floristic manual for Arizona and the absence of a Chihuahuan Desert flora publication. However, the species list in Table 1 is the result of a reasonable effort to consult the available literature and represents a majority of localized endemics in New Mexico.

### Patterns of New Mexico Endemism

Most of the features leading to vascular plant endemism in New Mexico have been thoroughly discussed by Spellenberg (1993). The influences of diverse geology, topography, and climate are given as the important features contributing to local speciation. We stress local speciation because none of the New Mexican narrow endemics can be classified as relictual paleoendemics. Paleoendemics are described by Stebbins and Major (1965) as

monospecific or small genera in small, phylogenetically old plant families. The monotypic *Apacheria chiricahuensis* C.T. Mason (Crossosomataceae) is an apparent paleoendemic; however, its 200-mile range excludes it from this study. If we grant ourselves greater latitude within larger families, there are two narrow endemics in the state that are relatively ancient. *Lepidospartum burgessii* B.L. Turner (Asteraceae) occurs in gypseous, dry lake beds of southeastern New Mexico and adjacent Texas. This unusual shrub is an isolated relict and the only species of this small genus to occur in the Chihuahuan Desert region (Turner 1977). *Ionactis elegans* (Soreng & Spellenberg) Nesom (Asteraceae) is a paleoendemic in the White Mountains of south-central New Mexico. Its closest relative occurs in the northwestern United States. Nesom's (1992) discussion of *Ionactis* speculates that the "widely disjunct distributions, and extremely narrow endemism ... suggest that the active evolutionary period for the genus occurred in a relatively ancient period." The remainder of our local New Mexico endemics are usually members of larger genera and appear to be relatively new species (neoendemics) in proximity to other related species.

Spellenberg (1993) states that "the species endemic in the state are representative of the composition of the state's flora." A majority of the New Mexico flora and the state's endemic species are perennial forbs. The relative numbers of succulents, shrubs, and annuals are also somewhat proportionate; however, no tree species are endemic to the state. The notion of representative composition is also true for the state's plant communities most frequently occupied by endemic species. The greatest species diversity occurs at intermediate elevations in Interior Chaparral (the shrubby ecotone above desert scrub), Piñon-Juniper Woodlands, and Montane Coniferous Forest (generally the ponderosa pine-Douglas fir association). The greatest number of New Mexico endemics also occupy these vegetation communities (Table 2). Few endemics occur in the low deserts and higher alpine communities. The alpine tundra in northern New Mexico has a few species endemic to the Southern Rocky Mountain region but has no very narrow endemics.

In a systematic sense, the concept of representative composition of endemics in the New Mexico flora is very uneven. The largest plant family in the state is the Asteraceae, which also contains the largest number (28) of the state's endemic species.

Table 1. Endemic vascular plants in New Mexico with total ranges of less than 100 miles.

Taxon	Habitat Substrate	Physical Habitat	Plant Communities
<b>Apocynaceae</b>			
<i>Amsonia fugatei</i>	Sedimentary conglomerate	Indeterminate	Chihuahuan Desert Scrub
<i>Amsonia tharpii</i>	Gypseous limestone	Indeterminate	Chihuahuan Desert Scrub
<b>Asteraceae</b>			
<i>Chaetopappa hersheyi</i>	Limestone	Cliffs	Interior Chaparral
<i>Chrysothamnus nauseosus</i> ssp. <i>texensis</i>	Limestone	Cliffs and boulders	Interior Chaparral
<i>Cirsium gilense</i>	Nonspecific	Riparian	Montane Conifer Forest
<i>Cirsium vinaceum</i>	Wet travertine	Wetland	Montane Conifer Forest
<i>Erigeron acomanus</i>	Sandstone	Shaded canyons and benches	P-J Woodland
<i>Erigeron bistiensis</i>	Sandy shale	Indeterminate	Great Basin Desert Scrub
<i>Erigeron hessii</i>	Andesite	Cliff and rock outcrop	Subalpine Conifer Forest
<i>Erigeron rhizonatus</i>	Seleniferous shale	Indeterminate	P-J Woodland
<i>Erigeron rybius</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Erigeron sivinskii</i>	Shale	Indeterminate	P-J Woodland
<i>Erigeron subglaber</i>	Granite	Indeterminate	Subalpine Conifer Forest
<i>Grindelia acutifolia</i>	Shale	Soil disturbance	P-J Woodland
<i>Haplopappus microcephalus</i>	Granite	Cliffs	Montane Conifer Forest
<i>Hymenoxys brachyactis</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Hymenoxys vasseyi</i>	Nonspecific	Indeterminate	P-J Woodland
<i>Ionactis elegans</i>	Granite	Cliffs and rock outcrop	Subalpine Conifer Forest
<i>Lepidospartum burgessii</i>	Gypsum	Playa bottom	Chihuahuan Desert Scrub
<i>Perityle cernua</i>	Granite	Shaded cliffs	P-J Woodland
<i>Perityle staurophylla</i> var. <i>homoflora</i>	Limestone	Shaded cliffs	Interior Chaparral
<i>Perityle staurophylla</i> var. <i>staurophylla</i>	Limestone	Shaded cliffs	Interior Chaparral
<i>Pinaropappus parvus</i>	Limestone	Shaded cliffs	Interior Chaparral
<i>Senecio cardamine</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Senecio cynthioides</i>	Rhyolite	Indeterminate	P-J Woodland
<i>Senecio quaerens</i>	Nonspecific	Riparian	Montane Conifer Forest
<i>Senecio neomexicanus</i> var. <i>metcalfei</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Senecio spellenbergii</i>	Calcareous balds	Indeterminate	Arid Grassland
<i>Senecio warnockii</i>	Gypsum	Indeterminate	Chihuahuan Desert Scrub
<i>Townsendia gypsophila</i>	Gypsum	Indeterminate	Arid Grassland
<b>Brassicaceae</b>			
<i>Draba mogollonica</i>	Rhyolite	Shaded cliffs	Montane Conifer Forest
<i>Lesquerella aurea</i>	Nonspecific	Soil disturbance	Montane Conifer Forest
<i>Streptanthus sparsiflorus</i>	Limestone	Canyon bottoms	Interior Chaparral
<b>Cactaceae</b>			
<i>Escobaria organensis</i>	Limestone	Gravelly benches	P-J Woodland
<i>Escobaria sandbergii</i>	Limestone	Gravelly benches	Interior Chaparral
<i>Escobaria sneedii</i> var. <i>sneedii</i>	Limestone	Cliffs and rock cracks	Interior Chaparral
<i>Escobaria sneedii</i> var. <i>leei</i>	Limestone	Cliffs and rock cracks	Interior Chaparral
<i>Escobaria villardii</i>	Limestone	Gravelly benches, N-facing	Interior Chaparral
<i>Mammillaria wrightii</i> var. <i>wilcoxii</i>	Nonspecific	Indeterminate	Interior Chaparral
<i>Opuntia arenaria</i>	Deep sand	Semistable dunes	Chihuahuan Desert Scrub
<i>Opuntia viridiflora</i>	Clayey sand	Indeterminate	P-J Woodland
<i>Pediocactus knowltonii</i>	Cobbly clayey sand	Indeterminate	P-J Woodland
<i>Sclerocactus mesae-verdae</i>	Shale	Indeterminate	Great Basin Desert Scrub
<i>Sclerocactus cloveriae</i> subsp. <i>brackii</i>	Shale	Indeterminate	Great Basin Desert Scrub
<b>Caryophyllaceae</b>			
<i>Stellaria porsildii</i>	Rhyolite	Indeterminate	Montane Conifer Forest
<b>Chenopodiaceae</b>			
<i>Atriplex griffithsii</i>	Alkaline silt	Playas	Chihuahuan Desert Scrub
<i>Atriplex pleiantha</i>	Shale	Indeterminate	Great Basin Desert Scrub
<b>Fabaceae</b>			
<i>Astragalus accumbens</i>	Sandy shale	Indeterminate	P-J Woodland
<i>Astragalus altus</i>	Nonspecific	Soil disturbance	Montane Conifer Forest
<i>Astragalus castetteri</i>	Limestone	Indeterminate	P-J Woodland
<i>Astragalus chuskanus</i>	Sandstone	Bare or disturbed	Montane Conifer Forest
<i>Astragalus cobrensis</i> var. <i>maguirei</i>	Rhyolite	Indeterminate	P-J Woodland
<i>Astragalus cyaneus</i>	Nonspecific	Indeterminate	P-J Woodland
<i>Astragalus eglestonii</i>	Nonspecific	Indeterminate	P-J Woodland



Table 1 (continued)

Taxon	Habitat Substrate	Physical Habitat	Plant Communities
<i>Astragalus feensis</i>	Nonspecific	Indeterminate	P-J Woodland
<i>Astragalus gypsodes</i>	Gypsum	Indeterminate	Chihuahuan Desert Scrub
<i>Astragalus humillimus</i>	Sandstone	Cracks and basins	Great Basin Desert Scrub
<i>Astragalus kerrii</i>	Granite	Soil disturbance	P-J Woodland
<i>Astragalus knightii</i>	Sandstone	Cracks and basins	P-J Woodland
<i>Astragalus neomexicanus</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Astragalus oocalycis</i>	Seleniferous shale	Indeterminate	P-J Woodland
<i>Astragalus ripleyi</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Astragalus siliceus</i>	Calcareous gravels	Indeterminate	Arid Grassland
<i>Astragalus wittmannii</i>	Limestone	Indeterminate	Plains Grassland
<i>Lupinus sierra-blancae</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Sophora gypsophila</i> var. <i>guadalupensis</i>	Gypseous sandstone	Indeterminate	P-J Woodland
<i>Trifolium longipes</i> var. <i>neurophyllum</i>	Nonspecific	Riparian	Montane Conifer Forest
<b>Hydrangeaceae</b>			
<i>Philadelphus argyrocalyx</i>	Nonspecific	Indeterminate	P-J Woodland
<b>Lamiaceae</b>			
<i>Agastache pringlei</i> var. <i>verticillata</i>	Monzonite	Shaded cliff bases	P-J Woodland
<i>Hedeoma apiculata</i>	Limestone	Shaded cliffs	Interior Chaparral
<i>Hedeoma pulcherrima</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<i>Hedeoma todsenii</i>	Gypseous sand	N-facing slope	P-J Woodland
<b>Liliaceae</b>			
<i>Zigadenus mogollonensis</i>	Nonspecific	Indeterminate	Montane Conifer forest
<b>Nyctaginaceae</b>			
<i>Abronia bigelovii</i>	Gypsum	Indeterminate	Arid Grassland
<b>Onagraceae</b>			
<i>Oenothera organensis</i>	Monzonite	Riparian	P-J Woodland
<b>Papaveraceae</b>			
<i>Argemone pleiacantha</i> ssp. <i>pinnatisecta</i>	Limestone	Soil disturbance	P-J Woodland
<b>Polemoniaceae</b>			
<i>Gilia formosa</i>	Shale	Indeterminate	P-J Woodland
<i>Ipomopsis sancti-spiritus</i>	Nonspecific	Soil disturbance	Montane Conifer Forest
<i>Phlox caryophylla</i>	Shale	Indeterminate	P-J Woodland
<b>Polygalaceae</b>			
<i>Polygala rimulicola</i> var. <i>mescalerorum</i>	Limestone	Shaded cliffs	Interior Chaparral
<i>Polygala rimulicola</i> var. <i>rimulicola</i>	Limestone	Shaded cliffs	Interior Chaparral
<b>Polygonaceae</b>			
<i>Eriogonum aliquantum</i>	Shale	Soil disturbance	Arid Grassland
<i>Eriogonum gypsophilum</i>	Gypsum	Indeterminate	Chihuahuan Desert Scrub
<i>Eriogonum jamesii</i> var. <i>wootonii</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<b>Ranunculaceae</b>			
<i>Aquilegia chrysantha</i> var. <i>chaplinei</i>	Limestone	Cliff seeps	Interior Chaparral
<i>Delphinium novomexicanum</i>	Nonspecific	Indeterminate	Montane Conifer Forest
<b>Rosaceae</b>			
<i>Potentilla sierra-blancae</i>	Granite	Indeterminate	Subalpine grassland
<b>Saxifragaceae</b>			
<i>Heuchera glomerulata</i>	Rhyolite	N-facing slopes	Montane Conifer Forest
<b>Scrophulariaceae</b>			
<i>Besseya oblongifolia</i>	Nonspecific	Indeterminate	Alpine Grassland
<i>Castilleja organorum</i>	Nonspecific	Indeterminate	P-J Woodland
<i>Penstemon alamosensis</i>	Limestone	Shaded cliffs and boulders	Interior Chaparral
<i>Penstemon cardinalis</i> var. <i>cardinalis</i>	Nonspecific	Shaded cliffs and boulders	P-J Woodland
<i>Penstemon cardinalis</i> var. <i>regalis</i>	Limestone	Shaded cliffs and boulders	P-J Woodland
<i>Penstemon neomexicanus</i>	Nonspecific	Indeterminate	P-J Woodland
<i>Penstemon pseudoparvus</i>	Rhyolite	Indeterminate	Montane Conifer Forest
<i>Scrophularia laevis</i>	Monzonite	Indeterminate	P-J Woodland
<i>Scrophularia macrantha</i>	Rhyolite	Shaded cliffs and boulders	P-J Woodland

Table 2. Habitat characteristics of New Mexico endemic plant species.

Habitat Characteristic	No. of Species
<b>Substrate</b>	
Limestone	23
Igneous	17
Shale	12
Gypsum and alkali lakes	10
Sandstone	4
Deep sand	1
Conglomerate	1
Nonspecific	29
<b>Habitat Features</b>	
Cliffs and shaded, rocky slopes	27
Soil disturbance	8
Wetland/Riparian	6
Playa bottom	2
Slick-rock basins	2
Dunes	1
Indeterminate	51
<b>Plant Communities</b>	
Chihuahuan Desert Scrub	8
Great Basin Desert Scrub	5
Arid Grassland	7
Interior Chaparral	15
Piñon-Juniper Woodland	34
Montane Coniferous Forest	24
Subalpine Forest and Meadows	4
Alpine Tundra	0

Twenty of these belong to the Astereae and Senecioneae (Table 3). The relatively large Heliantheae tribe has no narrow endemics in New Mexico.

The Poaceae is the second largest plant family in New Mexico, but it is not represented by any local endemic species. In fact, the entire Liliopsida of New Mexico has only a single narrow endemic (*Zigadenus mogollonensis* Hess & Sivinski: Liliaceae). Grasses, in general, form very few narrow endemic species as contrasted to large, western North American dicot families. Relatively large poaceous genera (*Poa*, *Muhlenbergia*, *Bouteloua*, etc.) share western North American habitats with rapidly speciating dicots and have been subjected to the same geologic and climatic regimes. So why are there so few narrow endemic grass taxa? A partial answer may be that the higher levels and frequency of ploidy deviations in monocots (Grant 1981) creates numerous locally adapted races or cryptic species that are morphologically indistinguishable to taxonomists.

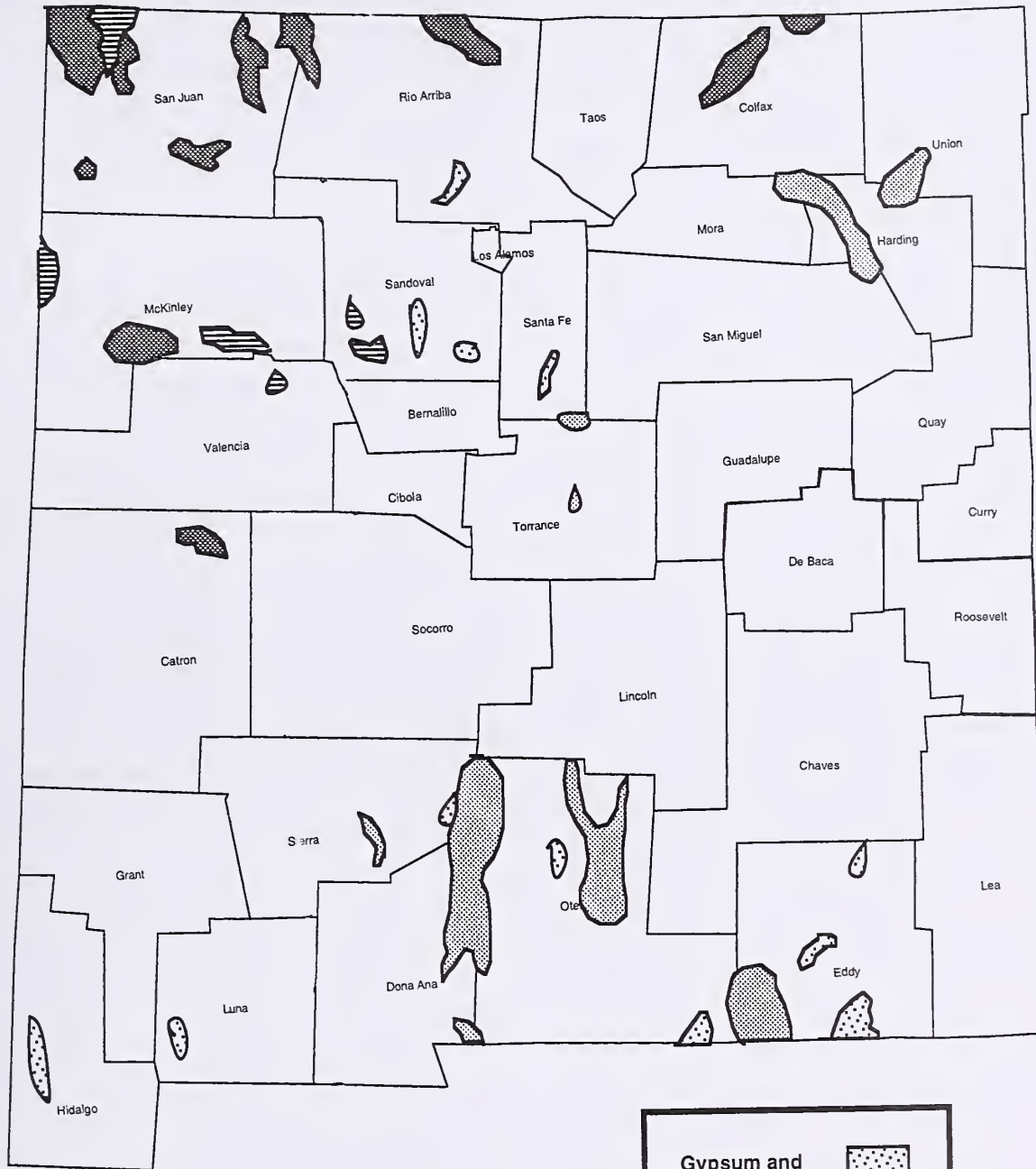
The narrow endemics of New Mexico represent 20 plant families, 49 genera, and 97 species. However, 42 percent of the total species occur in only five genera: *Astragalus*, *Erigeron*, *Senecio*, *Escobaria*, and *Penstemon* (Table 3). Shultz (1993) also identified *Astragalus*, *Erigeron*, and *Penstemon* as genera contributing large numbers of endemic species to the Utah flora. She reasoned that these genera are preadapted to arid climates and respond to desert expansion by speciating into new habitats. The genus *Astragalus* is especially important, contributing 17 narrow endemic species to New Mexico (Table 3) and 42 (usually narrow) endemic species to Utah (Schultz 1993). New Mexico's *Astragalus*, *Erigeron*, and *Penstemon* endemics are scattered throughout the state, and are not always associated with arid habitats. Our *Escobaria* endemics are strictly Chihuahuan Desert species, and most of the *Senecio* endemics occur in the mountains of the Mogollon Province.

The two factors with the greatest influence on endemism in New Mexico are geology and topography. The state's geological diversity produces numerous habitat substrates with unusual physical and chemical characteristics. These are often occupied by specialist plants that are occasionally endemic species. Table 2 presents a general summary of New Mexican substrate endemism. The distributions of endemic populations on sedimentary substrates are shown in Figure 1. Species counted as substrate nonspecific are those that occupy various substrate types or occur on mixed, nonresidual soils. Limestones support the largest number of state endemics. Numerous endemic plant populations occur on the limestone mountain ranges of southern New Mexico (Guadalupe, Sacramento, Organ, San Andres, and Caballo mountains). A few limestone endemics are restricted to bald, calcareous ridges and knolls in the shortgrass prairies of northeastern New Mexico (*Astragalus siliceus* Barneby, *Astragalus wittmannii* Barneby, and *Senecio spellenbergii* T.M. Barkley).

Igneous rocks are the next most frequent substrate occupied by endemic species in New Mexico. We have not listed the numerous rock types (rhyolite, granite, monzonite, etc.) for the Table 2 summary because the narrow endemics on igneous rocks in New Mexico do not appear to be substrate specialists (like the serpentine flora of California). A possible exception that may indicate mineral specificity is *Erigeron hessii* Nesom, which only inhabits andesite dikes that intrude through

# New Mexico

0 50 100 km



31°N  
109°W

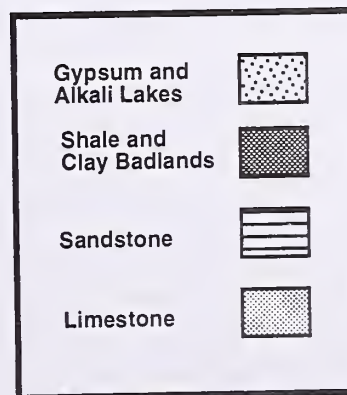


Figure 1. Distribution of endemic plant species on sedimentary substrates in New Mexico.

Table 3. Four vascular plant families with the greatest numbers of narrow endemics in New Mexico.

Family and Genera	No. of NM Endemics
Asteraceae	28
Astereae	13
Chaetopappa	1
Chrysothamnus	1
Erigeron	7
Grindelia	1
Haplopappus	1
Ionactis	1
Townsendia	1
Cardueae	2
Cirsium	2
Helenieae	5
Hymenoxys	2
Perityle	3
Lactuceae	1
Pinaropappus	1
Senecioneae	7
Lepidospartum	1
Senecio	6
Fabaceae	20
Astragalus	17
Lupinus	1
Sophora	1
Trifolium	1
Cactaceae	11
Escobaria	5
Mammillaria	1
Opuntia	2
Pediocactus	1
Sclerocactus	2
Scrophulariaceae	9
Besseyia	1
Castilleja	1
Penstemon	5
Scrophularia	2

the rhyolitic country rock of the Mogollon Mountains. Other endemics on igneous rocks may only require specific physical-textural qualities. For instance, *Haplopappus microcephalus* Cronquist is restricted to easily weathered (crumbly) forms of granite in north-central New Mexico.

The most specialized narrow endemics in the state occur on sedimentary formations of shale, gypsum, and sandstone. Shale habitats (including sandy clays and silts) have the next highest proportion of sedimentary substrate endemism after limestone (Table 2). Most occur on the numerous and varied shale formations in the northwestern quarter of New Mexico. Shales in the Mancos and

Fruitland formations in San Juan County and the Chinle Formation in McKinley County are important endemic plant substrates. *Astragalus oocalycis* M.E. Jones and *Erigeron rhizomatus* Cronquist can be further subdivided into seleniferous shale endemics. We base this conclusion on the consistent strong odor of selenium in their habitats and the presence of other common selenophiles (*Astragalus flavus* Nuttall, *Astragalus albulus* Wootton & Standley, etc.). Two endemics (*Eriogonum aliquantum* Reveal and *Grindelia acutifolia* Steyermark) occur on shale at the interface of the shortgrass prairie and the Rocky Mountains in northeastern New Mexico.

Central and southeastern New Mexico have several large gypsum formations that support numerous endemic plant species. A few endemic gypsophiles, such as *Astragalus gypsodes* Barneby and *Eriogonum gypsophilum* Wootton & Standley in the southeastern part of the state, occur on relatively pure forms of gypsum. Other gypsum endemics prefer gypseous soils with other sand or silt components. For instance, *Townsendia gypsophila* Lowrey & Knight in north-central New Mexico can occur on relatively pure Todilto Gypsum, but it reaches its greatest population densities on the sandy, gypseous soils at the interface of the Todilto and overlaying Morrison Formation. *Amsonia tharpii* on our southeastern limestones inhabits rare geologic transitions where gypsum grades into dolomite. Sometimes the gypsum component of these habitats is not evident. Previous laboratory analysis of *Sophora gypsophila* var. *guadalupensis* Turner & Powell substrates in the Guadalupe Mountains on the New Mexico/Texas border found no gypsum in the root zone of this endemic shrub (Northington 1976). Our New Mexico surveys for this plant found it in association with low densities of the strict gypsophile *Tiquilia hispidissima* (Torr. & Gray) A. Richardson and completely confined to fine sand, sandstone lenses that our laboratory determined to be 1 percent gypsum. Because gypsum is relatively insoluble, an increase of solid-phase gypsum beyond a few percent has no further effect on gypsum concentration of the soil solution (Meyer 1986). Therefore, this small gypsum content of the *Sophora gypsophila* var. *guadalupensis* habitat substrate may have played a significant role in the persistence of this disjunct variety in the Guadalupe Mountains.

New Mexico has numerous gypsophilic species that are regionally endemic, but are not included in this study because their range greatly

exceeds 100 miles. Of the few species with very small ranges, four occur on the Castle and White Horse formations in southeastern New Mexico, one on the south-central Yeso Formation, and two on the Todilto Formation in the north-central part of the state. Other gypseous habitats include alkaline playas in the dry beds of large, pluvial lakes. The previously mentioned *Lepidospartum burgessii* occupies the gypseous playas in the Alkali Lakes Basin in southeastern New Mexico and adjacent Texas. *Atriplex griffithsii* occurs in the alkaline Deming and Lordsburg playas of southwestern New Mexico and the Wilcox Playa in adjacent Arizona.

Only a few species are strictly endemic to sandstone (*Erigeron acomanus* Spellenberg & Knight, *Astragalus chuskanus* Barneby & Spellenberg, *Astragalus humillimus* A. Gray, and *Astragalus knightii* Barneby), and all four occur in northwestern New Mexico (Figure 1). The latter two *Astragali* are dependent upon cracks and shallow basins that collect soil and water on slickrock sandstones.

Six of our narrow endemic species occur on wet soils in wet meadows or riparian margins (Table 2). We have no endemic aquatic species. Soil disturbance is another habitat feature for eight of our endemic plants. Two of these plants require active soil erosion and deposition to maintain suitable habitats. *Astragalus kerrii* Knight et Cully in the Capitan Mountains and *Argemone pleiacantha* subsp. *pinnatisecta* Ownbey in the Sacramento Mountains are both inhabitants of the sand bars and banks that are deposited by floods in drainage channels that are otherwise dry. They appear to be preadapted to man-made disturbance as they frequently occupy the sides of roads that intersect their habitats. *Astragalus kerrii* was initially discovered in a disturbed site (Knight and Cully 1991) and the type collection was taken from a trailhead parking lot. Only one of our narrow endemics (*Opuntia arenaria* Engelman) occurs on deep sand or sand dunes, which are also unstable habitats.

The diverse topography of New Mexico includes many isolated mountain ranges and sheltered cliffs in numerous canyons and escarpments where we find many of our endemic species. More than a quarter of our narrow endemic plants occur on cliffs or steep, shaded slopes (Table 2). Sheltered topographic extremes probably serve as refugia for mesic habitat species during drying trends in the regional climate. This process results in isolated populations, which are susceptible to local speciation by mutation or genetic drift. Whole mountain ranges with their cooler, wetter

climates also serve as points of isolation in vast areas of surrounding deserts. Each major range of mountains in the arid, southern part of the state has two or more endemic plant species (Figure 2). The Guadalupe–Sacramento–Capitan chain of mountain ranges have sedimentary and igneous substrates with an especially large number (29) of locally endemic taxa, many of which are shared with Texas. The adjacent Organ–San Andres–Caballo mountains are less massive, but they also have sedimentary and igneous substrates with 13 endemic taxa (a few are shared with the Sacramento Mountains). The Mogollon province of western New Mexico and eastern Arizona is almost entirely volcanic in origin and is not conducive to substrate specialists. This province is, however, a large area of mountain ranges at the juncture of several floristic regions. These mountains are a melting pot for the flora of the southern Rocky Mountains, northern Sierra Madre Occidentale, upland Sonoran and Chihuahuan deserts, and the southern Great Basin Desert influence. The Mogollon Province in New Mexico has 12 endemic taxa, many of which are shared with Arizona.

Northern New Mexico has numerous large mountain ranges, but they support fewer endemic species. At the southern end of the Rocky Mountains, the massive Sangre de Cristo range has both limestone and igneous substrates, yet there are only three plants endemic to this range. The Zuni Mountains are a small, isolated sedimentary and igneous range that also have three endemic species.

The totals for occurrence by county of New Mexican endemic plants are shown in Figure 3. Because so few of these plants are ancient relicts, Figure 3 illustrates the general patterns of speciation into distributionally limited habitats. The greatest density of narrow endemic species is in south-central and southeastern New Mexico, mostly associated with isolated mountain ranges and pluvial basins that offer limestone, gypsum, and igneous substrates over a broad range of elevations. While substrate specialization may be important in this area, mountain range isolation is the key feature for endemism in the southern region of the state. The Mogollon Province of western New Mexico is the second most diverse area for narrow endemic plants. It is also a relatively isolated suite of mountain ranges. Most of our sandstone and shale endemics occur on the Great Basin and Colorado Plateau geology in the northwestern part of the state. This is consistent with the high level of recent speciation observed

# New Mexico

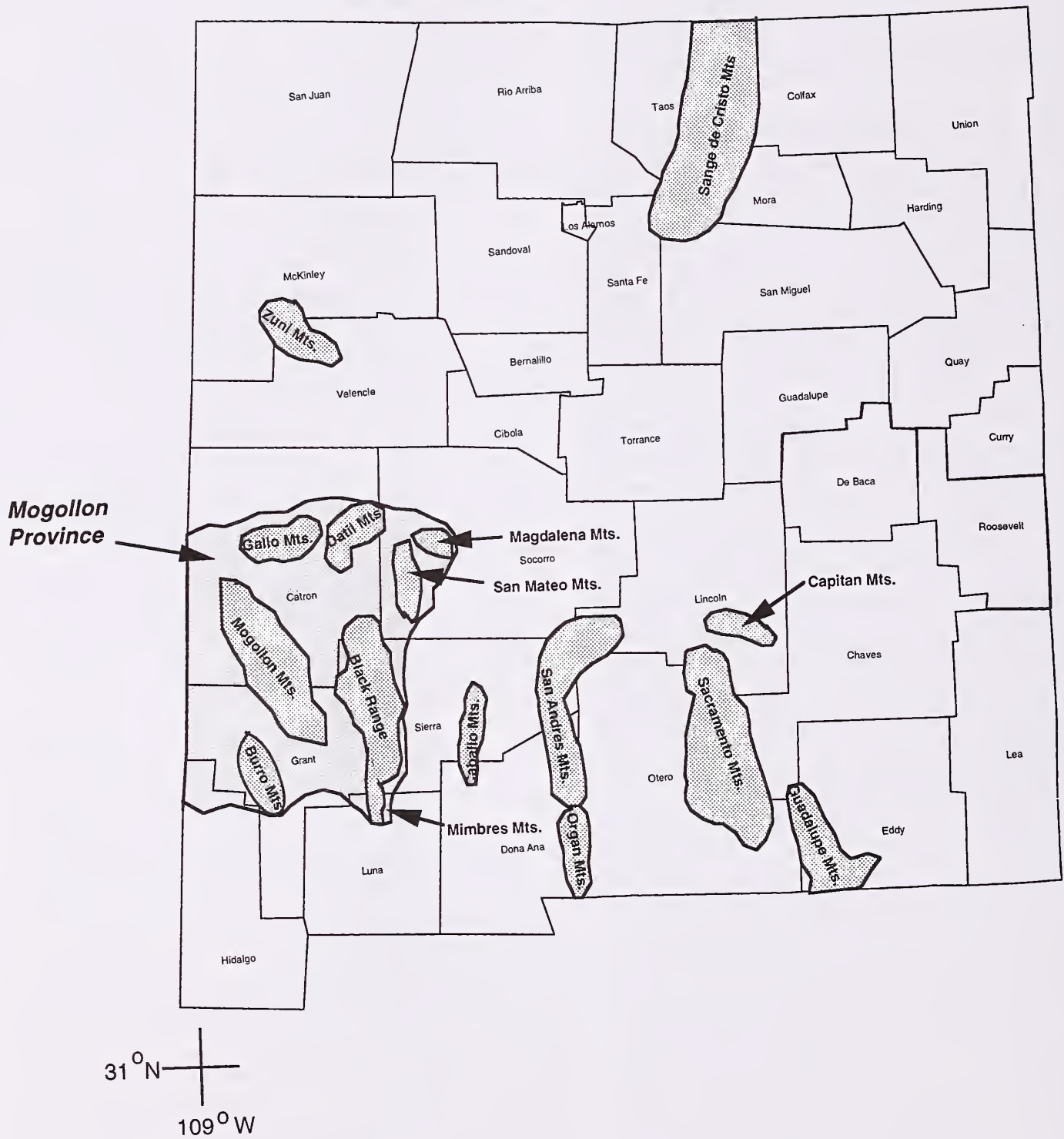
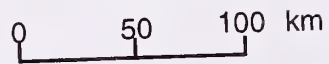


Figure 2. New Mexico mountain ranges with two or more narrow endemic plant species.

# New Mexico

0 50 100 km

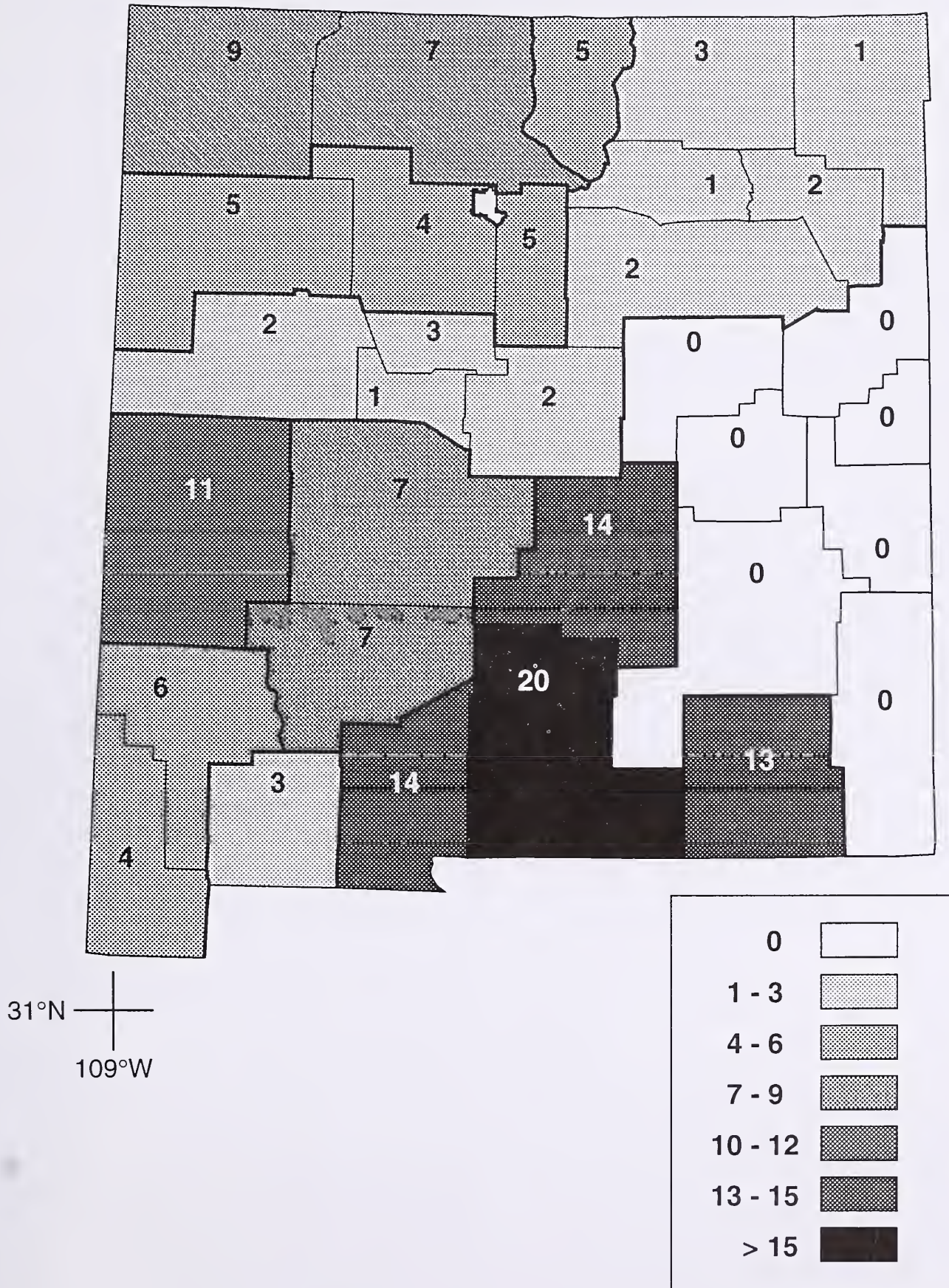


Figure 3. Numbers of endemic plant species by county in New Mexico.

on sedimentary substrates in adjacent Utah (Shultz 1993). Numbers of endemic plants taper off gradually to the northeast and abruptly at the east-central part of the state. In fact, east-central New Mexico has not a single endemic plant to call its own. The eastern area is mostly homogeneous prairie. However, it is also the least botanically explored area of the state. Perhaps, waiting to be found there, are species new to science. As New Mexico becomes better explored by botanists, we are certain that new endemic species will continue to come to light.

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# Preliminary Results of *Toumeyia Papyracantha* Surveys on the Lakeside Ranger District, Apache-Sitgreaves National Forests, Arizona

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**Abstract:** *Toumeyia papyracantha* (Engelm.) Britt & Rose was first documented on the Lakeside Ranger District (LRD) in the 1930's. In 1993 a concerted effort to determine its distribution and abundance on the LRD was undertaken. These efforts resulted in 26 new locations and 720 individuals. Fourteen 100-foot radius plots were established around previous cactus locations, and diameters and reproductive evidence were collected. Two plots were monitored in 1995 to gather reproductive information. Densities ranged from 3 to 161 individuals per plot. Diameters ranged from 2.9 mm to 32.4 mm. Reproductive characteristics are given by diameter size class. An average of 55 percent of all individuals of flowering size produced at least one bud. Fruiting bodies were produced by 71 percent of those individuals that produced buds. Occurrence of this cactus shows a strong correlation to soil mapping units on the LRD. Cactus locations have been subjected to juniper cabling, pushing, burning, and high-intensity, short-duration grazing in the past. Livestock grazing is substantially less than it was historically, but it still occurs.

## Introduction

The grama cactus (*Toumeyia papyracantha* (Engelm.) Britt & Rose) was first documented within the boundaries of the Lakeside Ranger District (LRD) in the 1930s (Kearny and Peebles 1942). It was not until 1993 that a concerted effort was directed towards determining the distribution and abundance on the LRD. This cactus has been considered a Forest Service sensitive species within Region 3 for some time (USFS 1990) and has received some type of federal classification by the U.S. Fish and Wildlife Service almost since the inception of the Endangered Species Act of 1973.

Intensive surveys were initiated in 1992 on the Heber Ranger District (adjacent and to the west of LRD) for this species along the Highway 277 right-of-way. Approximately 205 groups were found. This provided the impetus for attempting to determine its presence on the LRD. What began as an effort to simply determine presence evolved into a project to gather information regarding densities, diameters, reproduction, habitat characteristics, and range of this species on the LRD.

## Methods

The LRD had made cursory attempts to search for this cactus in the past to address project impacts, and intensive surveys for this species were undertaken in 1993. Between 1993 and 1995, three methods were used to search for this cactus: a grid of east-west transects, searching 6 feet to either side of randomly selected Parker 3-step range transects, and random searches.

East-west grid searches were established to specifically clear a juniper push project on 300 acres. Twenty variable-length transects (2500–7200 feet), approximately 200 feet apart, were established using a hip chain and compass. Surveyors searched 6 feet to each side of the transect line.

A modified Parker 3-step transect 100 feet in length was used to collect resource data for an ecosystem unit encompassing the west half of the LRD. Transects were located randomly, but were stratified to sample the various habitat types and vegetative structural stages within the unit. Starting coordinates for each transect were derived from the District's Geographic Information System (GIS) and then were located on the ground using a Geographic Positioning System (GPS). During the reading of these transects, a 6-foot area to each side of the transect was searched for this species. The crew surveying the Parker 3-step transects also conducted random searches for this species between transects, as well as in areas where no transects were scheduled, but which potentially provided habitat for this species.

Fourteen circular plots with a radius of 100 feet were established in 1994 around cactus locations found in 1993 or 1994. A "location" is a siting of one or more individuals that are not necessarily reproductively isolated from individuals in another location. Using GPS, previously found individuals were relocated and the plot center was established by placing a section of rebar 2 feet directly south of the first cactus relocated. Intensive searches were conducted at

approximately 8 degree intervals along a 100-foot tape stretched from the plot center. This resulted in approximately 45 transects. All cacti found were marked with a wire flag. At the completion of the search, the distance and compass bearing were recorded for each plant. The diameter (mm) was measured using calipers, and whether the plant had a bud, bloom, or fruiting body was recorded. Occasionally an individual was found immediately adjacent to the plot boundary. Information that was recorded for these individuals was used in the analysis of diameters and reproduction, but not included in the relative density estimates.

Data on reproductive status were collected in 1994 and 1995. Since some of the field work in 1994 was conducted outside of the normal reproductive season for this species, only those plots where data were collected between April 18 and June 30 are considered. Detailed information regarding flowering and production of fruiting bodies was collected for only one plot in 1994. In 1995, two plots were monitored specifically to obtain information regarding flowering and fruit production.

Habitat characteristics were summarized for the areas with grama grass cactus to determine if suitable habitat could be better defined. Both positive and negative searches conducted for this species between 1993 and 1995 were compared to mapping units described in the Terrestrial Ecosystems Survey (TES) of the Apache-Sitgreaves National Forests (USDA 1986). Four Parker 3-step transects for each of the 14 plots were used to collect vegetation data. Pinyon/juniper canopy cover, as determined from 1994 aerial photos, was determined for occupied areas.

District records were reviewed to determine past land-use history for two allotments in which grama grass cactus occurs.

## Results

### *Presence*

The search for grama grass cactus using the east-west grid method was negative. Based on our present knowledge, much of the area surveyed was not suitable grama grass cactus habitat due to the amount of pinyon-juniper canopy cover. The cactus was found on only 4 of the 40 transects that occurred within pinyon-juniper woodlands and grasslands. However, almost half of these transects were in areas that, due to the amount of pinyon-juniper canopy cover, were not cactus habitat.

Thirty-eight random surveys using the Parker 3-step transects resulted in eight cactus locations.

An additional 42 random searches conducted in 1994 resulted in 14 more locations. Eighteen of the 42 random transects were specifically targeted to sample other TES soil mapping units different from those that had been identified with grama grass cactus locations. Thus, between 1993 and 1995, a total of 26 new locations for the grama grass cactus were established for the LRD, representing a total of 720 individuals. Coordinates for all locations except one were recorded using GPS.

### *Diameters*

In total, 694 diameters from individual cacti were recorded, ranging from 2.9 mm to 32.4 mm, with the greatest number of individuals being between 5.0 and 9.9 mm (Table 1). The average diameter was 13.1 mm.

Phillips et al. (1982) reported diameters for 20 individuals, ranging from 4 mm to 15 mm with an average of 9 mm. Button (1987) provided diameters for 303 individuals in seven "subpopulations" on Bureau of Land Management land in Navajo County, Arizona. The average diameter for Button's plants (Table 2) and those we measured is similar (12.9 and 13.1 mm). However, Button's sample was composed of a significantly higher percentage of the smallest size class (24% to 4%) and a significantly lower percentage of both the 10-14.9 mm size class (16% to 30%) and the 15-19.9 mm size class (11% to 23%). Button also reported a smaller minimum diameter (1.0 mm) and a larger maximum diameter (40 mm). Additionally, none of Button's sub-populations had maximum diameters less than 20 mm, whereas 3 of the 14 plots in this study had maximum diameters smaller than 20 mm.

In general, there appears to be a normal distribution of size classes for the pooled individuals. One would expect higher representation of the smallest size class, but a lack in this group is probably attributable to the difficulty of finding them.

### *Density*

The grama grass cactus is colonial in nature and is found in small groups unevenly dispersed throughout what appears to be suitable habitat (Sivinski and Lightfoot 1994). Because of the highly cryptic nature of this cactus, reliable density estimates are extremely difficult to make, and published estimates are almost nonexistent. For example, two plots (9 and 14) had initial known populations of 58 and 100 respectively, but when monitored in 1995 for reproductive information, an additional 15 and 13 individuals were found.

Table 1. Summary of diameters (in mm) taken from 100-foot radius plots on Lakeside Ranger District, Navajo County in 1994–95 for *Toumeyia papyracantha*.

Plot No.	Min.	Max.	Avg.	0-4.9	5-9.9	10-14.9	15-19.9	20-24.9	25+	Total Cactus
1	7.9	28.1	14.7	0	2	5	2	1	1	11
2	5.8	25.2	15.6	0	5	9	5	6	2	27
3	7.5	17	12.6	0	3	3	3	0	0	9
4	7	16.9	11.1	0	4	4	2	0	0	10
5	4	27.1	16.5	1	4	10	12	9	3	39
6	2.9	30.1	14.3	1	12	15	14	7	2	51
7	5.2	25.9	13.6	0	27	30	26	9	2	94
8	4.1	29.9	13.6	3	13	13	10	4	2	45
9	3.1	26.5	10.2	9	36	11	10	5	2	73
10	3.2	32.4	11.4	7	63	50	35	3	2	160
11	5	26	12.2	0	10	8	5	1	1	25
12	5	18.9	14.2	0	1	0	2	0	0	3
13	4.8	25.1	11.8	1	13	14	3	2	1	34
14	3	27.7	13.6	3	31	37	27	12	3	113
Total Number of Plants				25	224	209	156	59	21	694
Average Number of Plants per Plot				1.79	16.00	14.93	11.14	4.21	1.50	49.57

Table 2. Summary of diameters (in mm) based on data collected by Claire Button (1987) on Bureau of Land Management lands for *Toumeyia papyracantha*.

Plot No.	Min.	Max.	Avg.	0-4.9	5-9.9	10-14.9	15-19.9	20-24.9	25+	Total Cactus
1.0	1.0	28.0	10.4	25	26	19	16	11	2	99
2.0	2.0	25.0	10.4	6	9	3	2	4	1	25
3.0	21.0	21.0	21.0	0	0	0	0	1	0	1
4.0	2.0	30.0	7.8	11	8	2	0	0	3	24
5.0	1.0	34.0	9.6	10	23	6	4	3	3	49
6.0	13.0	29.0	21.3	0	0	1	0	1	1	3
7.0	1.0	40.0	9.8	20	43	18	12	6	3	102
Total Number of Plants				72	109	49	34	26	13	303
Average Number of Plants per Plot				10.3	15.6	7.0	4.9	3.7	1.9	43.3

As such, the estimates given here as well as those used for comparison should not be considered definitive. The densities found in LRD are similar or higher than what has been found in other areas by other authors.

Densities ranged from a high of 5.52/100 m<sup>2</sup> to a low of 0.10/100 m<sup>2</sup> (Figure 1). Average density for all plots was 1.61/100 m<sup>2</sup>. Phillips et al. (1982) found 59 plants in an area of approximately 15,000 m<sup>2</sup> (0.39/100 m<sup>2</sup>). Fletcher and Moir (1993) re-

ported 54 plants from an area of 300 × 75 meters (0.24/100 m<sup>2</sup>). Sivinski and Lightfoot (1994) reported that 173 individuals were salvaged from a 200 m × 20 m area in New Mexico (1.5/100 m<sup>2</sup>). Wagner and Sabo (1977) reported finding 30 individuals in an area 15 m × 20 m (10/100 m<sup>2</sup>). Fletcher (1985) reported finding 26 plants in an area 25 m × 10 m and 20 plants in an area 12 m × 200 m for relative densities of 10.4/100 m<sup>2</sup> and 0.83/100 m<sup>2</sup> respectively.

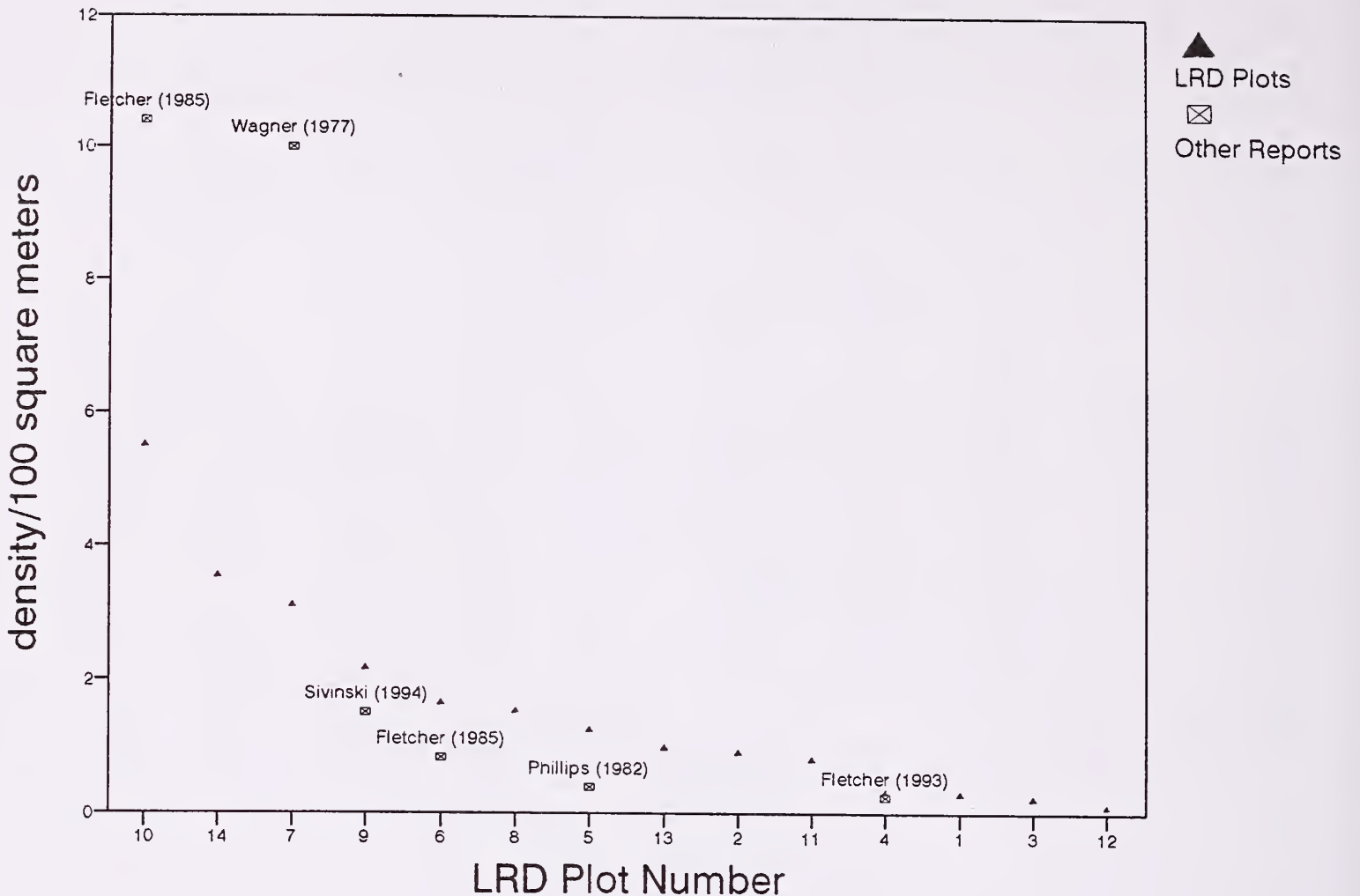


Figure 1. Comparison of LRD grama cactus densities to densities calculated from data presented in other reports (*Toumeyia papyracantha* density per 100 square meters).

Densities among plots varied widely. Three plots had densities lower than any derived from the literature and used for comparison. The average density for the pooled plots exceeded four of the six comparison densities. Two inherent problems exist in these density comparisons. First, it is unknown if the areas reported by the other authors represent bounds established by cactus found or area surveyed. The other problem lies in how our plots were established. By establishing the plot center at the first cactus encountered, it is possible that the plot could be centered on the perimeter, not the center, of a colony. The size of plot, however, was felt to compensate somewhat for this possibility.

#### Reproduction

The smallest individual to produce a bud was 5.7 mm in diameter, while two individuals with diameters of 30.1 and 25.9 mm produced five buds each (Table 3). Our data indicate that, in general, the smaller the individual the fewer buds pro-

duced. An average production of one bud per individual is not achieved until after reaching 15 mm in diameter. The percentage of individuals in a size class with buds also increased with size: 26 percent of 5–9.9 mm, 58 percent of 10–14.9 mm, 77 percent of 15–19.9, 98 percent of 20–24.9 mm and 92 percent 25 mm or greater. An average of 55 percent of all individuals of flowering size produced at least one bud. Reproduction at LRD was within the range of levels of reproduction presented by other authors. Phillips et al. (1982) reported only one of the 59 plants they measured was reproductive and 37 were non-reproductive; the rest were seedlings. They noted that the sampling dates did not allow for an accurate determination of reproductive status. Button (1987) found only one individual out of 297 had fruit and seed. She stated that plants as small as 14 mm in diameter were found with dried flowers. She felt that smaller plants tended to have fewer flowers, though this data was not recorded. Fletcher and Moir (1993) probably provide the most information regarding

Table 3. Summary of buds by size class, recorded from 100-foot radius plots on Lakeside Ranger District during 1994 and 1995 for *Toumeyia papyracantha*.

Size Class (mm)	Plot No.	Minimum Buds/Cactus	Maximum Buds/Cactus	Average per Cactus	Total No. of Buds	Total No. of Cactus	Average Diameter
0 to 4.9	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	1	4
	6	0	0	0	0	1	2.9
	7	0	0	0	0	0	0
	9	0	0	0	0	9	3.6
	14	0	0	0	0	1	2.9
Totals	SC-1	0	0	0	0	12	3.4
5.0 to 9.9	3	0	1	0.00	1	3	8.1
	4	0	1	0.50	2	4	7.8
	5	0	1	0.25	1	4	8.6
	6	0	2	0.25	3	12	7.2
	7	0	0	0	0	27	7.5
	9	0	1	0.03	1	36	7.4
	14	0	2	0.35	11	31	7.8
Totals	SC-2	0	2	0.16	19	117	9.1
10.0 to 14.9	3	0	2	1.00	3	3	13.1
	4	0	1	0.25	1	4	11.8
	5	0	1	0.80	8	10	11.7
	6	0	2	1.00	10	10	12.8
	7	0	2	0.57	17	30	12.3
	9	0	1	0.27	3	11	12
	14	0	2	0.78	29	37	12.5
Totals	SC-3	0	2	0.68	71	105	12.3
15.0 to 19.9	3	0	0	0.00	0	3	16.4
	4	0	2	1.00	2	2	16.2
	5	0	2	1.25	15	12	17.6
	6	0	2	1.00	14	14	17.1
	7	0	3	1.58	41	26	17.6
	9	0	1	0.40	4	10	17.1
	14	0	2	1.26	34	27	17.1
Totals	SC-4			1.17	110	94	17.0
20.0 to 24.9	3	0	0	0.00	0	0	0
	4	0	0	0.00	0	0	0
	5	1	3	2.22	20	9	22.2
	6	0	3	2.17	13	6	22.7
	7	2	4	3.00	27	9	22
	9	0	3	1.20	6	5	21.9
	14	1	4	2.00	24	12	22
Totals	SC-5			2.20	90	41	22.2
25 or greater	3	0	0	0.00	0	0	0
	4	0	0	0.00	0	0	0
	5	3	3	3.00	9	3	25.8
	6	4	5	4.50	9	2	27.6
	7	3	5	4.00	8	2	25.7
	9	0	3	1.50	3	2	26
	14	1	2	1.67	5	3	26.4
Total	SC-6			2.83	34	12	26.3

reproduction of this species. In 1986, 41 monitored plants of flowering age produced 23 flowers, all occurring singly except for one individual that had two. This would indicate that 54 percent of the population was capable of reproduction. Data for 155 individuals indicated an average flower production of 0.86 per plant in 1987 (Fletcher and Moir 1993). Our data for 369 individuals indicate 0.88 buds per individual.

For species that reproduce primarily through seed production, fruit development is important for the continued persistence of a population. In 1994 we only collected this information from one plot. We monitored two plots in 1995 to specifically gather this information (Table 4). Thirty-nine percent of all buds produced fruits. Of the 141 individuals that produced a bud, 100 produced fruits, or 71 percent. As with bud production, fruit

production per individual also increased with diameter. Fruit production ranged from 33 percent of bud production in the smallest diameter class producing buds to 81 percent in the largest. Over half of the buds produced by plants 10 mm in diameter and greater produced fruit. Fletcher and Moir (1993) found 68 percent of flowers with fruits in 1987 and 82 percent of flowers with fruit in 1992 for a wild population of this species.

In this study, the number of individuals producing buds is similar to that reported by other authors; however, the number of buds becoming fruits is considerably lower than that reported by Fletcher and Moir (1993). Obviously many things such as weather and pollinator activity can effect a population's reproductive capability, making it tentative at best to draw conclusions from one year of data on reproductive health based on fruiting.

Table 4. Summary of fruits produced by size class, recorded from 100-foot radius plots on Lakeside Ranger District during 1994 and 1995 for *Toumeyia papyracantha*.

Size Class (mm)	Plot No.	Minimum Fruit/Cactus	Maximum Fruit/Cactus	Average Fruit/Cactus	Total No. of Fruit	Total No. of Cactus	Average Diameter
0 to 4.9	7	0	0	0	0	0	0
	9	0	0	0	0	9	3.6
	14	0	0	0	0	1	2.9
Totals	SC-1	0	0	0	0	10	2.2
5 to 9.9	7	0	0	0.00	0	27	7.5
	9	0	0	0.00	0	36	7.4
	14	0	1	0.13	4	31	7.8
Total	SC-2	0	1	0.04	4	94	7.6
10 to 14.9	7	0	2	0.43	13	30	12.3
	9	0	1	0.09	1	11	12
	14	0	2	0.32	12	37	12.5
Totals	SC-3	0	2	0.33	26	78	12.3
15 to 19.9	7	0	2	0.92	24	26	17.6
	9	0	1	0.20	2	10	17.1
	14	0	2	0.81	22	27	17.1
Totals	SC-4	0	2	0.76	48	63	17.3
20 to 24.9	7	0	2	1.44	13	9	22
	9	0	2	0.60	3	5	21.9
	14	0	3	1.67	20	12	22
Totals	SC-5	0	3	1.38	36	26	22.0
25 or greater	7	1	4	2.50	5	2	25.7
	9	0	3	1.50	3	2	26
	14	1	2	1.67	5	3	26.4
Totals	SC-6	1	4	1.86	13	7	26.0

### Soil Mapping Units

Twenty-six locations for this cactus were recorded since 1993 and two historical locations occur on the District. Twenty-five of the new locations and one of the historical locations occur within TES mapping units 05+ and 04+ (Table 5). These soils are sandy loams derived from limestone parent material, occurring on the western portion of the District (Figure 2). Of the two locations found outside of these mapping units, one is suspected to be part of these same mapping units; the other (historical) is on private land. Several random searches as well as project-related intensive searches (Benda-Dodd 1992, Coffman Associates 1993, Klein 1995, SWCA, Inc. 1994, Wilhelm 1995) have occurred adjacent to this historical area with negative results. This area is located in mapping unit 504 which is described as a silty clay loam derived from cinder or basalt parent material. This is a fairly common mapping unit on the east half of the District. Nineteen random searches have been conducted in what appeared to be suitable habitat within and associated with this mapping unit, all with negative results.

Based on our information, there appears to be a strong relationship between occurrence of grama grass cactus on LRD and soil mapping units 05+ and 04+. This relationship is further supported by the locations on the Heber Ranger District, all of which are associated with these mapping units (K. Hughes, personal communication). There also appears to be a marked difference between occurrence within the two mapping units. Seventy-three searches were conducted within mapping unit 05+,

Table 5. Soil map units associated with grama grass cactus locations on the Lakeside Ranger District, Navajo County, Arizona.

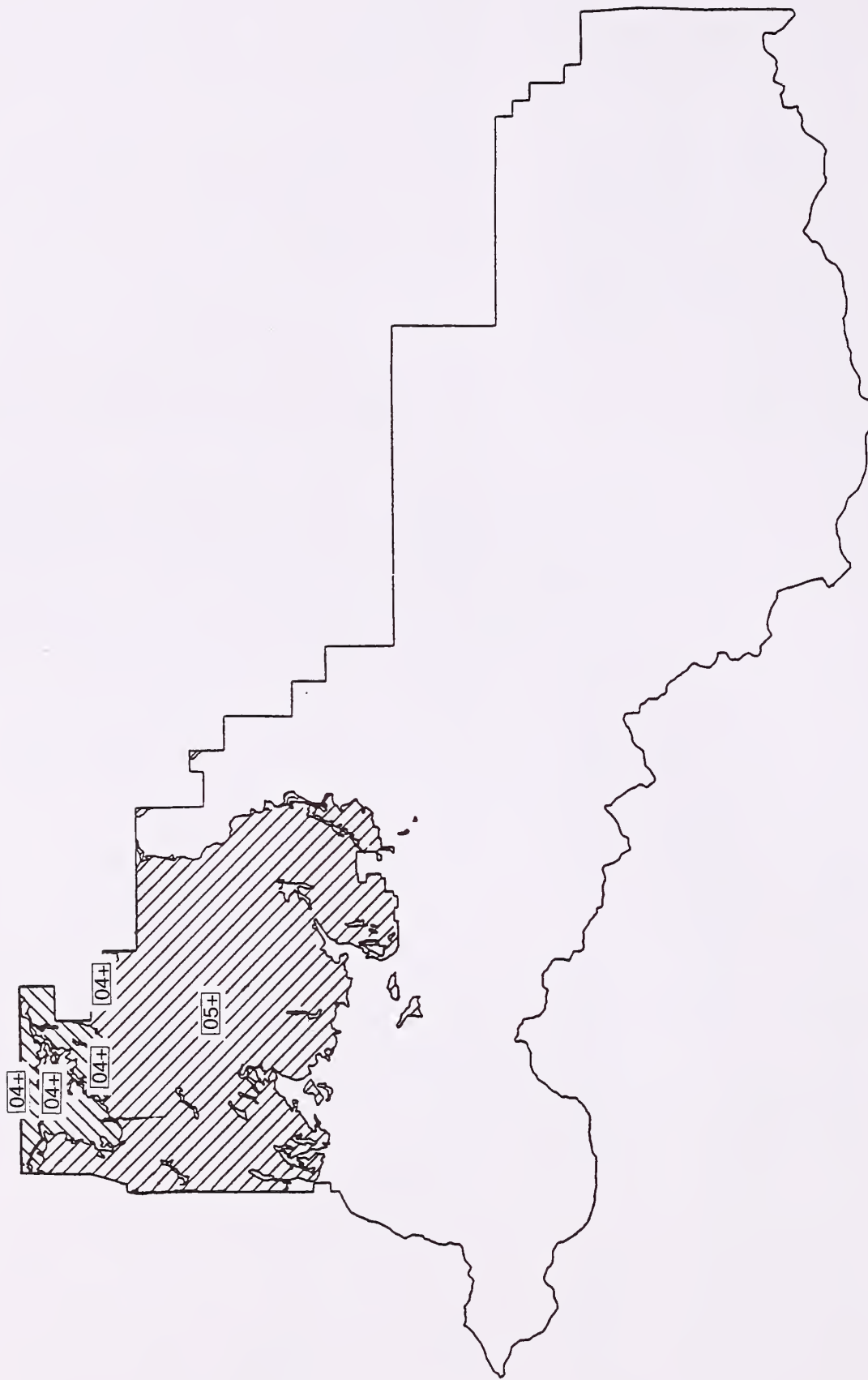
Unit	Searches	Locations	Individuals
043	18	10	272
044	6	5	266
051	3	1	5
052	1	0	0
053	58	8	172
054	10	0	0
058	1	1	2
504	10	1	3
503	1	0	0
531	3	0	0
580	5	0	0
Total	116	26	720

resulting in 10 locations or a 14 percent success rate. Twenty-four searches were conducted in mapping unit 04+, resulting in 15 locations or a success rate of 63 percent. Of the 717 individuals found within these mapping units, 25 percent were in 05+ with 75 percent occurring in 04+. Average density per plot was higher in 04+ (58.8) than in 05+ (33.2). However, both the highest and lowest densities per plot occurred within mapping unit 04+. Whether a key difference exists between these soil mapping units is not clear. It is possible that the noted difference is due to land-use history as discussed below.

### Vegetation

Twenty-five of the 26 grama grass cactus locations were compared to estimates of pinyon-juniper canopy cover estimated from 1993 aerial photos. Polygons (greater than 10 acres) were developed in GIS for grasslands, canopy covers of less than 5 percent, 5–39 percent, 40–69 percent and 70 percent and greater. All cactus locations occurred in areas of 39 percent canopy cover or less. Grasslands accounted for 60 percent (15) of locations and 44 percent of individuals. Canopy cover less than 5 percent accounted for 16 percent of locations and 12 percent of individuals. Canopy cover between 5 and 39 percent accounted for 24 percent of locations and 44 percent of individuals. The information collected from the Parker 3-step transects established for each plot can be used for a more site-specific analysis of cactus locations. For example, only 6 of 56 transects recorded pinyon-juniper overstory, occurring on 5 of the 14 plots. These hits can be interpolated as canopy cover ranging from 1 percent to 11 percent for individual transects. When averaged for individual plots, the maximum canopy cover was 3 percent (Table 6). Plot 10, which occurred at the landscape scale in a polygon of 5–39 percent canopy cover, averaged 0.75 percent at the site-specific scale canopy cover.

A preliminary analysis of the vegetation transects established in each of the 14 plots shows that an average of 60 percent of the hits on these transects were bare ground with a range of 2 percent to 90 percent. The transect that only showed 2 percent bare ground still only had 35 percent of hits on plants, the remainder being either rock or litter. The only comparable data was collected by Nabhan (1987) for areas with only one and two individuals. He found 40 percent and 60 percent bare ground in these areas.



Scale 1:240,000

Figure 2. Location of soil mapping units 04+ and 05+ on the Lakeside Ranger District, Navajo County, Arizona. Based on the Apache-Sitgreaves National Forests Terrestrial Ecosystem Survey (USDA 1986).



Only six of the individual transects had a range condition class of fair; the remainder were poor. Two plots had two of four transects that scored fair, but the number of cactus in these plots was low (9 and 10 individuals). The other two plots that had one transect that scored fair had 27 and 73 individuals. The dominant grass species in most transects was blue grama (*Bouteloua gracilis*).

#### Land-Use History

Twenty-two of the 26 locations of this cactus occur within two allotments, comprising approximately 29,000 acres. In the late 1800s and early 1900s uncontrolled use by cattle and sheep severely over-utilized the area. Cattle numbers have gradually decreased since 1920. Sheep grazing ceased in the 1930s. Today 135 head of cattle are grazed on one allotment and 199 head are grazed on the other. Based on a recent analysis by the District, one allotment was considered appropriately stocked and one allotment was considered understocked. Both allotments use a deferred rotation grazing system.

Approximately 10,800 acres of juniper were cabled from 1959 to 1960 by the permittees and the

Table 6. Polygon cover type, canopy cover and canopy cover for Parker three-step transects at locations of grama grass cactus on Lakeside Ranger District, Navajo County, Arizona.

Location No.	Polygon Cover Type	Polygon Canopy Cover	Transect Canopy Cover	Total Individuals
PL011	Grassland	0%	0%	25
RN893-2	Grassland	0%		1
PL007	Juniper	5-39%	0%	94
PL012	Grassland	0%	0%	3
RN893-5	Grassland	0%		2
RN893-6	Grassland	0%		1
PL013	Grassland	0%	0%	34
RN893-8	Grassland	0%		1
PL003	Juniper	5-39%	0%	9
PL002	Juniper	5-39%	1.5%	27
PL006	Grassland	0%	0%	51
PL001	Juniper	5-39%	3%	11
PL004	Grassland	0%	0%	10
PL005	Grassland	0%	0%	39
PL008	Grassland	0%	0.25%	45
PL009	Juniper	<5%	0.5%	73
PL010	Juniper	5-39%	0.75%	160
PL014	Grassland	0%	0%	113
RN994-19	Juniper	<5%		2
RN994-20	Juniper	5-39%		5
RN994-21	Juniper	<5%		1
RN994-22	Juniper	<5%		3
RN994-23	Grassland	0%		2
RN994-24	Grassland	0%		2
RN994-25	Grassland	0%		3
RN695-26	Grassland	0%	3	

Forest Service. Two cactus locations are found within these areas, accounting for 6 percent of individuals. An additional 8,000 acres have been pushed. Fourteen cactus locations are found within these areas accounting for approximately 35 percent of individuals. Portions of these treated areas were also burned. Nine cactus locations occur within these areas, accounting for approximately 33 percent of individuals.

The Dodson allotment was used for a pilot study of a short-duration, high-intensity grazing system for 7 years (1983-1990). Five locations subjected to this grazing system account for approximately 15 percent of plants found.

#### Discussion

Fletcher and Moir (1993) postulated that the lack of individuals with diameters exceeding 20 mm in Button's (1987) populations indicated that plant longevity was not sufficient to achieve full reproductive potential. They also speculated that many populations persist due to a large seed bank in the soil. Knowledge concerning the reproductive biology of wild populations is almost nonexistent. It is therefore extremely difficult to draw conclusions regarding grama grass cactus populations on the LRD. At this time, however, we feel that the populations on the LRD are healthy, based on the presence of various size classes, inferred reproductive capability, and high average densities. If indeed this cactus is associated with certain soils on the LRD as our data indicate, then it is also well distributed.

Though this cactus is found in pinyon-juniper, our data indicate that it occurs in the grassy openings within the woodland type and does not tolerate a high degree of canopy cover. Continued invasion of these grassy areas by pinyon-juniper could lead to reduced habitat for this species.

#### Acknowledgments

I would like to thank Bruce Palmer, U.S. Fish and Wildlife Service, for his field visits and advice in data collection. I would also like to thank the following U.S. Forest Service personnel: Terry Myers, Forest TES Specialist, for making funds available and for support of this project and review of this paper. Carl Vance Jr., Lakeside Ranger District, who conducted much of the field work that made this paper possible. Clay Baxter, Lakeside Ranger District, for his support of this project and collection of Parker 3-step data. There are many others who have helped along the way and I sincerely thank them all.

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# Surveys and Habitat Analyses of Five Rare Plant Species In the Organ Mountains of New Mexico

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**Abstract:** The Organ Mountains of south-central New Mexico support five rare plant species, four of them endemic to this small, isolated range. Rare plant surveys were conducted in the Organ Mountains on the Fort Bliss Military Reservation from 1990 to 1993. Distribution maps and habitat models were produced to aid Fort Bliss in planning for the preservation and habitat management of these rare species. New populations were documented for *Oenothera organensis*, *Perityle cernua*, *Scrophularia laevis*, *Draba standleyi*, and *Coryphantha organensis*. Quantitative data on habitat and population characteristics were collected, analyzed, and developed into habitat models that were verified during additional field surveys. Each species was dependent on its own unique mesic microhabitat. The potential effects of fires pose the greatest threat to the habitat.

## Introduction

The Organ Mountains form a relatively isolated small range in south-central New Mexico that has higher peaks (to 9012 feet) and more mesic canyons than the neighboring San Andres Mountains to the north and Franklin Mountains to the south. This has created an isolating effect and consequent endemism for plants that lack high dispersal capabilities (Figure 1).

The U.S. Army Fort Bliss Military Reservation is responsible for management of most of the Organ Mountains with the exception of the northwestern part, which is managed by the Bureau of Land Management (BLM). Military uses and lightning strikes have resulted in frequent fires in these mountains. A lightning fire burned the central part of the range in 1994. Fire management in the Organ Mountains has the potential to enhance the habitats of species of special interest. Thus, there is a need to know the distribution of sensitive species in order to manage fire in the mountains in ways that address their distribution and habitat requirements.

The New Mexico Natural Heritage Program conducted surveys for rare and endemic plants in the Organ Mountains from 1990 to 1992 and tested the habitat models with further surveys in 1993. Potential habitat and distribution for each species were determined by applying the habitat model.

Seven of the plant species in the Organ Mountains on Fort Bliss were either endemic, federal candidates for protection, or state endangered at the time of this research. Two state sensitive species and two other rare species were also found in the Organ Mountains, but were not endemic. This paper presents the results of surveys and habitat

analyses for the five species that were federal category 2 candidates for protection and/or endemics: *Oenothera organensis* Munz (Organ Mountain evening primrose), *Perityle cernua* (Greene) Shinnery (nodding cliff daisy), *Scrophularia laevis* Woot. & Standl. (Smooth figwort), *Draba standleyi* Macbr. & Payson (Standley's whitlowgrass), and *Coryphantha organensis* A.D. Zimmerman (Organ Mountain pincushion cactus).

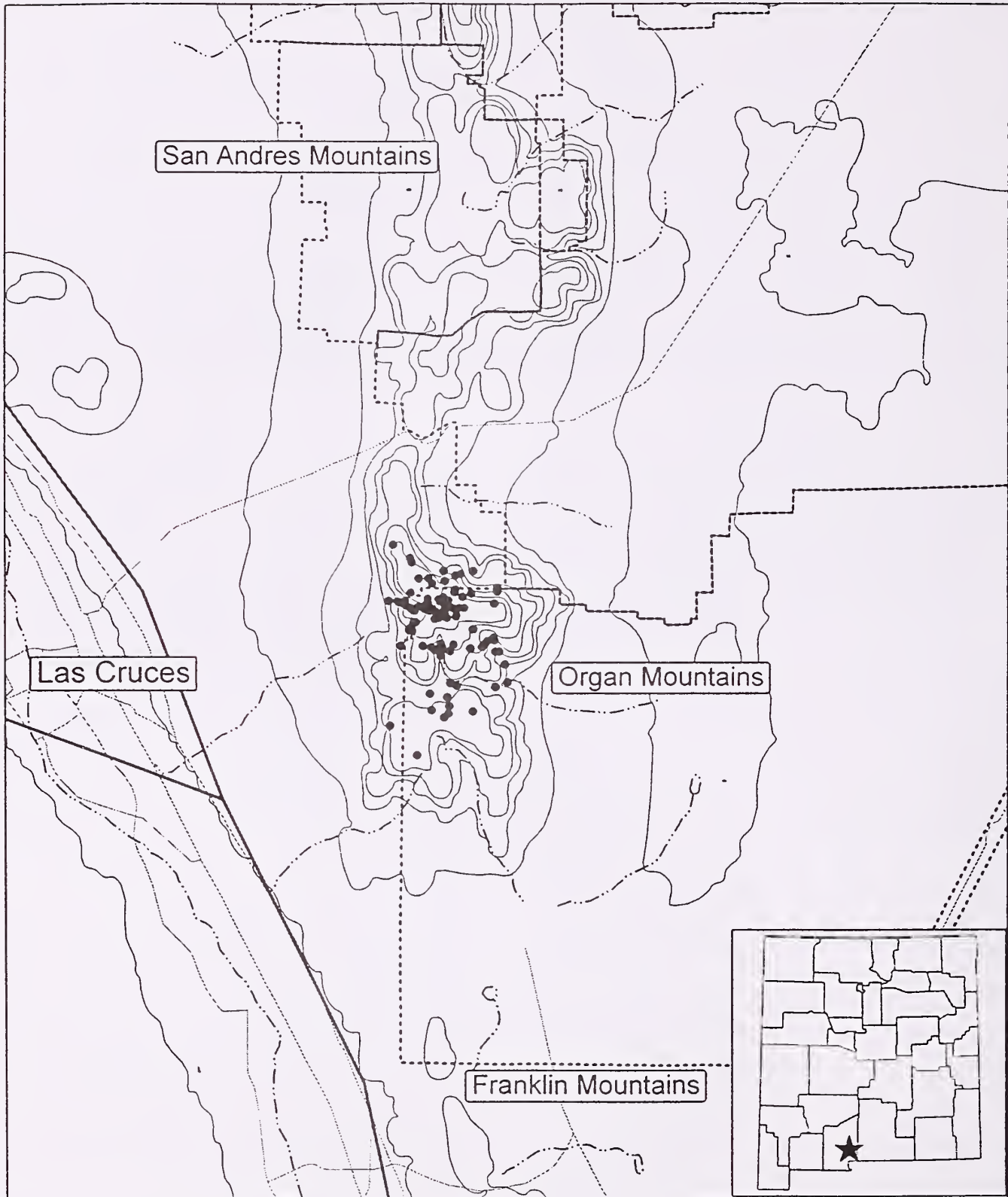
## Species Backgrounds

*Oenothera organensis* (Onagraceae) is endemic to the Organ Mountains. It is closely related to three other species that have scattered relictual distributions in central and northern Mexico (Dietrich et al. 1985). It is a bushy perennial herb, initiating from a slender taproot with a single rosette. Numerous adventitious shoots then arise from the taproot and lateral roots, with the root system appearing fibrous; stems are numerous, many branched, weakly erect to sprawling, growing to 0.6 m tall and 1.5 m in diameter. The basal leaves form a rosette. Flowers have a uniquely long floral tube, 10–19 cm long. The four petals are creamy yellow, obovate, 3.5 to 5 cm long, and almost as wide.

Other yellow evening primroses in the Organ Mountains have a more erect, less bushy habit and smaller flowers.

*O. organensis* is known only from the northern two-thirds of the Organ Mountains. Its distribution was published in a status report by Worthington in 1981. Results of recent surveys on BLM lands were presented by Skaggs at the first Southwestern Rare and Endangered Plant Conference in 1993.

# Rare Plants of the Organ Mountains



- Legend
- ▭ Military Boundaries
  - Rare Plant Populations
  - - - Streams
  - · - · - NM Highways
  - 500ft Contours

Miles  
0 2 4

September 1995  
New Mexico Natural Heritage Program

Figure 1. Rare plant populations in the Organ Mountains of New Mexico.

Flowering occurs from June to September. Plants are self-incompatible. Seeds that are passed in deer feces may have enhanced germination (Worthington 1981). Research has been focused on the genetics of this species, but has not addressed life cycles.

*Perityle cernua* (Asteraceae) is endemic to the Organ Mountains. The species is very distinct from any others in the genus (Niles 1970). It is a low tufted perennial from a woody base, and the stems are leafy, to about 10 cm long. Leaves are dark green, petiolate, with the blades broadly rounded-triangular, thick, fleshy, 2–3 cm wide, margins toothed, and old leaf bases persistent. Flower heads are yellow, rayless, solitary and terminal on peduncles to 2 cm long, held upright just above the leaves while flowering, and nodding when seeds mature. Ray flowers are absent and disk flowers are many (50–75) with yellow corollas about 4 mm long.

There are no similar species in the same habitat. On the high cliffs, *P. cernua* can be distinguished by the deep green color of the compactly tufted plants and the showy yellow flowers above the leaves.

*P. cernua* is known to occur only in the Organ Mountains. Previous collections by Spellenberg, Todsén, Castetter and others (DeBruin et al. 1994) were from two main populations in two separate canyons on Fort Bliss. Three small population have been found on BLM land, two of them reported by Skaggs (1993). Flowering continues from June to October.

*Scrophularia laevis* (Scrophulariaceae) is endemic to the Organ Mountains. It is a perennial herb having single stems, mostly unbranched, slender, bright green, to 120 cm tall. Petioles are slender, 2–3 cm long. Leaves grow to 7 cm long, 3.5 cm wide, broadly lance shaped with serrate margins, thin, bright green, glabrous except sparsely glandular-puberulent along the main veins. Flowers occur in sparse clusters of five or fewer branches from leaf axils near the top of the stem, pedicels slender sepals 3–4 mm long, triangular to lanceolate. Corolla is 7–12 mm long, pale carmine below, the upper lobes dark carmine, the reflexed lower midlobe white to pink, the throat glabrous (Todsén 1978).

Todsén (1978) published a revised description of the species that details its distinction from *S. montana* and *S. parviflora*. Plants differ from *S. montana* in their slender habit and doubly serrate leaf margins and from *S. parviflora* in their completely

deflexed lip and limited pubescence.

*Scrophularia laevis* is known only from the northern half of the Organ Mountains. Flowering occurs in August and September. By September many seeds have set, insect herbivory is high, and the elongated stems have become brittle and are easily broken.

*Draba standleyi* (Brassicaceae) is known in New Mexico only from the Organ Mountains. It is a small yellow-flowered mustard; a tufted perennial herb from a thick root, leaves mostly basal, narrowly oblanceolate, long-petiolate, 1.5–8 cm long, 2–7 mm wide, hairs strigillose to ciliate, stem leaves 1–8, smaller, toothed or entire. Stems are 3–15 cm tall, few and slender, erect to partially decumbent, simple or few-branched, glabrous to softly pubescent below, glabrous above. Racemes are elongate, 5–20 flowered, sepals ca. 2.25 mm long, glabrous to pilose. Flower petals are yellow, 4–6 mm long, seed pods are linear-elliptic, glabrous, 8–12 mm long, 1.5–2.5 mm broad, flattened, and twisted (Hitchcock 1941).

Leaves are longer, more slender, and more strongly basal than in *D. helleriana*; hairs are almost never stellate or cruciform as in *D. petrophila*.

It is known to occur in the Chiricahua Mountains of southeastern Arizona, the Organ Mountains in New Mexico, the Davis Mountains of western Texas, and Canon el Moreno in northeast Coahuila, Mexico. The populations are small, uncommon, and widely dispersed. Only one population on BLM land in the Organs was known prior to 1993.

Flowering occurs from June through August in Arizona and Mexico and continues into September in the Organ Mountains. Pollination by flies was observed at one location in Arizona (Malusa et al. 1992).

*Coryphantha organensis* (Cactaceae) synonym *Escobaria organensis* (D. Zimmerman) Castetter, Pierce & Schwerin is endemic to the Organ Mountains. It is anticipated that Zimmerman will eventually publish his revision of the species, naming it as a variety of *C. sneedii*.

*C. organensis* stems grow in loosely packed clumps, usually 10 or more per cluster, 2–7 cm in diameter, to 12 cm tall; central spines are tan to golden yellow, tips often reddish brown, about 10–20 mm long, inner central spines 0 to 4 (usually 1) per areole, outer central spines 8 to 14 per areole, subequal. Radials are white, spines (total) 37 to 49 per areole, spines are usually not completely obscuring underlying tubercles. Flowers are pink,

about 1.5 to 2.5 cm long, outer perianth parts fringed; fruits are cylindrical, yellowish green, to about 14 mm long (Zimmerman 1985).

*Coryphantha sneedii* var. *sneedii*, which has whiter spines and stem diameters 1.4 to 4.5 cm, grows on limestone south of the Organ Mountains. All of the clustered *Coryphantha* seen in the Organ Mountains fit the description of *C. organensis* with central spines less white and a larger average stem diameter.

Prior to 1990, only a few collections documented the locations of this cactus on Fort Bliss (DeBruin et al. 1994). Flowering occurs from May to June. Plants are easily observed throughout the year, because they are not hidden by heavy grass cover. Fruits are persistent into autumn.

### Methods

Prior to starting field surveys the available information on species and their locations in the Organ Mountains was compiled from the literature, herbaria, and personal communications. In 1990, general reconnaissance surveys of potential habitat were conducted for target species. Locations of populations were recorded on topographic maps and on the survey forms used for the Biological Conservation Database (BCD) by the New Mexico Natural Heritage Program (NMNHP). During the preliminary surveys of 1990, the distribution of each species was delineated and mapped on a canyon-by-canyon basis for most parts of the Organ Mountains that were accessible and relative measures of habitat and population size were recorded.

In 1991 quantitative data on habitat and population characteristics were collected from sample areas that were stratified to represent the range of potential habitat variables: substrate, slope, aspect, slope position, and elevation. Within each sample area, 10 potential data collection sites of uniform size were selected as they were encountered in the field. Five of the sites that corresponded to previously chosen random numbers were sampled. At each site individual plants were counted and slope, aspect, elevation, substrate, slope position, phenology, and associated species were recorded. A condition rating was assigned to the site based on the number and vitality of plants, the quality of the habitat, and its vulnerability to potential threats. Each data collection site was used as a sample unit for statistical analysis of habitat. The collection sites were grouped by sample area. Each sample area was entered into the BCD at the NMNHP as one "element occurrence." The occur-

rences were mapped on topographic quadrangle maps at NMNHP.

In 1992 the field data were used to develop predictive habitat models for each species. The models were tested in the field by sampling the predicted habitat in the same manner as the original samples. The sampling plan was adapted to the unique habitat of each species, as described in the following paragraphs.

*Oenothera organensis* individuals were counted at most of the small population sites. Subsamples were selected from extensive populations by assigning a number to each group of six or more plants separated along the streambed from the next group by 20 or more meters. The sample groups were chosen by random numbers. The area counted covered 20 meters downstream from the first plants. Individuals were counted in four size classes: rosettes, less than 0.5 m in diameter, 0.5–1 m, and greater than 1 m in diameter.

*Perityle cernua* individuals were counted with binoculars on all of the cliffs where they were found except at the large populations where the canyon walls were divided into numbered sections and the numbers were chosen at random. Plants within the sections were counted with binoculars from the best available vantage point. The plants are easily seen against the rock walls, but the counts are conservative because the very young plants and those hiding in crevices cannot be seen at a distance.

*Coryphantha organensis* plants were counted in small clusters when they were scattered along ridgelines. On canyon sides where they grew in larger patches, sample patches were selected by the same random number method used for other species. Individuals were counted within 20 square-meter samples.

*Scrophularia laevis* grew in thick patches. In small patches, all plants were counted. In large patches, we counted a 10-meter-square section and estimated the number of sections within the population. In extended populations, we numbered the patches as they were found and counted those that matched randomly selected numbers. The samples were used to estimate the population size.

*Draba standleyi* were found in only a few limited areas; all plants were counted.

Statistical analyses of habitat preference were performed using the sample data for the four endemic plant species. *Draba standleyi* was not analyzed because there were too few samples. Regression models of the independent variables of elevation, slope, position and aspect were con-

structed for the dependent variables area, number of individuals, and density.

## Results

The total population numbers given in Table 1 do not include the Organ Mountains north of the Fort Bliss boundary. The counts are individual plants recorded during our surveys. The estimates include ocular estimates in the field and extrapolations from our sample sites onto entire sample areas mapped. Total estimates include extrapolations onto additional potential habitat plus estimates given by other sources for sites that we did not resurvey. Each category includes the number from the column to the left.

Table 1. Population summaries for plant species in the Organ Mountains on Fort Bliss.

Species	Count	Estimate	Est. Total
<i>Oenothera organensis</i>	718	1960	2300
<i>Perityle cernua</i>	2822	5121	6800
<i>Scrophularia laevis</i>	764	864	2000
<i>Coryphantha organensis</i>	326	500	5000
<i>Draba standleyi</i>	610	650	850

The regression approach to predicting the area, number of individuals, and density of the four endemic species in relationship to elevation, slope, aspect, and position was unsuccessful. No statistically significant models were found and the marginally significant models only predicted about 25 percent of the variation in plant abundance. The relationships of the five rarest species to the important elements of their habitat are presented graphically in Figures 2–6.

*Oenothera organensis* plants were found only in streambeds or on adjacent hillside seeps where surface water is present for at least part of the growing season. The amount and duration of surface water required is unknown. The largest populations occurred around pools of water that persist in both spring and fall. Stray individuals were found in dry streambeds a short distance up or down stream from flowing water. Robust populations have been found in all aspects and on all of the substrates present in the Organ Mountains at elevations from 4860 feet to 7800 feet. Plants are usually in open sun or partially shaded by riparian trees and shrubs. Most plants are rooted in coarse, wet sand and gravel in sheltered pockets along the bank or on the downstream side of rocks and boulders.

Associated trees and shrubs in the riparian community were *Pinus ponderosa*, *Cercocarpus montanus*, *Symphoricarpos*, *Vitis*, *Quercus arizonica*, *Q. grisea*, *Fraxinus velutina*, *Baccharis glutinosa*, *Chilopsis linearis*, *Ptelea angustifolia*, *Fallugia paradoxa*, *Juniperus deppeana*, *Acer negundo*, and *Salix gooddingii*.

*Oenothera organensis* has been found in the wet sections of most of the canyons in the northern half of the Organ Mountains. The populations seem to shift with available water, judging by historical records (Worthington 1981), so new sites could be found in any of the occupied canyon bottoms. We found populations that were more extensive than those reported by Worthington (1981) in three of the wettest canyons. Plants were reported scoured by flooding from one canyon in 1991 (Skaggs 1993), where a few scattered adults are now regrowing from roots. The effects of fire and erosion on *O. organensis* habitat will be monitored by NNMHP in 1996.

*Perityle cernua* was found on previously unrecorded cliff sites near the known locations, but in more open canyons on sunnier aspects. The substrate was always rhyolite, andesite, or Achenback tuff (Seager 1981). Surveys for *P. cernua* were negative in six canyons on quartz-monzonite cliffs.

Habitat for *P. cernua* on Fort Bliss was restricted to rhyolite cliffs, which may provide a more mesic substrate than granite (Skaggs 1993). Known locations are at elevations from 5400 to 7800 feet. Plants are most abundant in areas shaded by cliffs or in recesses. They are not restricted to deep shade, however, but have also been found on open cliffs with varied aspects including direct southern exposure. Roots are anchored in crevices of the rock and plants are found most frequently along fissures and other indentations on the cliffs where the microhabitat is more mesic and roots can be established.

Associated species are chasmophytic plants including *Silene plankii*, *Coryphantha organensis*, *Aletes filifolius*, *Selaginella*, *Dasylyrion*, *Sedum*, and *Yucca baccata*. The vegetation at the base of the cliffs ranges from Montane Scrub and Montane Riparian to Lower Montane Coniferous Forest.

Wrens were observed taking mature seeds. There was herbivory by caterpillars on many plants in 1991. Stems of the plants break easily and seeds may be distributed down the cliff by falling stem fragments.

Most of the occupied sites are inaccessible to hikers or livestock. Rock climbers could destroy many of the fragile plants if they were allowed

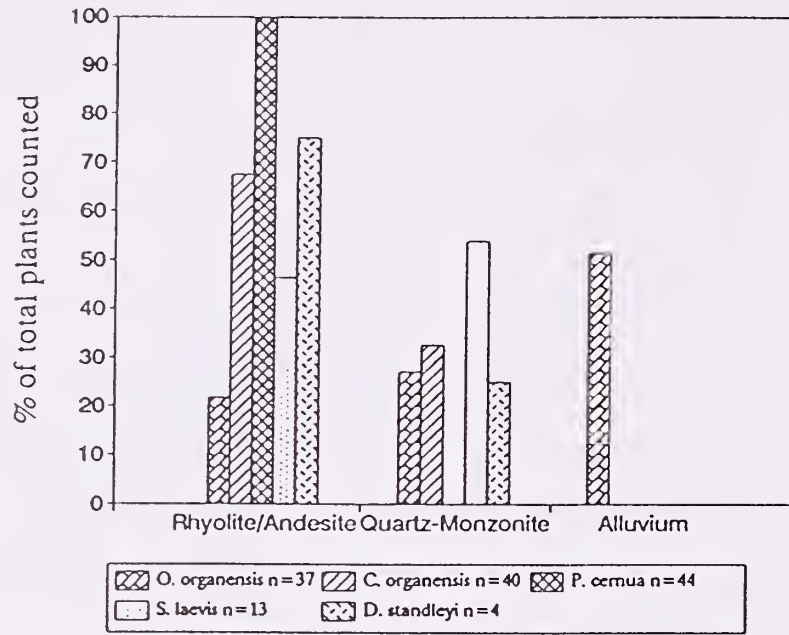


Figure 2. Substrate affinities of rare plant species in the Organ Mountains on Fort Bliss.

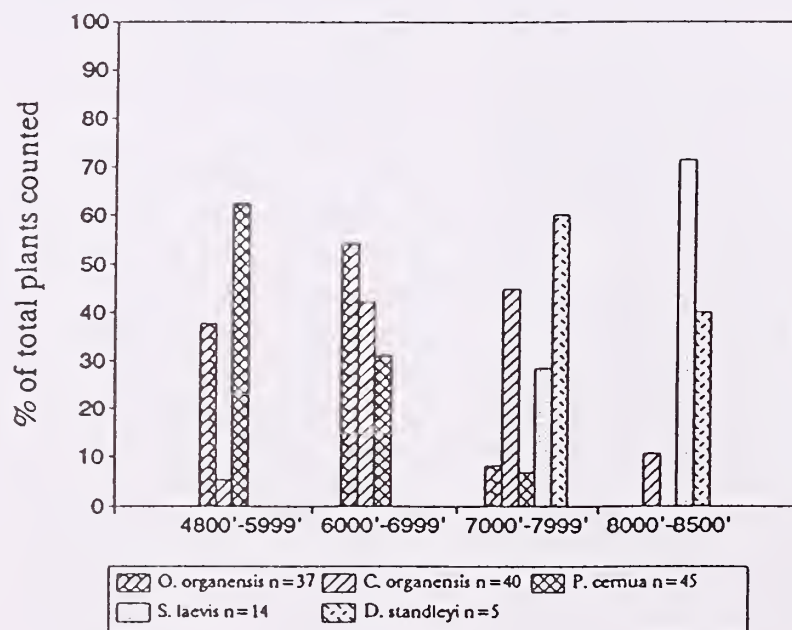


Figure 3. Elevation affinities of rare plant species in the Organ Mountains on Fort Bliss.



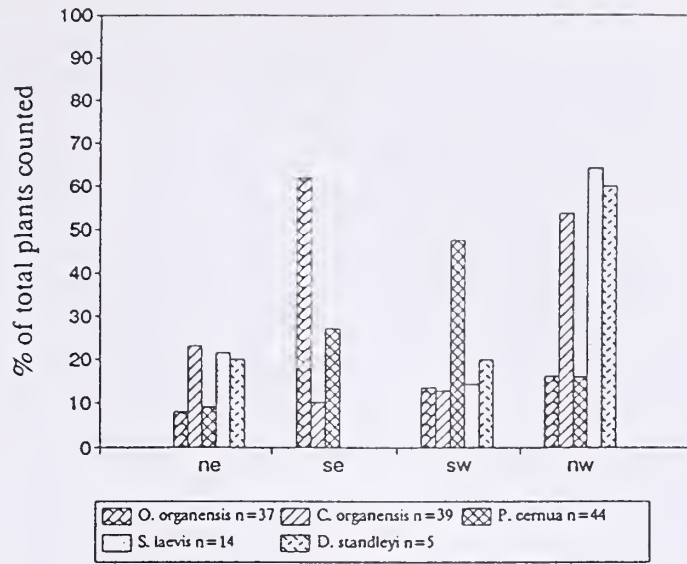


Figure 4. Aspect affinities of rare plant species in the Organ Mountains on Fort Bliss.

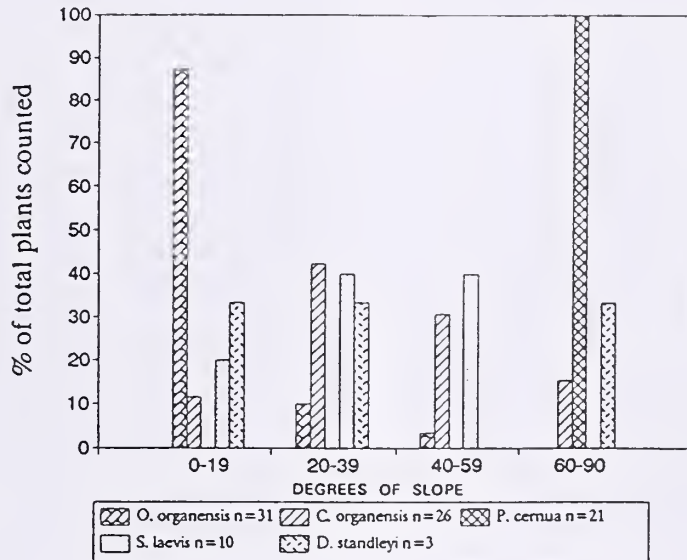


Figure 5. Slope affinities of rare plant species in the Organ Mountains on Fort Bliss.

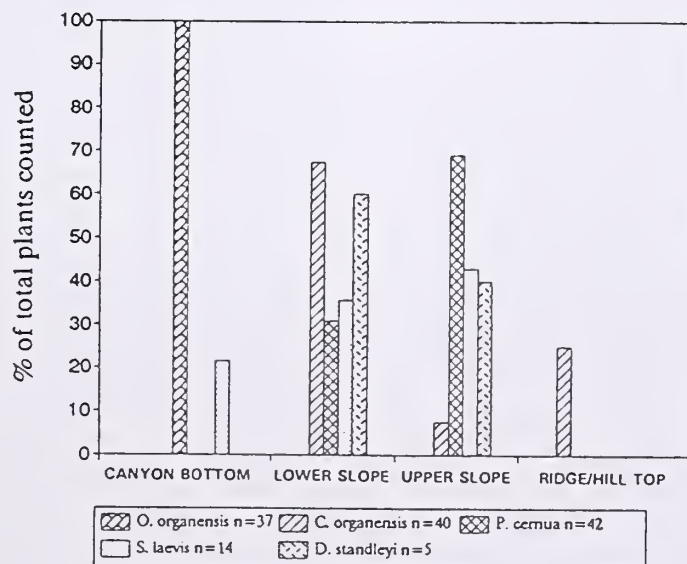


Figure 6. Position affinities of rare plant species in the Organ Mountains on Fort Bliss.

access. The best habitat in terms of numbers and size of plants and naturally protected location is in one short section of a deep canyon that should be set aside from human disturbance. Monitoring the population trends and any effects of the fire on *P. cernua* is planned for 1996.

*Scrophularia laevis* was found only in the northern third of the Organ Mountains. There is potential for many more populations in the canyons on the north side of the range on BLM land.

*S. laevis* grows in the understory of Gambel oak stands in dark organic soil and litter on north-facing slopes and in uneroded canyon bottoms. Elevations range from 7120 to 8400 feet. Substrates were rhyolite, andesite, and quartz-monzonite on slopes from 5 to 40 degrees with aspects mostly northwest to northeast between 250 degrees and 45 degrees. The best populations found during 1990–1992 in terms of size, condition, and quality of habitat were on the north slopes of the highest peaks and ridges.

Associated species under stands of Gambel oak and ponderosa pine included Douglas fir, stinging nettle, New Mexico locust, *Ptelea*, *Thalictrum*, *Geranium*, *Symphoricarpos*, and *Ribes*.

The smooth figwort is probably palatable to cattle and would be impacted if its habitat were open to grazing on a regular basis. The sporadic grazing that occurs now did not appear to affect this species during the three autumn seasons of our surveys. Since it grows in the understory on organic soil and litter, an intense fire could destroy its habitat for a time. Recovery potential of a population after fire might depend on the degree of damage to the oak overstory and the amount of soil loss from erosion following the fire. Monitoring of these effects will start in 1996.

*Draba standleyi* was found on only three sites in two canyons on the north side of the highest peaks and ridges of this rugged mountain range. Across its range, it grows at elevations from 6000 to 9400 feet in the mixed conifer vegetation zone on cliffs and large boulders, almost always in mesic, north-facing shaded locations on rhyolite or other igneous substrates. In the Organ Mountains it was found on small and large rhyolite boulders, as well as cliffs from 6500 to 8450 feet on northerly aspects.

Associated species were *Coryphantha organensis*, *Heuchera rubescens*, *Sedum*, mosses, and lichens.

There are no apparent threats to the species. It was found in a low-intensity burn area at one location in the Chiricahua Mountains of Arizona

(Malusa et al. 1992) The known populations will be monitored for effects from the fire starting in 1996.

*Coryphantha organensis* was found in all but the most open and dry canyons in the northern half of the range. It was not found in the southern part of the Organ Mountains and does not appear to form a continuous population with the endangered *Coryphanth sneedii* var. *sneedii* that has been found on the limestone ridges of the next mountains to the south.

*C. organensis* was found growing on rocks at elevations of 5750–8500 feet from the pinon-juniper to mixed conifer vegetation zones. It was most abundant on rhyolitic substrates, but was also found on granitic substrates at a few of the sites surveyed. Slope positions ranged from ridge crests down to the base of canyon side slopes, and degree of slope seemed insignificant as long as stable rocks were present. All aspects were occupied, but the lowest potential for this species occurred on the most exposed south-facing slopes. Habitat with highest potential was on the north side of open rocky ridges. Plants were found most frequently in mesic microhabitats with strong light. They were frequently on the north side of boulders, in *Selaginella* mats, and on canyon sides in crevices, soil pockets, and ledges on the rock. On wooded hillsides they appeared in small clearings among the trees.

Associated species included *Pinus edulis*, *Juniperus deppeana*, *Quercus gambellii*, *Heuchera*, *Selaginella*, *Garrya wrightii*, *Cercocarpus montanus*, *Yucca baccata*, and *Echinocereus triglochidiatus*.

Plants grow in patches scattered over rugged canyon sides that are nearly inaccessible on Fort Bliss. Collection may occur around the BLM recreation areas. No signs of herbivory or disease were observed. The 1994 fire burned across most of the range for *C. organensis*. Mortality and fire effects on the habitat will be monitored starting in 1996.

## Discussion

The highest impact to plant populations from public recreational use is along the shared Fort Bliss/BLM boundaries on the west and north sides of the Organ Mountains. Cooperation on management and monitoring programs between the two agencies' biologists would be beneficial to the rare plant populations that occur in these high-use areas. The Dripping Springs boundary area has small populations of four of the rare species.

Monitoring the impact of public use in that area compared to control sites that are not accessible to the public may provide useful information for management of the species.

Fillmore Canyon and its watershed contains healthy populations of all five rare and endemic plant species. It is one of the most outstanding natural botanical areas in New Mexico. The condition of the riparian area and the *Oenothera organensis* population there could be improved by fencing the boundary to exclude the cattle that are grazing there.

*Oenothera organensis* may be the most threatened of the Organ Mountain endemic plants because it depends on surface water. Drawing down of the water level by new wells or diversion of springs and runoff water would probably eliminate most of the extant populations.

Fort Bliss is responsible for stewardship of all or most of the habitat for the four endemic plant species in the Organ Mountains. If this critical habitat became subject to major disturbances or water loss, one or more of these four species would probably be proposed for listing as endangered. These rare species have not been proposed for federal endangered or threatened status because the U.S. Fish and Wildlife Plant Recovery Team and the public believe that the species are secure within the military reservation portion of the Organ Mountains. At the same time, the Fort Bliss Natural Resources office needs public support for preservation of these species to justify its continued special management of the habitat.

Following our surveys, the New Mexico Natural Heritage Program recommended monitoring to document the population trends of the species at risk in the Organ Mountains and the changes in their habitat over time. The 1994 lightning fire spread across most of the habitat covered by our plant surveys. In 1996, we will again have the support from Fort Bliss to establish a long-term monitoring program for burned and unburned samples of the populations that were surveyed previously on Fort Bliss. We will also complete the surveys for these species on BLM land in the Organ Mountains in 1995 with support from the National Biological Service. Together the information from Fort Bliss and the BLM portions of the Organ Mountains will allow us to assess the global status of the four endemic species and the New Mexico status of *D. standleyi*.

## Acknowledgments

Support for this research was provided by the Directorate of Environment, Cultural/Natural Resources Division, Fort Bliss, Texas; we thank Kevin Von Finger for his assistance. The Dripping Springs Natural Area staff also were generous with their assistance.

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# Monitoring and Population Biology of *Talinum validulum* on the Kaibab National Forest, Arizona

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**Abstract:** *Talinum validulum*, the Tusayan flameflower, has received considerable attention since the late 1980s due to several large-scale development plans proposed for the Tusayan area, adjacent to Grand Canyon National Park. Initially, very few sites were documented. Surveys since 1990 on the Tusayan, Chalender, and Williams ranger districts, as well as the Prescott National Forest, have revealed many new localities for the species. Four monitoring plots established in August, 1990, and read monthly during the growing season until October, 1994, have provided much information about the phenology and population biology of the species.

## Introduction

Field investigations on the distribution and ecology of *Talinum validulum* Greene (Tusayan flameflower) were carried out from August, 1990 to September, 1994 under contract with the Kaibab National Forest. Studies included the establishment of four monitoring plots to study phenology and population biology, surveys to determine the range and distribution of the species on the Forest, and comparisons with similar species (Phillips 1993).

*Talinum validulum*, a member of the Portulacaceae, occurs in Coconino and Yavapai counties, Arizona, and is known from four widely separated areas: throughout most of the Tusayan Ranger District and in limited areas of the Chalender and Williams districts of the Kaibab National Forest, on the Chino Valley and Walnut Creek districts of the Prescott National Forest, and on private lands near Grand Canyon Caverns. A 1916 collection by Bertaud near Santa Fe, New Mexico, has been neither verified nor relocated. It was first collected by Leiberg on 23 September 1901 from cinder slopes at Rattlesnake Tank, northwest of the San Francisco Peaks, a locality that has not been relocated. The type specimen was collected by R.R. Hill in the Tusayan Forest Reserve in August, 1912, and the species was described by E.L. Greene (1912) (Reichenbacher 1986). The species is currently listed as a U.S. Fish and Wildlife Service category 2 candidate species (U.S. Fish and Wildlife Service 1993, Rutman 1992) and a Forest Service Region 3 sensitive species (USDA Forest Service 1990).

The plant is a low perennial herb with fleshy leaves and a tuberous root. The stems die back to the root crown each fall, breaking dormancy in

May and remaining active until entering fall dormancy between mid-September and mid-October. Plants have one to several branches, the larger individuals tending to have elongated horizontally spreading branches. Flowering may occur soon after the plants break dormancy in the spring or it may be postponed until mid-summer if spring conditions are dry. In most years two flowering seasons occur, separated by a period of semi-dormancy in June and July during the hot, dry period preceding the onset of summer rains.

Tusayan flameflower occurs on soils of both limestone and volcanic origin. More important than substrate is the microhabitat of shallow, gravelly soils in small depressions in bedrock. Such sites may occur over an open area of several acres, as on a basalt flow at the Pine Flat site, or in extremely local, limited areas as in many Kaibab Limestone canyon rim sites in the Tusayan area. The shallow soils preclude the establishment and survival of most shrubs and many other herbs, reducing competition from other species and making the microsites with their comparatively barren appearance stand out from surrounding areas.

Previous recent field surveys for the plant include a status report prepared by Reichenbacher (1986) which included surveys in the Pine Flat area south of Williams and at Grand Canyon Caverns and file reports of field surveys by Galeano-Popp (1988, 1989) in the Pine Flat area. Plants were relocated for the first time in 77 years in the Tusayan area near Grand Canyon Airport by B. Phillips and S. Treptow in 1989 while carrying out a survey for a proposed Grand Canyon Railroad spur alignment. As a result of intensive surveys carried out from 1990 to 1992 by the authors of this report, Kaibab National Forest personnel, and others,

many additional locations and thousands of plants have been found.

## Methods

Studies on *Talinum validulum* included field searches throughout its range on the Kaibab National Forest to determine its range, abundance, and habitat characteristics, and monitoring of selected sites to collect data on its phenology, ecology, and life history.

Surveys carried out for this project were designed to cover a wide geographical range in order to document the distribution of *Talinum validulum* on the Tusayan District. Field surveys were carried out in most areas of the district, with stops made to check for plants when apparently suitable habitat was observed.

Searches were initially carried out using known sites as a center of occurrence and moving out in all directions, surveying for suitable habitat and sites that have plants. The species is very habitat specific and it is found only in areas having the necessary substrate characteristics. Surveying for proper habitat is the most efficient procedure in view of the small size, local occurrence, and difficulty in finding the plants. Because of development pressures, surveys were carried out much more intensively near Tusayan and Grand Canyon Airport.

Most of the time spent on the Williams District was used for monitoring studies of the population at Pine Flat. Other previously reported sites were relocated or verified. The full extent of the population at Pine Flat was not determined; however, a number of negative searches were carried out in the general vicinity of the known sites.

Forest Service personnel have searched for *Talinum validulum* in several parts of the Kaibab National Forest. This information has been made available and is included in order to make this report as complete as possible. These searches were made on selected parcels proposed for exchange east of Grand Canyon Airport, and on isolated offered inholdings of private land located throughout the Tusayan District.

Four 1 m<sup>2</sup> monitoring plots were set up in August, 1990, to determine the seasonal phenology, ecology, and life history of the species, and assess characteristics for its accurate separation from the closely related *Talinum parviflorum*. Plots 1–3 were established at Pine Flat on the Williams District and Plot 4 was set up southeast of Grand Canyon Airport on the Tusayan District.

A one-square-meter wooden plot frame was constructed with a 10 cm grid system that allowed accurate mapping of individual plants within each plot. The location of each plant was recorded by grid square, and if more than one plant was present in a square, each plant was also recorded by coordinates within the square to 0.1 cm. Each plant was numbered at the initial reading or at its initial appearance and the same number remained with the plant throughout the project. If a plant died, its number was not reassigned.

The plots were established in August, 1990, and were read approximately once per month during the growing season (April or May through September or October) through the 1994 season. At each of the readings the following parameters were recorded: plant height, plant width, number of branches, number of inflorescences, and number of buds, flowers, and fruits.

At the time plots were established, there were many seedlings present at the Pine Flat sites. Seedlings in some grid squares were initially given plant numbers and their grid locations were recorded, as with adult plants. Seedlings in other squares were simply counted. Surviving seedlings from 1990 that had not been numbered initially were given numbers in May, 1992. Seedling counts by grid square were continued throughout the project.

## Results and Discussion

Surveys for *Talinum validulum* have provided a much clearer picture of its distribution and abundance than was known previously (Phillips 1993). On the Tusayan District, the plant was unreported for 77 years until a few small sites were discovered in 1989, followed by intensive and extensive surveys that have documented its distribution at dozens of sites with thousands of individuals throughout the district. It has also been located at new sites on the Chalender and Williams districts, and recent surveys have extended its distribution to the Prescott National Forest where numerous populations and thousands of individuals have been discovered (Baker and Wright 1993). Plants closely resembling *T. validulum* were reported from the Blue Ridge District in the southeastern part of the Coconino National Forest by C. Franz in September, 1995 (B. Phillips, personal communication); these have not been verified in the field.

Monitoring plots established at Pine Flat and near Tusayan in August, 1990, and read monthly during the growing season through 1994, have

provided information on the ecology and phenology of the species. This has given land managers information useful for managing the species and carrying out surveys during optimum times of the year. Problems with the separation of *T. validulum* from the similar species *T. parviflorum* have been addressed and guidelines have been developed for separating them in the field.

#### General Ecology

*Talinum validulum* is narrowly habitat specific and its limitation to a specialized habitat occurring in small, disjunct patches over a wide area is the reason for its distribution in isolated dispersed colonies. The colonies may be fairly dense when substrate conditions are favorable. The plants occur in thin, gravelly soils in shallow depressions in bedrock along the rims of drainages, where shallow gravel pockets occur at the tops of outcrops and walls, and along the spines of broad, low, gently sloping ridges where bedrock occurs at the surface. Sites visited on the Kaibab National Forest during this study range in elevation from 1870 to 2180 m. General vegetation ranges from open ponderosa pine forest to pinyon-juniper woodland, with an understory of sagebrush at lower sites.

The microsites where the plants occur are generally flat or nearly flat, although the general surrounding area may be steeply sloping, especially in canyon rim sites. Soils at the microsites are sufficiently shallow so that few other plants can survive. *Talinum* is apparently quite tolerant of the harsh microenvironment of alternating wet soils when rains fill the bedrock basins with water, and exaggerated drought when the pockets dry out and are isolated from deeper subsurface moisture. Sites where the plants are not usually found include areas with deep alluvial soils, as along drainages, under trees where thick litter is present, and on slopes with dense vegetation such as oak thickets along the sides of drainages.

The presence of comparatively barren openings in areas with sagebrush, grama grass, and other dense herbaceous or shrubby ground cover vegetation is a field clue for a potential *Talinum* occurrence; it does not compete well with other species. The plants tolerate moderate shade, but do not grow under the canopy of trees or shrubs. Trees and large shrubs are rarely rooted in the pockets, although the boundary between a pocket and an adjacent area of deeper soils is sometimes so abrupt that *Talinum* may occur near species in the general area with little or no transition, and

probably very minimal substrate competition. While *Talinum* does not tolerate competition from other species, it appears to be moderately tolerant of habitat disturbance, as sites were found in old two-track roadways, in areas that received sporadic disturbance from camping, and in areas adjacent to moderately used cattle watering or feeding sites where other vegetation was removed by grazing and trampling.

One curious aspect of *Talinum validulum* distribution is its occurrence in similar habitats in very different substrate types. All of the occurrences at Tusayan are associated with Kaibab Limestone bedrock, while the sites in the Pine Flat area and on the south Chalender are on substrate of volcanic origin. The shallow bedrock depressions are the common denominator, rather than the rock type. Rhyolite or basalt substrate is absent at Tusayan, and limestone, at least at the surface, is not present in areas where the plants occur south of Williams. Sites on the Prescott National Forest, some 20 miles south of Pine Flat, are reported by Baker and Wright (1993) to occur exclusively on limestone substrate; the plants apparently are lacking from nearby basaltic soils.

#### Distribution

There are two main centers of occurrence for *Talinum validulum* in the Kaibab National Forest: the Tusayan District and the southeastern corner of the forest between Garland Prairie and Perkinsville Road (Figure 1). Although apparently suitable habitat is present between these areas, no plants have been found.

Table 1 presents a summary of *T. validulum* sites found on Kaibab National Forest lands from August, 1990 to September, 1992 by the authors of this report and associates and by Kaibab National Forest personnel. Additional sites found on the Prescott National Forest by M. Baker and within Grand Canyon National Park by the National Park Service are not included in this table. These are the main surveys carried out during the period. The Kaibab National Forest surveys have resulted in nearly 16,000 plants actually counted in a total occupied area of approximately 20 ha. Estimates and extrapolations are not included in this compilation and many more plants at uncensused sites certainly exist. Eighty-four sites were documented on the Kaibab National Forest and 11 sites were mapped on the Prescott.

The documented distribution of sites is in part a result of the need for surveys in response to various land exchange and development proposals.

### Mean Widths and Mean Number of Inflorescences for *Talinum* at Plot 3

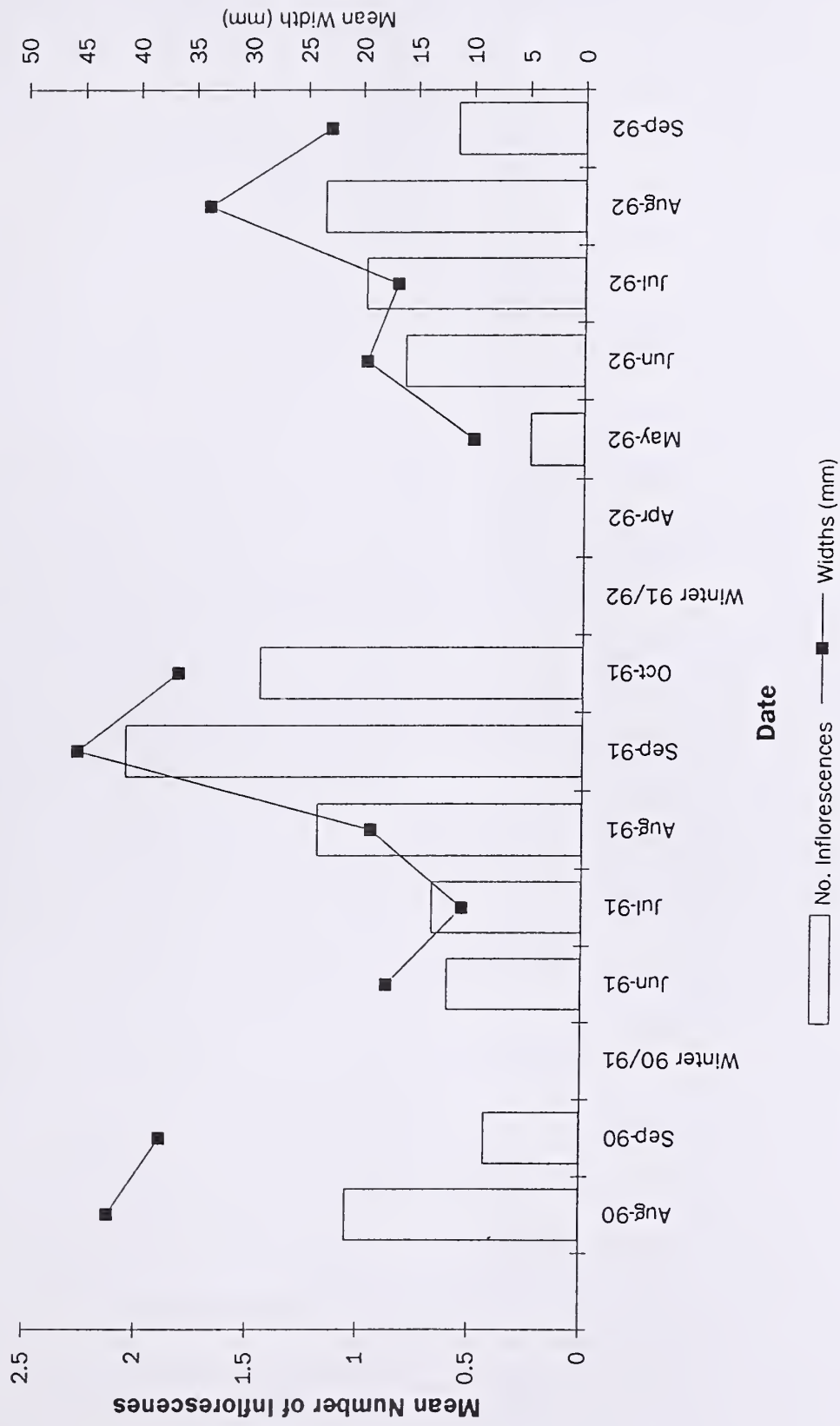


Figure 1. *Talinum validulum* localities on the Kaibab National Forest.

Table 1. Summary of *Talinum validulum* sites found on Kaibab National Forest lands during surveys by Forest Service personnel and A. Phillips and Associates, August 1990–September 1992.

Area	No. Sites	Size (m <sup>2</sup> )	No. Plants
<b>Tusayan Ranger District</b>			
Dodd and Bass Tanks	3	1721	456
Brush Tank	1	3720	20
Sheep and Sam Moore Tanks	3	1683	327
South of GC Airport	14	48,221	3884
West of Tusayan	11	453	1066+
East of GC Airport	24	85,950	5299
Tusayan	5	—	729
L. Red Horse Wash	1	0.8	8
Upper 10X Tank	3	158	563
East of Red Butte	2	950	150+
Northeast of Red Butte	1	279	60+
Russell Wash	5	270	100+
Willows	4	8,370+	240+
Tappan Wash	1	9	50
Lee Canyon	1	—	500
<b>Chalender Ranger District</b>			
White Horse Lake Rd.	1	40,500	200+
Frenchy Can.	5	—	785
N. of Big Spring Can.	9	—	1060
LO Spring	1	—	200
<b>Williams Ranger District</b>			
Pine Flat (Plots 1–3)	1	3	128
<b>Kaibab National Forest</b>			
Total plants counted at 96 sites: 15,697			
Total area recorded: total plants counted at 192,288 m <sup>2</sup> = 19.2 ha			

Thus, the area around Tusayan and Grand Canyon Airport has been more intensively surveyed than other portions of the range of the species and appears to be a center of distribution for the species with many colonies of plants located within a relatively small area. This area contains an abundance of suitable microsites; whether a similar density of sites exists elsewhere has not been documented as surveys of comparable intensity have not been done.

Accessible terrain away from the Tusayan village area was surveyed to check for suitable habitat. The plants were usually found in areas that appeared to the investigator to have the right conditions. In the summer of 1990, Forest Service personnel surveyed small, isolated parcels of private inholdings offered for exchange; populations of *Talinum validulum* were found on several of these, with plants located at nearly extreme east and west ends of the District. Our surveys during the 1992 season were designed to ascertain whether these were isolated occurrences or whether the plants are found more or less continuously throughout the district where suitable microsites exist. The latter appears to be the case, except for

the southern boundary area and the northeast corner of the district, where suitable habitat is rare and conditions are probably unfavorable due to low elevation.

On the Williams District, surveys were carried out east, south, and west of the Pine Flat site in an effort to locate additional sites in the area. Although several additional localities for *T. parviflorum* were found, no *T. validulum* was located. Suitable habitat appears to be present in scattered areas but all sites visited were smaller than the Pine Flat meadow, generally less rocky and with better developed soils. These surveys were spot checks rather than systematic, complete searches; we can conclude only that *T. validulum* is not widespread and that its occurrences are local, not that it is present only at the known site.

In the Chalender District, a search for a previously known site (Galeano–Popp 1988) was relocated on the north side of the White Horse Lake Road (FR 110) in a rocky, open area about 4 ha in size. The site is flat and very rocky with thin soils and basalt bedrock outcrops and strikingly fewer ponderosa pine than in the surrounding forest. It resembles the site at Pine Flat. Several hundred plants were present when the site was visited in August, 1991.

A number of additional sites within the Chalender District were found by Forest Service personnel conducting wildlife surveys in August, 1992 in the Frenchy Canyon area and between KA Hill and the Sycamore Canyon vista parking area. Some 2000 plants were located and mapped during this survey. Both species were reported at some of the sites (see Table 1).

#### *Comparison of Talinum validulum and T. parviflorum*

Throughout much of the range on the Kaibab National Forest, both species of *Talinum* are often found occurring together or at least in some proximity. As they are difficult to distinguish without close examination, this has caused some problems in the past.

Differences between the two species given in keys (e.g., Kearney and Peebles 1960) are not necessarily consistent in the field. Stamen number is one separation, cited as 10 or more for *T. validulum* and 4–8 for *T. parviflorum*. For *T. validulum*, our field counts on the Tusayan District included a site near Sheep Tank where plants had a mean of 10.2 stamens with a range of 6–13 for 43 flowers and a site west of Rain Tank Wash with a mean of 13.4 and a range of 9–17 for 24 flowers. A count of



13 flowers near Plot 3 at Pine Flat showed a mean of 8.6 and a range of 7–10. Number of stamens on 24 *Talinum parviflorum* flowers at a site east of Pine Flat had a mean of 4.96 with a range of 4–6; all but three flowers had five stamens. This small sample indicates that stamen counts may be diagnostic, but within ranges differing from those previously published. They are difficult to obtain as flowers do not appear until mid to late afternoon and may not be present during significant portions of the growing season, depending on precipitation.

Another separation in the key is the combined length of stems and scapes, given as less than 10 cm for *T. validulum* and "commonly at least 10 cm long" for *T. parviflorum* (Kearney and Peebles 1960). In the field, stem and scape lengths are heavily dependent on seasonal and rainfall variations and do not provide an accurate separation between the species.

In areas where the two species occur together, growth form is a fairly consistent differentiating character, although it is often difficult to interpret. *T. validulum* plants may develop one to several elongated, horizontal leafy stems, especially in late summer, which die back to an overwintering bud at the top of an enlarged more or less vertical storage root at or below the soil surface when they enter dormancy in the fall. *T. parviflorum* produces short, leafy tufts at the tips of perennial above-ground stems each season, with the leaves but not the stem dying in dormancy. Older plants thus have an elongated, horizontal tuberlike prostrate stem on the soil surface. The anatomical root/stem characteristics of these storage structures was not investigated; however, their behavior in the two species is quite different. Difficulties in using this characteristic arise in particularly rocky areas with soils so shallow that *T. validulum* roots are partially exposed above the surface and with juvenile plants in which the elongated stems of *T. parviflorum* have not yet developed.

There are also significant and consistent differences in pigments present in the two species. *T. validulum* has a higher proportion of red pigments, giving the leaves a dark green or gray-green color, often with red tips. The leaves turn bright red (like tiny flames, hence the common name, "flameflower") for a brief period when entering dormancy in the fall and do not exhibit obvious yellow coloration. The leaves of *T. parviflorum* are yellow-green through the growing season and turn yellow with a speck of red at the very tip in the terminal 0.5 mm or less. The entire leaf does not turn red.

Differences in the scapes, or flowering stems,

are also consistent. Scapes of *T. validulum* are proportionately thicker and softer, making them more pliable. *T. parviflorum* scapes are stiffer and thinner. These characteristics appear to be consistent regardless of length of the inflorescence, which is influenced by rainfall.

### Phenology

Plants in the plots exhibited growth from late April or early May, when they first broke dormancy, until a point between mid-September and mid-October when they ceased activity and entered winter dormancy. The onset of spring growth is probably dependent on a combination of favorable soil moisture conditions and warm temperatures. Flowering was recorded in May, so under the proper conditions reproduction may occur soon after spring growth commences. Spring flowering was suspected, but had not been documented prior to this study; it is possible that it may not occur every year if spring conditions are severely droughty. Spring growth and flowering occurred during each of the four years of this study.

Following a spring growth and flowering season of several weeks, flowering ceases, fruits are formed and dehisce, and the plants enter a secondary dormant period during the hot, arid pre-summer rain period of June and early July. The leaves are generally retained, but they lose turgidity, fold upward and inward over the tip of the stem, shrivel, and turn an ashen gray-green color. They do not turn red as they do at the onset of winter dormancy. During the early summer inactive period the plants are very inconspicuous and field surveys are difficult. In 1992, unusually heavy rains in late May and cool weather in June prolonged the spring growth period and the early summer dormancy did not occur until July.

The start of the second growth period is determined entirely by the onset of the summer rainy season. This may occur any time between late June and late July, and may vary by days or even weeks from place to place within a short distance. It is unknown how much rain is necessary to trigger growth, but the plants resume growing rapidly and with adequate soil moisture and higher humidity they soon begin flowering and resume stem elongation. The leaves regain turgidity and turn bright green. Additional stems may begin to grow and large plants spread horizontally and develop numerous inflorescences. This is the main growth and reproductive period of the year and it is the period during which field surveys are most accurate. It also appears to be the time when most

germination occurs.

The double peak in growth for 1991 and 1992 in Plot 3 at Pine Flat is illustrated by Figure 2 and the same pattern is exhibited by the two species of *Talinum* present in Plot 2 (Figure 3). Plant width and number of inflorescences are correlated and both respond to seasonal moisture availability, as represented by data from Plot 3 (Figure 4).

The summer rains did not begin until July 30th in 1993, after a 6-week drought, causing all but one plant at the Tusayan plot to enter a winter-like dormancy, losing all leaves formed during the spring growth period. The leaves did not turn red, as in the fall; they simply wilted and fell off. Once the rains began, the plants rapidly revived, although flowering was light. One of the interesting aspects of the 1993 late summer growing season at Tusayan was the germination of 30 seedlings in August and September. These were the first seedlings found at this plot during the study.

The onset of winter dormancy is probably triggered by a combination of decreasing day length and lower humidity and soil moisture after the cessation of the summer rains. It is quite variable, depending upon how long the summer rainfall pattern is prolonged into September, or upon the movement of Pacific tropical storms into the Southwest, an event that occasionally produces abundant rain in early fall. The extreme dates for plants entering dormancy observed during this study were in Plot 4 at Tusayan, on September 10, 1992 and about October 15, 1994, as determined by the leaves turning bright red.

The survivorship of adult plants during the five seasons of this study was rather high and the plants may be relatively long lived for an herbaceous perennial species. In Plot 1, 87.5 percent (14 of 16) of adult plants plotted in August, 1990, were still present in September, 1994. In Plot 2, the survivorship was 100 percent (6 of 6) for individuals of *T. validulum* and 68.2 percent (15 of 22) for *T. parviflorum*. In Plot 3, 66.7 percent (14 of 22) of the original adult plants survived and in Plot 4, 82.6 percent (38 of 46) of the original plants were still alive after 4 years.

#### *Germination and Seedling Survival*

Seedlings proved difficult to track, and since they were not all located with coordinates, it was not possible to follow them except for the few that were initially mapped. Germination events were noted every year during the project, in August and/or September 1990, 1991, 1993, and 1994, and in May, 1992. The May event is probably unusual

and followed a rainy period occurring during a usually dry time of year. Warm, wet periods in late summer are probably the normal germination time, but the May event shows that the plants can be opportunistic and germinate when conditions are favorable. Of the five events noted, the first in 1990 was most successful; nearly all germinants of the other four events died within a few months after germinating. Desiccation during periods of drought stress was an important cause of death, but most seedling losses apparently occurred during the winter.

Of the 36 seedlings of the 1990 cohort mapped in Plots 1–3 in August, 1990, three were still alive in September, 1994, a survival rate of 8.3 percent. During 1992, 66 additional plants in the three plots were mapped and assigned plant numbers; these were seedlings, mostly from 1990, that had become well established by the 1992 season. In 1994, seven of the 35 plants that survived flowered, while 47.0 percent of the original 66 were dead. It apparently takes at least 4 years for seedlings to reach reproductive size.

No seedlings were noted for the first 3 years in the Tusayan plot. In 1993, 30 seedlings germinated in August and September; all had died by September, 1994. A few new germinants were noted in late summer, 1994.

Many questions remain about the germination process. It is not known if seeds must overwinter before germinating or if the seedlings first observed in August were from seeds produced that year. The seeds that germinated in May, 1992 must have come from seeds that were produced the previous summer. Several more years would be needed to determine at what age plants first flower and to develop a survival rate from germination to reproduction.

## Conclusions and Management Recommendations

*Talinum validulum* is a narrow edaphic micro-site endemic found in two disjunct areas on the Kaibab National Forest. Intensive surveys since 1990 have greatly extended the known range and abundance of the species, especially on the Tusayan Ranger District. In 1992, Baker and Wright extended the distribution to the Prescott National Forest, reporting thousands of plants, and reporting a possible new population from the southeastern part of the Coconino National Forest in 1995.

Several major proposed developments and projects in the Tusayan area have the potential to

**Comparison of *Talinum* Mean Widths (mm) at Plot 3 for Three Different Years**

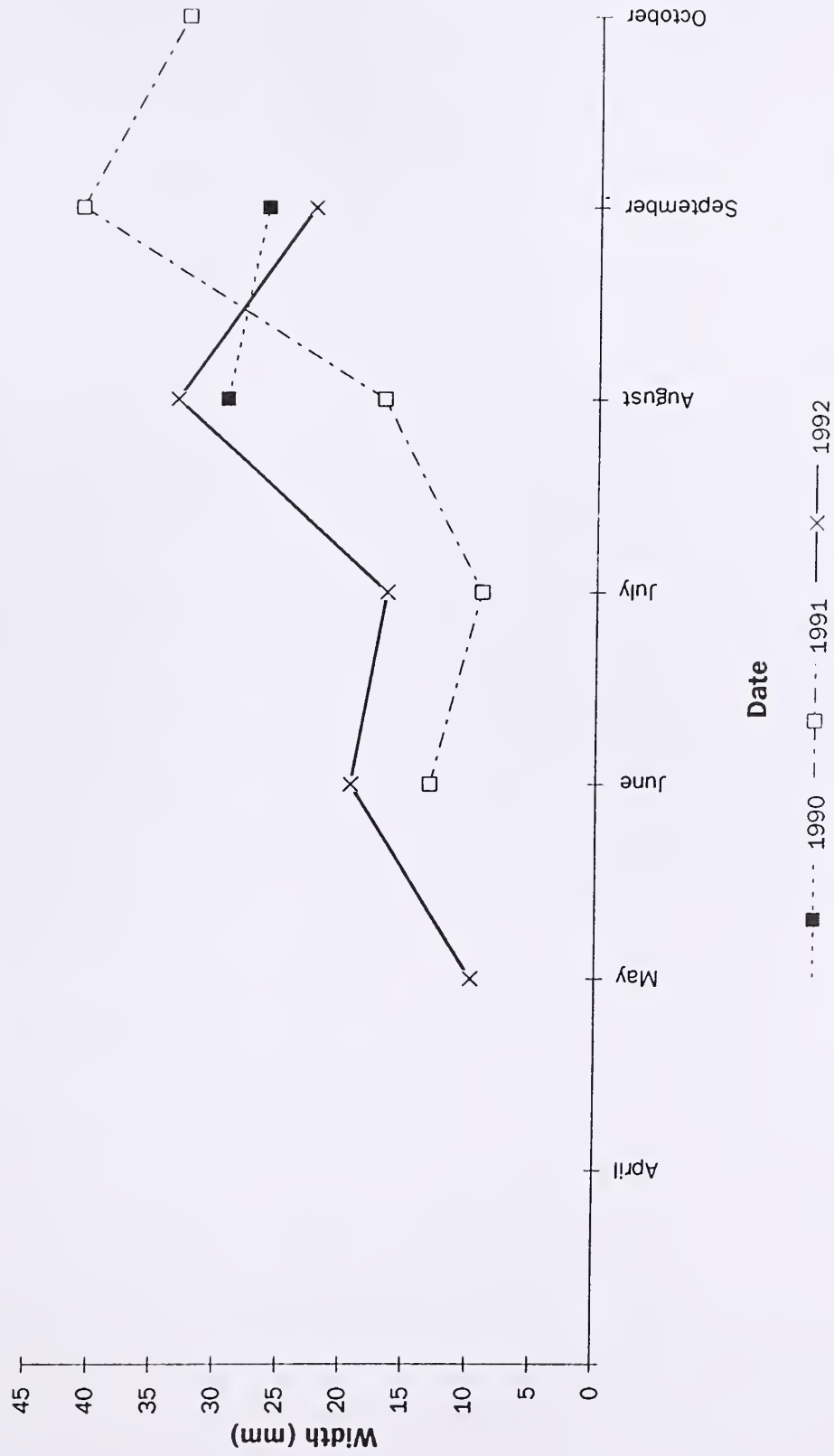


Figure 2. Mean widths of *Talinum validulum* in Plot 3, Pine Flat, 1990-92.

### Comparison of *Talinum* Mean Widths (mm) at Plot 2

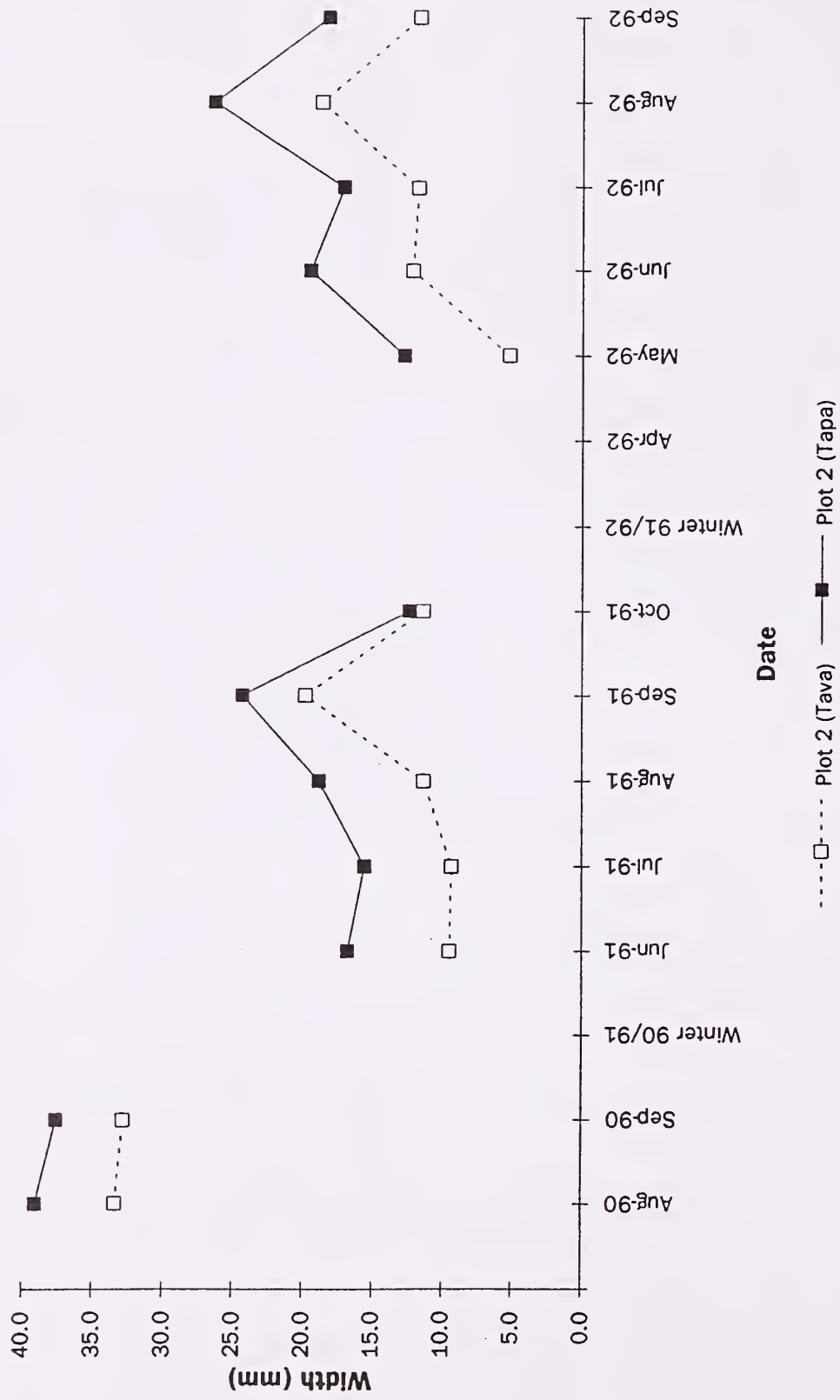


Figure 3. Mean widths of two species of *Talinum* in Plot 2, Pine Flat, 1990-92.

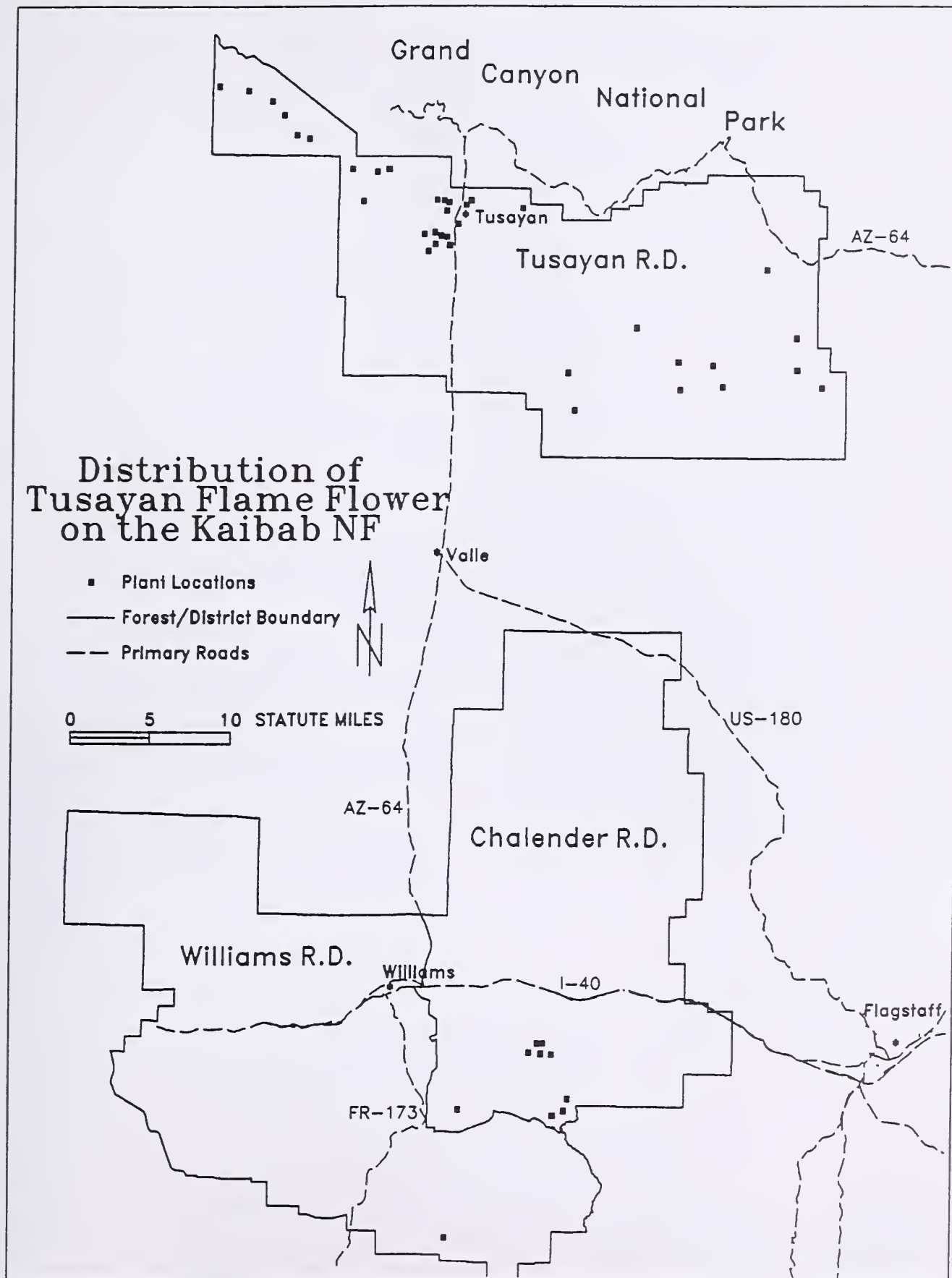


Figure 4. Monitoring and population biology of *Talinum validulum* on the Kaibab National Forest, AZ.

affect populations of the species. These include land exchanges, expansion of the Grand Canyon Railroad, development in the Tusayan village area, and campground and road construction.

While surveys have shown that *T. validulum* is far more abundant than was believed 5 years ago, it remains a narrow endemic restricted to a specialized and very localized habitat. Its abundance on the Kaibab alone is probably not sufficient to remove it from consideration as a Candidate and Forest Service Sensitive species; however, enough plants are known that impacts on a small portion of the range probably will not have a detrimental effect on the species as a whole. Loss of a few individuals is probably insignificant; unavoidable loss of a few hundred will not jeopardize the species; loss of thousands should be avoided. Cumulative effects must be considered with each assessment because of the numerous potential threats to documented sites. Left undisturbed, *Talinum validulum* is likely to remain healthy, vigorous, and naturally self-sustaining.

Because the species is so habitat specific, broad extrapolations of potential population levels over large areas based upon a few plants in a small area should be avoided. If extrapolations are necessary, they should be made conservatively and should be based upon censuses at a number of sample points within the area over which the plants are assumed to occur.

Continued monitoring at the four plots would provide information on the species response to variations in seasonal weather and life history information requiring more years than the length of the current study.

### Acknowledgments

This project was carried out under purchase orders issued by the Kaibab National Forest to Arthur M. Phillips, III. Keith Menasco was project COR and Dr. Barbara Phillips, Forest Service Zone Botanist, supervised the project on an ongoing basis. Sandra Dannenfeldt, Barbara Phillips, Ruth

Phillips, Dr. Clark Franz, and William Schuchman assisted with plot readings. Mimi Murov carried out surveys in the Tusayan and Williams districts. Noel Fletcher shared survey information collected by Tusayan Ranger District staff. Barbara Phillips made available survey data from the Chalender District and Prescott National Forest. Figure 1 was generated by the Kaibab National Forest GIS office; Figures 2–4 were produced by Ken Walters.

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