

Hybridization as a source of evolutionary novelty: leaf shape in a Hawaiian composite

Stacy Jørgensen^{1,2} & Rodney Mauricio³

¹Department of Geography, University of Georgia, Athens, GA 30602, USA; ²Current address: Department of Geography, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA (Phone: +808-956-7526; Fax: +808-956-3512; E-mail: jorgy@hawaii.edu); ³Department of Genetics, University of Georgia, Athens, GA 30602, USA

Received 1 May 2002 Accepted 4 December 2003

Key words: adaptation, Asteraceae, AFLP, gene complexes, Hawaiian Islands, leaf shape, *Lipochaeta tenuifolia*, *Lipochaeta tenuis*, morphological variation, hybridization

Abstract

Hybridization is increasingly recognized as a significant creative force in evolution. Interbreeding among species can lead to the creation of novel genotypes and morphologies that lead to adaptation. On the Hawaiian island of O'ahu, populations of two species of plants in the endemic genus *Lipochaeta* grow at similar elevations in the northern Wai'anae Mountains. These two species represent extremes of the phenotypic distribution of leaf shape: the leaves of *Lipochaeta tenuifolia* individuals are compound and highly dissected while leaves of *L. tenuis* are simple. Based primarily on leaf shape morphology, a putative hybrid population of *Lipochaeta* located at Pu'u Kawiwi was identified. Individuals in this population exhibit a range of leaf shapes intermediate in varying degrees between the leaf shapes of the putative parental species. We analyzed individuals from pure populations of *L. tenuifolia*, *L. tenuis* and the putative hybrids using 133 AFLP markers. Genetic analysis of these neutral markers provided support for the hybrid origin of this population. The correlation between genetic background and leaf morphology in the hybrids suggested that the genome of the parental species with simple leaves might have significantly contributed to the evolution of a novel, compound leaf morphology.

Introduction

The diverse flora and fauna of remote island chains have been studied by evolutionary biologists for many decades (e.g., Darwin, 1859; Mayr, 1942; Carson, 1996; Grant & Grant, 1996). Geographic isolation and founder-mediated speciation have historically been emphasized as the driving forces behind adaptive radiation on these islands (e.g., Weller, Sakai & Straub, 1996). However, there has long been interest in the role of interbreeding among species, or hybridization, as a creative force in evolution (Anderson & Stebbins, 1954; Lewontin & Birch, 1966). Hybridization is increasingly recognized as an evolutionary force that can lead to adaptation through the creation of

novel genotypes and morphologies (Rieseberg, 1995; Arnold, 1997).

Despite its recognition as a recurrent process in the diversification of flowering plants, the importance of hybridization as a general mechanism of evolution driving speciation and adaptation has been and remains unclear (Heiser, 1973; Levin, 1979; Rieseberg, 1991). Many workers have pointed to the fact that early-generation hybrids often exhibit significant reductions in viability and fertility (Barton & Hewitt, 1980; Templeton, 1981), thought to be caused by the disruption of coadapted gene complexes (Dobzhansky, 1951; Mayr, 1963) or by the introduction of maladapted genes (Waser & Price, 1991). Additionally, hybridization may result in the creation of morphologically

intermediate offspring, adapted to neither parental habitat and outcompeted by non-hybrid individuals (Arnold & Hodges, 1995).

Given these findings, it is perhaps not unexpected that the role of hybridization in speciation on islands has historically been considered minor (Humphries, 1979; Ganders & Nagata, 1984; Francisco-Ortega, Jansen & Santos-Guerra, 1996). In fact, contemporary examples of hybridization in the Hawaiian flora, for example, appear to be rare, presumably because the allopatric distribution of species prevents pollen flow (Mayer, 1991). However, there are reasons to suspect that hybridization may, indeed, play a role in plant speciation on oceanic islands. For example, within the Hawaiian flora, a high rate of fertility is often observed in artificially induced interspecific and intergeneric hybrids (Carr, 1995). Examples include a number of groups within the Asteraceae: *Bidens* (Gillet & Lim, 1970), *Tetramolopium* (Lowrey, 1986), and the silversword alliance (Carr & Kyhos, 1981), which are known to hybridize freely in the few locations where different species co-occur (Caraway, Carr & Morden, 2001). Furthermore, non-concordance between nuclear- and organelle-derived phylogenies of groups such as the silversword alliance (Baldwin, Kyhos & Dvorák, 1990) and the Drosophilidae (DeSalle & Giddings, 1986) are generally interpreted as indicative of a role for hybridization in the diversification of these groups. These findings, along with the general lack of post-zygotic genetic barriers to hybridization among congeners, makes the fact that hybridization has been generally discounted as a factor in adaptive radiation in island settings surprising (Crawford, Whitkus & Stuessy, 1987).

In this study, we examined a putative example of natural hybridization in plants from the Hawaiian Islands. On the island of O'ahu, two species of plants in the Hawaiian endemic genus *Lipochaeta* (family Asteraceae) grow in the northern Wai'anāe Mountains: *Lipochaeta tenuifolia* and *L. tenuis*. Both species are found at similar elevations in mesic forest, with *L. tenuifolia* found in the extreme northern portion of the mountain range and *L. tenuis* known from locations to the south. Individual species of *Lipochaeta* have diverged in a number of vegetative and floral traits, including leaf shape. *Lipochaeta tenuifolia* and *L. tenuis* represent the extremes in the genus with regard to leaf

shape: the leaves of *L. tenuifolia* are compound and highly dissected, while the leaves of *L. tenuis* are simple. A population of *Lipochaeta* in the northern Wai'anāe Mountains has been hypothesized to be of hybrid origin because individuals within the population possess a variety of leaf morphologies intermediate between those characteristic of *L. tenuifolia* and *L. tenuis* (J. Lau, Hawai'i Natural Heritage Program, pers. comm.). Our primary objective in this study was to use genetic markers to test the hypothesis that the population of *Lipochaeta* in the northern Wai'anāe Mountains is of hybrid origin. Furthermore, within this putative hybrid population, we were interested in identifying correlations between leaf shape and the parental origin of our genetic markers.

Materials and methods

Study species

Lipochaeta DC (Asteraceae) is an endemic Hawaiian genus of about 20 species of primarily suffruticose perennials (Wagner, Herbst & Sohmer, 1990); two sections, based on morphology and cytology (*Lipochaeta*, $n = 26$, four-petaled disk florets; *Aphanopappus*, $n = 15$, five-petaled disk florets), are recognized within the genus. Artificial hybrids can be induced in crosses within and between sections (Rabakonandrianina, 1980), and between *Lipochaeta* and *Wollastonia biflora* ($n = 15$), the presumed progenitor of *Lipochaeta* (Rabakonandrianina & Carr, 1981). Although the exact relationship between the two sections is unclear, section *Lipochaeta* likely arose from a hybridization event involving a member of section *Aphanopappus* and another member of the genus *Wollastonia* (Gardner, 1977; Chumley et al., 2002).

Members of section *Aphanopappus* ($n = 14$, of which 11 are extant) are distributed in a classic adaptive radiation pattern; all but two species are single-island endemics (Wagner & Robinson, 2001). Individual species have diverged in vegetative and floral morphology including leaf shape, growth habit, and the color, number, and size of ray florets. Natural hybridization within the group appears to be uncommon (Gardner, 1979) but not unknown (Wagner Herbst & Sohmer, 1990). Heretofore, reports of natural hybridization within *Lipochaeta* were based solely on morphological

descriptions of intermediacy rather than the genetic criteria we are employing.

Field sampling and laboratory techniques

Individuals were sampled from naturally growing populations of *L. tenuifolia*, *L. tenuis*, and the putative hybrid population, which was assumed to be composed entirely of hybrid individuals (Figure 1); sample sizes were five, three, and 13 individuals, respectively. Two leaves were collected per individual and placed in plastic bags with desiccating silica gel. Each individual collected in the hybrid population was assigned to a leaf shape class (Figure 2): 1, *L. tenuis*-type, deltate; 2, deltate with basal lobes; 3, deltate with several distinctive

lobes; 4, deltate with numerous lobes and some further dissection of lobes; 5, very highly dissected with numerous lobes and sub-lobes, but less dissected than the parental species *L. tenuifolia*.

Leaves were crushed by vortexing with ball bearings (Colosi & Schaal, 1993), and total genomic DNA was extracted according to a standard phenol-chloroform procedure (Sambrook, Fritsch & Maniatis, 1989). Following phenol extraction, DNA was precipitated with ethanol and resuspended in deionized water to an approximate concentration of 50 ng/μl. Amplified fragment length polymorphism (AFLP) fragments (Vos et al., 1995) were detected using standard kits available from Applied Biosystems (ABI). A restriction-ligation was conducted with the enzymes *EcoRI* and *MseI* and enzyme-specific ligators from the preselective amplification kit (ABI part # 402004). Following ligation, two rounds of PCR were conducted. During preselective amplification, a single nucleotide was added to the 3' end of the primers; the preselective product was diluted to serve as the template for the subsequent selective amplifications. During selective amplification, two additional nucleotides were added to the primers, and the *EcoRI* primer was fluorescently labeled to permit fragment detection. Six *EcoRI-MseI* primer-pair combinations were used for selective amplification (listed by the additional nucleotides added): ACA-CAT, ACA-CTT, ACG-CTG, ACT-CTG, ACC-CAT, and, AGG-CTT.

Fragments were separated by electrophoresis using 4.75% polyacrylamide gels on an ABI 377 sequencer. A ROX-500 fluorescently labeled size standard was loaded with each sample during electrophoresis to permit fragment-size determination. The software package GeneScan® (version 3.1, Applied Biosystems) was used to visualize the gels and determine fragment size by interpolating to the ROX-500 standard (ABI #401734) loaded

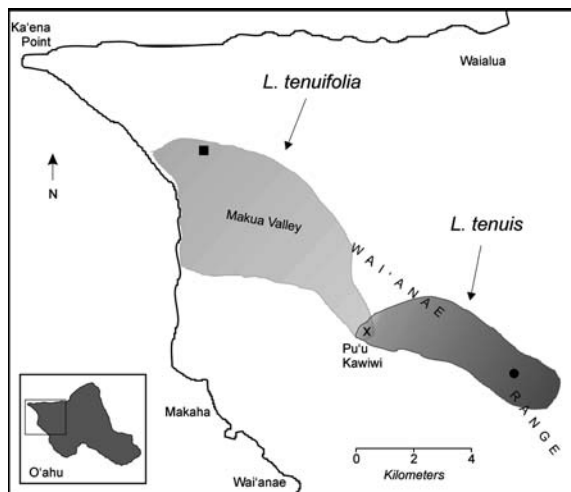


Figure 1. Distribution of *Lipochaeta tenuifolia* and *L. tenuis* and the location of a putative hybrid population in the northern Wai'anae Mountains, O'ahu. The locations of the populations sampled from the parental taxa are indicated by a closed square for *L. tenuifolia* and a closed circle for *L. tenuis*; the hybrid population is indicated by a \times . Species distributions were extrapolated from occurrences in the Hawai'i Natural Heritage Program database.

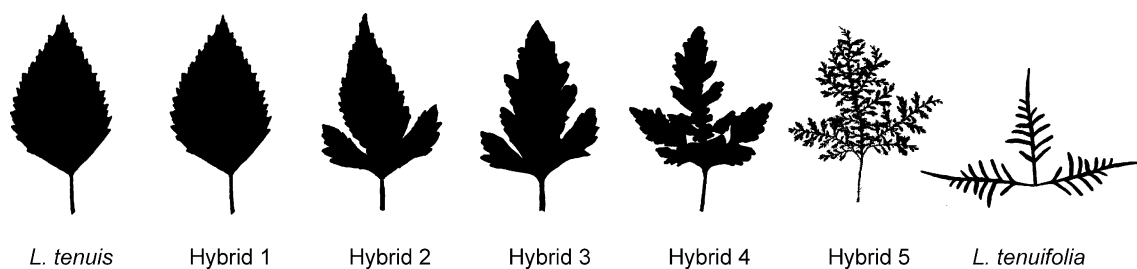


Figure 2. Variation in leaf shape among *L. tenuifolia*, *L. tenuis* and their putative hybrids.

with each sample, which permitted the analysis of fragments between 70 and 450 bp. Each differentially sized fragment was considered a single gene locus, and individuals were scored by the presence or absence of the indicated fragment.

Data analysis

We analyzed the AFLP data to quantify genetic diversity in the parental and putative hybrid populations and to examine individual plant genotypes for correlations among fragments and overall genetic similarity among individuals. Three assumptions were necessary for these analyses: (1) Mendelian segregation of polymorphic fragments, (2) allelic identity of same-size fragments, and (3) the existence of a single dominant (amplified) and recessive (null) allele at each locus. The calculation of standard measures of genetic diversity and structure required the additional assumption of Hardy–Weinberg proportions within populations (Travis, Maschinski & Keim, 1996). Genetic diversity within each of the three groups was assessed by the percentage of polymorphic loci (P) and heterozygosity (H). A locus was considered polymorphic if its associated fragment did not occur in every individual analyzed. Heterozygosity at each locus was estimated from the equation $H = 1 - [(1 - q)^2 + q^2]$ where q^2 is the frequency of individuals in which a fragment was absent; total heterozygosity was calculated as the mean heterozygosity among loci.

When a large number of loci are examined, there are likely to be non-independent associations

among loci. Traditional analyses of genetic structure, which are based on a locus-by-locus approach, are unlikely to reveal the effects of such associations or linkages (Edwards, 2003); also, traditional analyses of genetic structure require *a priori* divisions into groups. Therefore, the relationships among individuals sampled from the three populations were analyzed via principal components analysis (PCA). This analysis was selected because the components generated by the analysis will reflect correlations among fragments in their presence or absence (i.e., non-independence) and because divisions into groups are not required (Wiley, 1981; Caraway, Carr & Morden, 2001). All loci were used for the analysis; however, only those individuals/samples for which all six primer-pair combinations were resolved were included in the PCA analysis; calculations were conducted with the software package PC-ORD (McCune & Mefford, 1999).

Results

The six primer pairs yielded 133 AFLP fragments among all individuals. Well over half (61%) of the fragments were shared by the parental species (Table 1). Four unique fragments (i.e., also absent from hybrids) were detected in each *L. tenuifolia* and *L. tenuis*. Fixed differences between the parental species were detected at only two loci; in both cases, the fragments were present in *L. tenuis* and absent in *L. tenuifolia*. Twenty-two fragments were detected in only *L. tenuifolia* and the hybrids, and nine were shared by only *L. tenuis* and the

Table 1. Summary of AFLP markers analyzed in *Lipochaeta tenuifolia*, *L. tenuis*, and their putative hybrids. One hundred thirty-three markers were detected among all sampled individuals

AFLP markers	<i>L. tenuifolia</i> × <i>L. tenuis</i>		
	<i>L. tenuifolia</i>	<i>L. tenuis</i>	<i>L. tenuis</i>
Total number ^a	107	94	124
Constant markers	17	47	15
Polymorphic markers	90	47	109
Shared by both parental species	81	81	–
Constant in both parental species	12	12	–
Shared by parent and hybrid	102	89	–
Absent in other parent	22	9	–
Unique to species or hybrid	4	4	13

^aNumber of fragments present in at least one individual of the group.

hybrids. A single fragment was shared by the parental species but was absent in the hybrids. In contrast, 13 fragments detected in the hybrids were absent from both parental species.

The number of polymorphic markers varied substantially between the parental species. Ninety (84%) of the fragments detected in *L. tenuifolia* were polymorphic, while only 47 (50%) polymorphic fragments occurred in *L. tenuis*. The putative hybrids possessed the greatest number (109) of polymorphic fragments. Heterozygosity, as averaged across all 133 loci, also was greatest in the putative hybrid population ($H = 0.30$). Heterozygosity in *L. tenuifolia* was, at $H = 0.24$, almost twice the level observed in *L. tenuis* ($H = 0.13$) (Table 2).

The three groups largely segregated into discrete groups along the first two principal

component axes (Figure 3), which accounted for 27 and 18% of the variance observed in the total data set, respectively. Individuals of the two parental species largely segregated from individuals from the hybrid population along the first principal component. Notably, this division was not complete: individuals of the most highly dissected leaf shape (hybrid 5) clearly segregated with individuals of the simple-leafed parent. Individuals of the two parental species segregated from one another along the second principal component. Again, hybrid 5 individuals, which are morphologically most similar to *L. tenuifolia*, segregated with *L. tenuis*.

Discussion

Evidence for hybridization

The AFLP data presented here strongly suggest a *L. tenuifolia* \times *L. tenuis* hybrid origin for the population at Pu'u Kawiwi. As would be expected in a hybrid population, the Pu'u Kawiwi population contained a mix of the AFLP fragments detected in the parental taxa (Rieseberg, 1991); in fact, virtually all the fragments detected in the parental species were also found in the hybrid population.

Only 13 of the 133 fragments detected in the putative hybrids were absent from both parental species, although it is likely that sampling error could explain this discrepancy. Only one population each was sampled from the parental species, and these populations were located well away from Pu'u Kawiwi; it is possible that populations of *L. tenuifolia* and *L. tenuis* closer to the hybrid population might contain these fragments. The failure to detect these fragments could also indicate another species of *Lipochaeta* has been involved in the formation of the hybrid population. Two other species of diploid *Lipochaeta* are known from extreme northwestern O'ahu locations; however, these species are known from coastal (*L. integrifolia*) and lowland (*L. remyi*) locations fairly removed from the mesic forest locales of *L. tenuifolia* and *L. tenuis*.

In addition to possessing fragments from both the parental species, the greater percentage of polymorphic loci and higher levels of heterozygosity found in the putative hybrid population are

Table 2. Sample sizes, percent polymorphic loci, and heterozygosity calculated in *L. tenuifolia*, *L. tenuis*, and their hybrids determined from 133 AFLP loci

Species	<i>N</i>	<i>P</i>	<i>P'</i>	<i>H_e</i>
<i>L. tenuifolia</i>	5	67.7	84.1	0.238
<i>L. tenuis</i>	3	35.3	50.0	0.131
Hybrids	13	82.0	87.9	0.300

The percentage of polymorphic loci was calculated using all fragments (*P*) and only those fragments actually occurring within each group (*P'*).

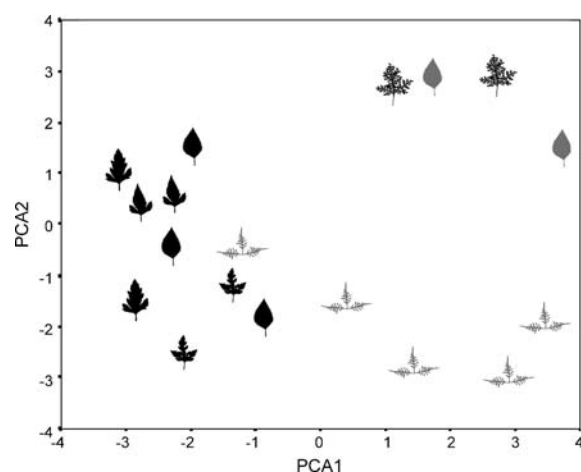


Figure 3. PCA of AFLP data using all scored fragments. Individuals are depicted by their leaf shape as shown in Figure 2; hybrids are shown in black, while individuals of the parental species are shown in gray.

also consistent with the hybrid origin hypothesis. Although there were virtually no apparent fixed differences between the parental species, *L. tenuifolia* and *L. tenuis* have diverged in their allele frequencies at many loci. Crosses of the two parental taxa would result in a greater number of polymorphic loci, more even allele frequencies and therefore higher levels of heterozygosity in populations consisting of hybrid individuals.

Fixed differences between parental species would have made possible a determination of whether the population at Pu'u Kawiwi consists of early or late generation hybrids, backcrossed individuals or some combination of these crosses. For example, fixed differences between the silver-sword alliance members *Dubautia ciliolata* and *D. scabra* allowed Caraway, Carr and Morden (2001) to conclude that many individuals in a hybrid population from lava flows on the island of Hawai'i represented later generation backcrosses to *D. ciliolata*. The lack of fixed differences between *L. tenuifolia* and *L. tenuis*, precludes this analysis, however. Genetically, most individuals in the population appear intermediate or equally similar to *L. tenuifolia* and *L. tenuis*, which would seem to argue for a large occurrence of F₁ individuals. However, the varying leaf morphologies found in the population and the genetic identities of the hybrid individuals with the most highly dissected leaf pattern are inconsistent with this explanation.

Morphology and genetics uncoupled?

The hybrid individuals show a variety of intermediate leaf morphologies that are distinctly different from those of the parental species. In an F₁ hybrid population, a single, intermediate leaf morphology would be expected (Rieseberg, 1991) if loci contributing to leaf shape act additively. Later generation hybrid crosses or backcrosses could generate a variety of leaf forms as segregation occurs among loci. Overall, the hybrid individuals were genetically intermediate to the parental species, but there was variation in the degree of genetic similarity to the parental species with regard to the various leaf morphologies. Most strikingly, those individuals with the most highly dissected leaf morphologies, that is, most resembling *L. tenuifolia*, were genetically very similar to *L. tenuis*. In other words, a *L. tenuifolia*-like leaf

morphology was present with a *L. tenuis*-like genetic background. Obviously, this conclusion is tempered by the very small number of hybrid five individuals we were able to sample from this small, natural population. However, cautiously taking the result at its face value, it suggests that genes from a simple-leafed parent, segregating in novel hybrid genomes, might play a role in generating a highly dissected leaf shape.

Such uncoupling of genetics and morphology is not unusual in hybrids. For example, present-day varieties of cultivated cotton are tetraploid, but are derived from two distinct diploid parental species (Jiang et al., 1998). Surprisingly, QTL that contribute to fiber quality were found to come from the diploid parent species that possesses no spinnable fiber on its seeds, suggesting a non-additive interaction between the two parental genomes affecting seed fiber quality. As in the cotton example, our present study illustrates that the merger of genomes with divergent evolutionary histories can produce 'unique avenues' for selection (Anderson & Stebins 1954; Jiang et al., 1998; Wright et al., 1998).

Backcrossing to the *L. tenuis* parent could explain how individuals within the hybrid population have become genetically almost identical to that parental species. Although their status as 'pure' may be questionable, populations identified as *L. tenuis* do occur near Pu'u Kawiwi; pollen flow from these populations is a likely mechanism of backcrossing. Although individuals genetically similar to *L. tenuis* could theoretically arise by later generation crosses among hybrids (i.e., not involving backcrossing), this mechanism seems unlikely given the very small hybrid population size (tens of individuals). Only a very small percentage of late-generation filial hybrids would randomly end up with a predominantly *L. tenuis* genetic make-up, and there is no reason to expect that all these individuals would possess the dissected leaf morphology similar to that of *L. tenuifolia*. In fact, one would predict such advanced generation hybrid individuals to possess an external morphology virtually indistinguishable from *L. tenuis*. It is highly unlikely, then, that the pairing of the external morphology of *L. tenuifolia* with the genetic background of *L. tenuis* would arise by chance alone, making selection the best explanation for this pattern.

In fact, different classes of hybrids may have varying levels of fitness (Arnold & Hodges, 1995),

with selection often favoring the native phenotype (Nagy, 1997). Based on this prediction and given populations of *L. tenuis* near Pu'u Kawiwi, hybrid individuals with entire leaves would be expected to possess the highest levels of fitness. However, native phenotypes do not always possess the highest fitness, and there are scenarios under which a non-native phenotype could be selected. For example, Nagy (1997) examined a variety of morphological traits, including leaf shape, petal shape, and petal color, in F₂ individuals created by crossing individuals from two subspecies of the annual plant *Gilia capitata* occurring in coastal and inland habitats in California. For all traits except leaf shape, native phenotypes were favored; the inland leaf shape, with fewer lobes or dissection, was favored at both locations.

Adaptive significance of leaf shape

Leaf shape itself has long been recognized as a trait of adaptive significance (e.g., Raschke, 1960; Givnish, 1979). In particular, leaf dissection appears correlated with environmental characteristics, with highly dissected leaves often favored in dry, sunny habitats, because the leaves are less likely to become overheated (Gurevitch, 1988). In addition to having significance with regard to the abiotic environment, leaf shape has also been shown to have adaptive significance with regard to interspecific interactions. For example, differences in leaf shape among closely related species with similar geographic ranges may be a response to avoid predation by herbivorous insects (Gilbert, 1975). Rausher (1978) demonstrated that females of the pipevine swallowtail butterfly *Battus philenor* discriminated between broad- and narrow-leaved *Aristolochia* when searching for specific plants on which to oviposit.

It is worth noting that hybrid individuals of *Lipochaeta* with the most highly dissected leaves were clearly morphologically distinct from both of the typical parental leaf morphologies. Furthermore, that such variation in leaf shape occurs between the parental species, which occur in similar habitats (i.e., mid-elevation mesic forest), suggests a selective pressure other than simple environmental conditions. Arthropods comprise over 75% of the Hawaiian fauna, and many are highly host specific (Roderick & Gillespie, 1998). Co-evolution with arthropods has been suggested as an important factor in the diversification of the

silversword alliance (Roderick, 1997). If leaf shape in *Lipochaeta* is, in part, driven by herbivory a novel leaf shape might have a selective advantage over either parental phenotype. Concordant with this hypothesis is the leaf shape variety of *Lipochaeta* present when multiple diploid species occur on a single island. For example, on Kaua'i *L. fauriei* (entire, deltate), *L. waimeaensis* (entire, elongated), and *L. micrantha* (highly dissected) all have very different leaf morphologies, and these morphologies are not consistent with the general predictions based on the physical environment alone: *Lipochaeta waimeaensis* occurs on dry, exposed slopes within Waimea Canyon while *L. micrantha* is a forest species.

Although the diversity of leaf shape in the hybrid population seems remarkable, the genetic basis of transition between simple and compound leaves is well understood (Sinha, 1997). In fact, the transition between simple and compound leaves in the hybrid population is remarkably similar in appearance to induced mutants in leaf morphology known in the cultivated tomato, *Solanum esculentum* (Kessler et al., 2001). In the tomato model system, whether a plant makes complex, divided leaves or simple ones is controlled by KNOTTED1-like (KNOXI) homeobox genes (Bharathan & Sinha, 2001). This group of genes is found in most plants; they are switched on in the leaves of all plants with complex leaves but are inactive in plants with simple leaves (Bharathan et al., 2002). A single gene, *PHANTASTICA* (PHAN) controls whether a leaf is pinnate or palmate (Kim et al., 2003). Although the genetic basis of leaf shape seems remarkably simple considering the complexity of the phenotype, even simple leaves can begin development as 'complex' primordia (Bharathan et al., 2002). Certainly, the molecular genetic studies of leaf shape illustrate that small genetic changes can lead to the generation of great morphological diversity. It seems likely that an analysis of KNOXI gene expression in *Lipochaeta*, and in the hybrid population specifically, would yield interesting results.

Conclusions

DNA markers are powerful tools for the confirmation of hybridization within plant species, and, in fact, are necessary to assess the contribution of each parental taxon to the hybrid population. The

leaf morphologies of *L. tenuifolia* and *L. tenuis* represent the ends of a continuum found within the genus, and hybrids between the two species yield individuals with a variety of intermediate morphologies. In fact, the variety of leaf morphologies found in the *L. tenuifolia* × *L. tenuis* hybrid population at Pu'u Kawiwi is indicative of later generation hybrids or backcrosses. The genetic composition of the hybrid individuals could not be predicted from their vegetative morphology. Further studies of this hybrid population should include controlled crosses between the parental taxa; these crosses could yield important information about the number of genes controlling leaf morphology and whether epistatic interactions among loci may affect leaf morphology.

Acknowledgements

We thank K. Kawelo, J. Lau and J. Rohrer for assistance with field collections. M. Arnold, A. Bouck and K.C. Parker provided helpful comments that improved the manuscript. This research was supported by grants from the National Science Foundation (9901022) and the Biogeography Specialty Group of the Association of American Geographers to SJ and National Science Foundation (0129191) to RM.

References

- Anderson, E. & G.L. Stebbins, 1954. Hybridization as an evolutionary stimulus. *Evolution* 8: 378–388.
- Arnold, M.L., 1997. *Natural Hybridization and Evolution*. Oxford University Press, New York.
- Arnold, M.L. & S.A. Hodges, 1995. Are natural hybrids fit or unfit relative to their parents? *Trends Ecol. Evol.* 10: 67–71.
- Baldwin, B.G., D.W. Kyhos & J. Dvorák, 1990. Chloroplast DNA evolution and adaptive radiation in the Hawaiian silversword alliance (Asteraceae-Madiinae). *Ann. MO. Bot. Gard.* 77: 96–109.
- Barton, N.H. & G.M. Hewitt, 1980. Hybrid zones and speciation, pp. 109–145 in *Evolution and Speciation. Essays in Honor of M.J.D. White*, edited by W.R. Atchley & D.S. Woodruff. Cambridge University Press, Cambridge.
- Bharathan, G. & N.R. Sinha, 2001. The regulation of compound leaf development. *Plant Physiol.* 127: 1533–1538.
- Bharathan, G., T.E. Goliber, C. Moore, S. Kessler, T. Pham & N.R. Sinha, 2002. Homologies in leaf form inferred from KNOX1 gene expression during development. *Science* 296: 1858–1860.
- Caraway, V., G.D. Carr & C.W. Morden, 2001. Assessment of hybridization and introgression in lava-colonizing Hawaiian *Dubautia* (Asteraceae: Madiinae) using RAPD markers. *Am. J. Bot.* 88: 1688–1694.
- Carr, G.D., 1995. A fully fertile intergeneric hybrid derivative from *Argyroxiphium sandwicense* ssp. *macrocephalum* × *Dubautia menziesii* (Asteraceae) and its relevance to plant evolution in the Hawaiian Islands. *Am. J. Bot.* 82: 1574–1581.
- Carr, G.D. & D.W. Kyhos, 1981. Adaptive radiation in the silversword alliance (Compositae-Madiinae) I. Cytogenetics of spontaneous hybrids. *Evolution* 35: 543–556.
- Carson, H.L., 1996. Pacific Basin biotas and evolutionary theory, pp. 7–17 in *The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes*, edited by A. Keast & S.E. Miller. SPB Academic Publishing, Amsterdam.
- Chumley, T.W., S.C. Keeley, J. Panero & R.K. Jansen, 2002. A phylogeny of *Lipochaeta* (Asteraceae) inferred from the internal and external transcribed spacers of nuclear ribosomal DNA. Supplement to *Am. J. Bot.* 89(6).
- Colosi, J.C. & B.A. Schaal, 1993. Tissue grinding with ball bearings and a vortex mixer. *Nucl. Acids Res.* 21: 1051–1052.
- Crawford, D.J., R. Whitkus & T.F. Stuessy, 1987. Plant evolution and speciation on oceanic islands, pp. 183–199 in *Differentiation Patterns in Higher Plants*, edited by K. Urbanska. Academic Press, London.
- Darwin, C., 1859. *On the Origin of Species by Means of Natural Selection*. John Murray, London.
- DeSalle, R. & L.V. Giddings, 1986. Discordance of nuclear and mitochondrial DNA phylogenies in Hawaiian *Drosophila*. *Proc. Natl. Acad. Sci. USA* 83: 6902–6906.
- Dobzhansky, T., 1951. *Genetics and the Origin of Species*, Third edition. Columbia University Press, N.Y.
- Edwards, A.W.F., 2003. Human genetic diversity: Lewontin's fallacy. *BioEssays* 25: 798–801.
- Francisco-Ortega, F., R.K. Jansen & A. Santos-Guerra, 1996. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *Proc. Natl. Acad. Sci. USA* 93: 4085–4090.
- Ganders, F.R. & K.M. Nagata, 1984. The role of hybridization in the evolution of *Bidens* on the Hawaiian Islands, pp. 179–194 in *Plant Biosystematics*, edited by W.F. Grant. Academic Press, Toronto.
- Gardner, R.C., 1977. Chromosome numbers and their systematic implications in *Lipochaeta* (Compositae: Heliantheae). *Am. J. Bot.* 64: 810–813.
- Gardner, R.C., 1979. Revision of *Lipochaeta* (Compositae: Heliantheae) of the Hawaiian Islands. *Rhodora* 81: 291–339.
- Gilbert, L.E., 1975. Ecological consequences of a coevolved mutualism between butterflies and plants, pp. 210–240 in *Coevolution of Animals and Plants*, edited by L.E. Gilbert & P.H. Raven. University of Texas Press, Austin.
- Gillet, G.W. & E.K.S. Lim, 1970. An experimental study of the genus *Bidens* (Asteraceae) in the Hawaiian Islands. *Univ. Calif. Publ. Bot.* 56: 1–63.
- Givnish, T.G., 1979. On the adaptive significance of leaf form, pp. 375–401 in *Topics in Plant Population Biology*, edited by O.T. Solbrig, S. Jain, G.B. Johnson & P.H. Raven. Columbia University Press, New York.

- Grant, P.R. & B.R. Grant, 1996. Speciation and hybridization in island birds. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351: 765–772.
- Gurevitch, J., 1988. Variation in leaf dissection and leaf energy budgets among populations of *Achillea* from an altitudinal gradient. *Am. J. Bot.* 75: 1298–1306.
- Heiser, C.B., 1973. Introgression re-examined. *Bot. Rev.* 39: 347–366.
- Humphries, C.J., 1979. Endemism and evolution in Macaronesia, pp. 171–199 in *Plants and Islands*, edited by D. Bramwell. Academic Press, New York.
- Jiang, C.X., R.J. Wright, K.M. El-Zik & A.H. Paterson, 1998. Polyploid formation created unique avenues for response to selection in *Gossypium* (cotton). *Proc. Natl. Acad. Sci. USA* 95: 4419–4424.
- Kessler, S., M. Kim, T. Pham, N. Weber & N.R. Sinha, 2001. Mutations altering leaf morphology in tomato. *Int. J. Plant Sci.* 162: 475–492.
- Kim, M., S. McCormick, M. Timmermans & N. Sinha, 2003. The expression domain of *PHANTASTICA* determines leaflet placement in compound leaves. *Nature* 424: 438–443.
- Levin, D.A., 1979. Introduction, pp.1–6 in *Hybridization: An Evolutionary Perspective*, edited by D.A. Levin. Dowden, Hutchinson, and Ross, Stroudsburg.
- Lewontin, R.C. and L.C. Birch, 1966. Hybridization as a source of variation for adaptation to new environments. *Evolution* 20: 315–336.
- Lowrey, T.K., 1986. A biosystematic revision of Hawaiian *Tetramolopium* (Compositae: Astereae). *Allertonia* 4: 203–265.
- Mayer, S. S., 1991. Artificial hybridization in Hawaiian *Wikstroemia* (Thymelaeaceae). *Am. J. Bot.* 78: 122–130.
- Mayr, E., 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.
- Mayr, E., 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge, Massachusetts.
- McCune, B. & M.J. Mefford, 1999. PC-ORD. Multivariate Analysis of Ecological Data. Version 4.01. MjM Software, Gleneden Beach, Oregon.
- Nagy, E.S., 1997. Selection for native characters in hybrids between two locally adapted plant subspecies. *Evolution* 51: 1469–1480.
- Rabakonandrianina, E., 1980. Intrageneric relationships and the origin of the Hawaiian endemic genus *Lipochaeta* (Compositae). *Pac. Sci.* 34: 29–39.
- Rabakonandrianina, E. & G. D. Carr, 1981. Intergeneric hybridization, induced polyploidy, and the origin of the Hawaiian endemic *Lipochaeta* from *Wedelia* (Compositae). *Am. J. Bot.* 68: 206–215.
- Raschke, K., 1960. Heat transfer between the plant and the environment. *Annu. Rev. Plant Physiol.* 11: 111–126.
- Rausher, M.D., 1978. Search image for leaf shape in a butterfly. *Science* 200: 1071–1073.
- Rieseberg, L.H., 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *Am. J. Bot.* 78: 1218–1237.
- Rieseberg, L.H., 1995. The role of hybridization in evolution: old wine in new skins. *Am. J. Bot.* 82: 944–953.
- Roderick, G.K., 1997. Herbivorous insects and the Hawaiian silversword alliance: coevolution or cospeciation? *Pac. Sci.* 51: 440–449.
- Roderick, G. K. & R. G. Gillespie, 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. *Mol. Ecol.* 7: 519–531.
- Sambrook, J, E.F. Fritsch & T. Maniatis, 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.
- Sinha, N., 1997. Simple and compound leaves: reduction or multiplication? *Trends Plant Sci.* 2: 396–402.
- Templeton, A.R., 1981. Mechanisms of speciation – a population genetic approach. *Annu. Rev. Ecol. Syst.* 12: 23–48.
- Travis, S. E., J. Maschinshi & P. Keim, 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. *Mol. Ecol.* 5: 735–745.
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijtens, J. Pot, J. Peleman, M. Kuiper & M. Zabeau, 1995. AFLP: a new technique for DNA fingerprinting. *Nuc. Acids. Res.* 23: 4407–4414.
- Wagner, W.L. & H. Robinson, 2001. *Lipochaeta* and *Melanthera* (Asteraceae: Heliantheae subtribe Ecliptinae): establishing their natural limits. *Brittonia* 53: 539–561.
- Wagner, W.L., D.R. Herbst & S.H. Sohmer, 1990. *Manual of the Flowering Plants of Hawai'i*. University of Hawaii Press and Bishop Museum Press, Honolulu.
- Waser, N.M. & M.K. Price, 1991. Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes, and seed set. *Ecology* 72: 171–179.
- Weller, S.G., A.K. Sakai & C. Straub, 1996. Allozyme diversity and genetic identity in *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae) in the Hawaiian Islands. *Evolution* 50: 23–34.
- Wiley, E.O., 1981. *Phylogenetics. The Theory and Practice of Phylogenetic Systematics*. John Wiley and Sons, New York.
- Wright, R.J., P.M. Thaxton, K.M. El-Zik & A.H. Paterson, 1998. D-subgenome bias of *Xcm* resistance genes in tetraploid *Gossypium* (cotton) suggests that polyploid formation has created novel avenues for evolution. *Genetics* 149: 1987–1954.