THE URBAN WILDLANDS GROUP

Captive Rearing of Lange's Metalmark Butterfly, 2011–2015

Jana J. Johnson[1,2], Tyler Wilson[2], Cory Taylor[2], Yunfei Xia[2], Jane Jones,[2] Ken Osborne,[3] and Travis Longcore[1,4]

[1] *The Urban Wildlands Group*

[2] *The Butterfly Project, Moorpark College*

[3] *Osborne Biological Consulting*

[4] *University of Southern California*

February 10, 2016

Report to: United States Fish and Wildlife Service, CVPIA Habitat Restoration Program

Project: *Captive Rearing and Release to Support Recovery of Lange's Metalmark Butterfly at Antioch Dunes National Wildlife Refuge*

Dates: October 2011–September 2015

Agreement No.: F11AP00168

The Urban Wildlands Group
P.O. Box 24020
Los Angeles, California 90024-0020

Table of Contents

Abstract

The federally endangered Lange's metalmark butterfly underwent a substantial population decline at its sole habitat at the Antioch Dunes National Wildlife Refuge (ADNWR) leading up to starting a captive breeding program for the species in 2007. Despite some success and release of captive-bred butterflies back to the wild, the population has not yet recovered and the captive breeding program continues as insurance against extinction and to augment and reintroduce butterflies in habitats at the ADNWR. This report documents the captive breeding program undertaken from fall 2011 through fall 2015, through the U.S. Fish and Wildlife Service's (USFWS), Central Valley Project Improvement Act, Habitat Restoration Program (HRP) Grant No. F11AP00168. During this time, adult butterflies were collected from the wild each year. Captive breeding was attempted and mating was observed but a self-sustaining captive population has not been achieved. In 2011, 5 female metalmarks were captured, 2 larvae released, and 4 females mated in captivity. In 2012, 5 female metalmarks were captured, 79 pupae and 23 adults were released, and 9 females mated in captivity. In 2013, 3 females were obtained, 28 pupae and 15 adults released, and 4 females mated in captivity. In 2014, 4 females were captured, 1 larvae, 7 pupae, and 2 adults were released, and 3 females were mated in captivity. The production of the program seems to be related to the ongoing drought because it depends on the capture of wild butterflies to provide eggs for each year. The drought is probably affecting both the fecundity of wild metalmarks and the timing of their flight because foodplants and nectar sources have been growing and senescing earlier in the season. Through the captive program we have learned that: under laboratory conditions male butterflies emerge slightly before females (protandry), female butterflies weigh slightly more than males, and hatch rate for

eggs from wild-caught females (foundresses) was higher than for captive-bred females. The hatching of eggs occurs in February (not fall as suggested by the literature), this leaves November and December as prime time to be active in the habitat as all live individuals are eggs. Captive mating has only been successful in small containers, with multiple aging males and a single newly eclosed female. We have attempted multiple different sizes of cages. Until the wild population exceeds 500 on the peak survey date (most recently it was 44), a captive program should be maintained as insurance against catastrophic loss of the wild population.

Glossary of Terms

Allee effects – adverse effects on population growth rate of a species at a small population size

Autecology – the study of the relationship between a species and its environment

Captive breeding – maintaining a species in captivity in a manner that includes multiple generations resulting from successful mating

Captive propagation – maintaining a species in captivity through an extended period of time but not including mating and resulting offspring

Diapause – a period of physiological dormancy and delay in development in response to adverse environmental conditions

Eclosion – the act of an insect emerging from its pupal case

Foodplant – the plant species required as sustenance for the larvae of an insect species

Frass – excrement produced by insects

Imago – the final, sexually mature, stage of development of an insect, usually with wings

Instar – a stage of development of a larval insect between molts, often referred to by number (first instar, second instar, etc.)

Oviposit – lay an egg or eggs, usually by an insect

Petiole – the stalk that attaches a leaf to a stem

Protandry – in insects, the appearance of males of a species appearing earlier in a season than females of the same species

Senesce – to deteriorate with age

Trichomes – fine hairs on a plant

Walkabout – informal term for a larva that crawls around, leaving its foodplant

Introduction

The Lange's metalmark butterfly, *Apodemia mormo langei* (Lepidoptera: Riodinidae) (Figure 1), was placed on the federally endangered species list in 1976. The species is restricted to the ADNWR (narrow distribution), is monophagous (has extremely restricted habitat specificity), and suffers from a deplorably low abundance. The maximum number of adults observed on any one day in 2011 was 32, down from 2,300 in 1999. These three factors place Lange's metalmark in Rabinowitz's most rare (and therefore endangered) category of rarity (Rabinowitz 1981, Rey Benayas et al. 1999).

Figure 1. A captive reared Lange's metalmark butterfly is released in the Antioch Dunes National Wildlife Refuge in 2008.

Lange's metalmark was recognized as a subspecies by Comstock (1938), although recent genetic investigations provide "conflicting" evidence of its evolutionary significance when compared with other isolated populations of the species (Proshek et al. 2015). It is univoltine, breeding once per year in synchronization with the flowering of their only host plant, Antioch Dunes naked-stemmed buckwheat (buckwheat), *Eriogonum nudum* var. *psychicola*, which is a unique variety endemic to the ADNWR (Reveal 2007). Adult butterflies eclose from their pupae in late July to September (Arnold 1981, 1983, Davenport et al. 2011). During their roughly one week lifespan in the wild, the butterflies mate and the females oviposit their eggs in small clusters directly onto the buckwheat. The eggs are thick-walled and it is difficult to differentiate between fertile and infertile eggs. The larvae develop within the egg and most of the hatch occurs in the early spring (Figure 2), while others have reported the fall (Arnold 1981). The first instars will feed on new, tender plant growth. After hatching in the early spring, we have observed the larvae to come in and out of dormancy to feed as temperature allows. Through the spring and summer, the larvae resume feeding and mature before metamorphosing into pupae. About two weeks later, the adult butterflies emerge and begin the cycle anew (Arnold 1981, 1983).

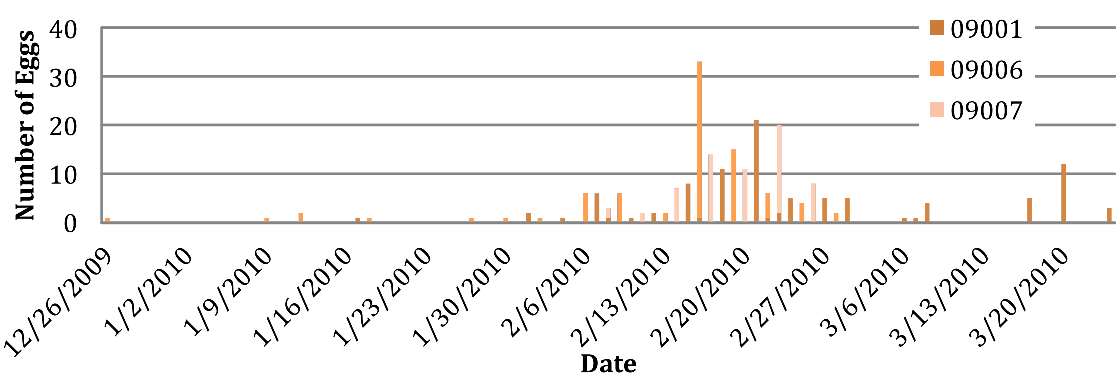


Figure 2. Hatch date for Lange's metalmark butterfly eggs laid in captivity in the summer of 2009 (Johnson et al. 2011).

In 2007, a captive rearing and propagation program for Lange's metalmark was established to guard against extinction, augment the dwindling wild population, and increase knowledge of the autecology of this species. These efforts have been funded by the CVPIA Habitat Restoration Program, which continues to fund the rearing project, most recently through the 2016 season. Rearing efforts focused on production the first two years and on data gathering the second two years (Johnson et al. 2011). This report covers captive rearing efforts from October 2011 through September 2015.

Study Area

The ADNWR is located on the south bank of the San Joaquin River east of the city of Antioch in Contra Costa County, California approximately 40 miles northeast of San Francisco. ADNWR is also home to two endemic endangered plant species, the Contra Costa Wallflower (*Erysimum capitatum* var. *angustatum*) and the Antioch Dunes Evening Primrose (*Oenothera deltoids* ssp. *howellii*). These plants are addressed in the same recovery plan with the Lange's metalmark butterfly (U.S. Fish and Wildlife Service 1984).

The rare and endemic arthropods of the Antioch Dunes have been a focus of public interest and conservation attention since the publication of a multi-page feature in *Life* magazine in the 1950s (Feininger et al. 1955). As a riverine dune system of limited geographic extent, the Antioch Dunes are a ready-made locus for speciation and endemicity for arthropods, and are recognized as center of biological diversity by the independent scientists assessing the Bay Delta Conservation Plan (Spencer et al. 2008). It is type locality for 27 insect species, four of which are already extinct (Feininger et al. 1955, Bettelheim 2005). Rare and/or endemic insects include the giant flower-loving fly (*Raphiomidas trochilus*), anthicid beetle (*Anthicus antiochensis*), San Joaquin dune beetle (*Coelus gracilus*), Hurd's robberfly (*Cophura hurdi*), Antioch robberfly (*Efferia antiochi*), Antioch yellow-banded adrenid bee (*Perdita hirticeps luteocincta*), Antioch adrenid bee (*Perdita scituta antiochensis*), Pacific velvet ant (*Myrmosula pacifica*), sphecid wasp (*Philanthus nasalis*), and Middlekauf's shieldback katydid (*Idiostatus middlekaufii*).

The ADNWR is divided into two sites, the 41-acre Stamm Unit and the 14-acre Sardis Unit (Figure 3). The Pacific Gas and Electric Company owns an additional 12 acres of Lange's metalmark habitat on either side of the Sardis Unit. These private lands are within the ADNWR boundary.

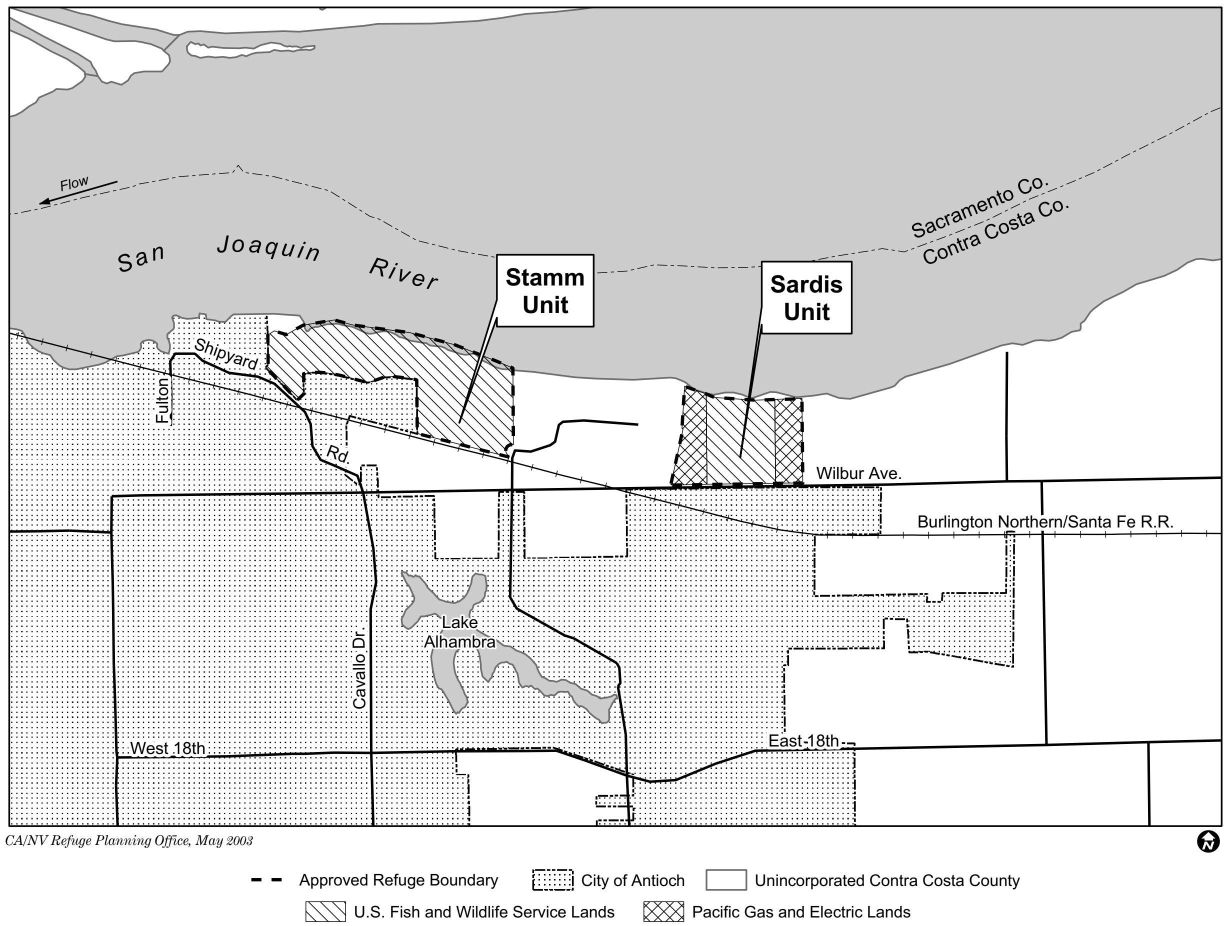


Figure 3. Location of the two Antioch Dunes National Wildlife Refuge units along the San Joaquin River, separated by industrial land uses.

Methods

The methods used for captive breeding and release were adaptively managed to account for the difficulties and opportunities encountered over the four years of the project. This section describes the chronology and techniques involved in the process, which generally followed guidelines we previously established for propagation of this species (Johnson et al. 2007), for protecting genetic integrity (Johnson and Longcore 2008), and for augmentation of the wild population (Longcore et al. 2008). Captive breeding involves the following steps each year: collection of adult female butterflies, maintenance of females and their eggs in captivity, maintaining larvae and pupae, eclosion of adults and attempted captive breeding, and habitat assessment and release of captive propagated or captive bred stock.

Collection of Wild Females

Around the first weekend of September during each year (Labor Day weekend), female Lange's metalmarks were collected at the ADNWR. Females were confined over live foodplants and provided with honeywater solution. They were transported to the Moorpark College (Moorpark, Ventura County, California) captive breeding site (America's Teaching Zoo/The Butterfly Project) after dusk to minimize heat stress.

Egg Maintenance

At the Moorpark College captive propagation facility, leaves with eggs affixed to them were enclosed by plastic containers (called "mondos") on living plants. Generally, during the early winter the containers were built with ventilation holes covered with mesh, which allows greater air circulation. When larvae began to hatch, finer grain organza fabric (a plain-weave, sheer fabric) was used for ventilation so that the small larvae could not escape. When it was cool, the plants with enclosing plastic containers were kept inside a greenhouse with excellent sun exposure and a heater to keep ambient temperatures above 45° F (Figure 4). If the plant that the female oviposited directly onto began to die, the parts of that plant that had the eggs were relocated onto a new plant and cradled in an "egg hammock" and leaves were used as bridges between the hammock and live growth to insure first instars could walk easily across to the new living plant (Figure 5). When eggs were relocated, past data on egg locations was used to decide where to place them and careful records maintained about the contents of each container with eggs or larvae.

Figure 4. Eggs and early instar larvae of Lange's metalmark in plastic containers over living plants in: a) the greenhouse and b) inside multi-plant boxes outdoors.

When weather warmed, the eggs (in their containers) on living plants were relocated from inside of the greenhouse to freestanding boxes that contained multiple plants (known as "multis"). The move outside was motivated by the need for increased ventilation and sun exposure (associated with higher survivorship in plants and larvae). The legs of the box sat in soapy water baths to prevent non-target insects gaining access to the box. A plexiglass sheet on the top protected against rain (Figure 4). It was necessary to protect the eggs and larvae from the elements during winter storms. To do this, blanket covers were made that could be easily deployed during inclement weather (Figure 6). These were placed over the boxes, as needed, to guard against wind-driven rain and overnight low temperatures below 50° F.

Figure 5. Plastic container in which a dead leaf with Lange's metalmark eggs on it is cradled in an "egg hammock" (yellow arrow) and connected to fresh plant growth with leaves (blue arrow).

Figure 6. A multi-plant box containing Lange's metalmark eggs and larvae on *Eriogonum* foodplant prepared for a winter storm with a blanket cover.

Loops and a penlight were carefully used to search for eggs inside the container to minimize any possible disturbances to the eggs. Egg leaves were labeled, sketched, and recorded in the Egg/Larval Log to easily keep track of eggs' live status for future observation. When exhaustive egg counts were done, three separate people checked all vegetation in each container.

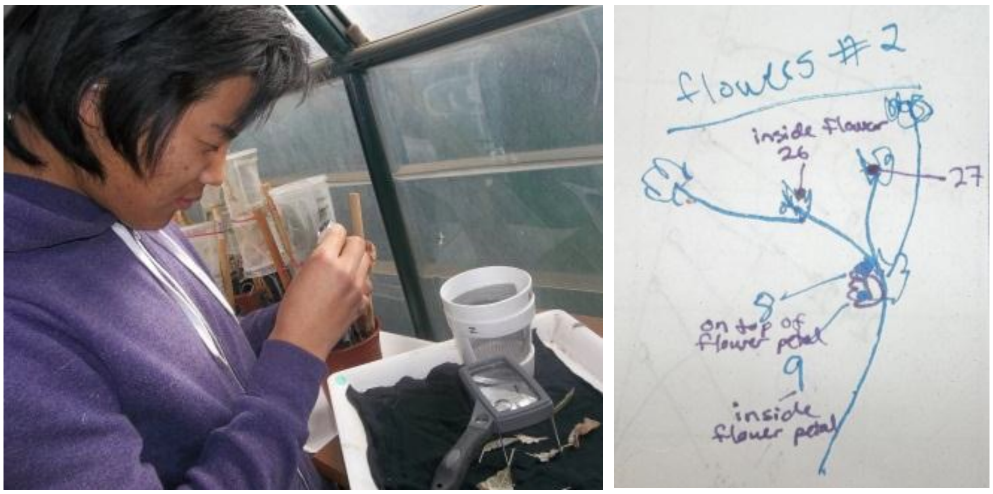


Figure 7. Searching leaves carefully for eggs and resulting sketch showing location of eggs on flowerheads and plant.

Over time, we switched to a less thorough check for eggs to minimize disturbance to the plants and microclimatic conditions. In 2014 we shifted our emphasis to an egg count summary rather than 100% accurate (and therefore unavoidably high disturbance rate) egg count to reduce the risk of losing eggs in the manipulation of the plant. The strategy minimized the risk of losing eggs due to plant manipulation while still providing data on rate and quantity of oviposition. An additional precaution added in 2014 was the use of soil covers to prevent the loss of any eggs falling into the soil of the plant. When mapping eggs attached to leaves of the plant, a clock map was used (Figure 8a); with detached leaves the clock map was ignored and leaves were identified by the recorded egg locations and kept in an egg leaf hammock (identified by an arrow in Figure 8b). When mapping attached leaves, the leaves were noted as being so many leaves up from the bottom and located at a certain numerically clock based position. Before manipulating the plant, a visual inspection was done by slowly rotating the whole plant from the pot, to find any obvious eggs. Next the leaves were moved by carefully grasping them with spoon-billed forceps. Before grasping with the forceps the leaf was visually inspected thoroughly to ensure that no eggs were crushed. Eggs were drawn on the map with a description of their leaf's location and were numbered continuously through the whole plant.

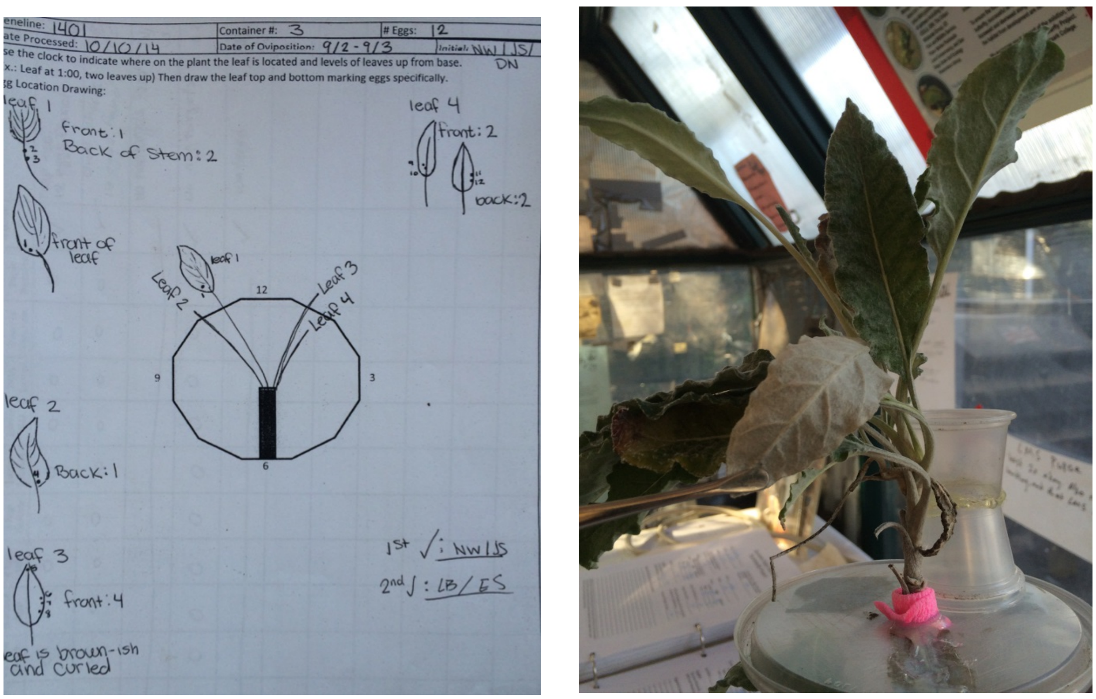
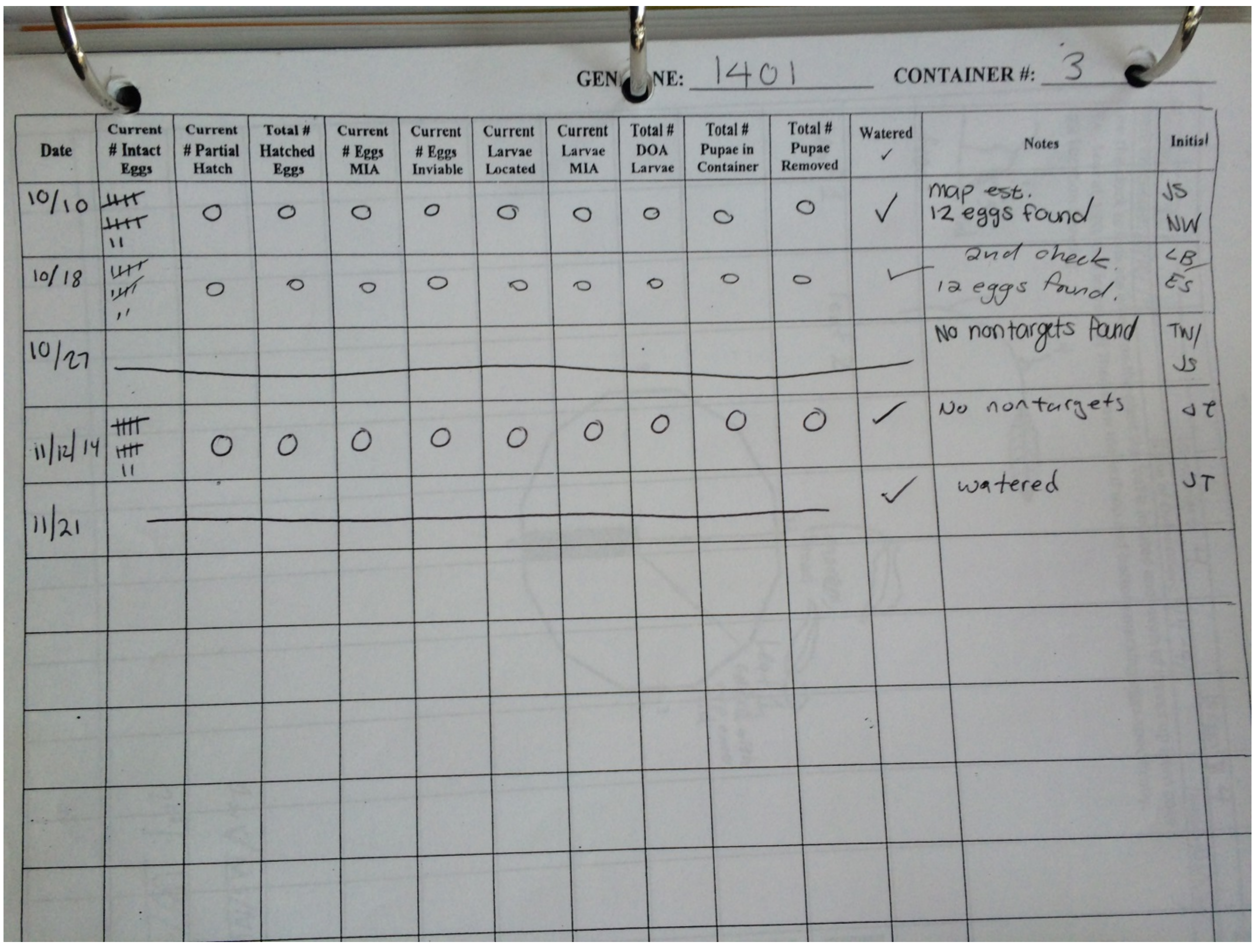


Figure 8. Left: Clock map for recording the location of eggs and egg leaves on a living plant. The circle is the lid around the stem of the plant, 6 P.M. position is the sealed cut used to get the lid around the stem. Right: A plant ready for egg mapping, 6 P.M. is towards the position of the photographer.

If a plant was unhealthy (including infestation with non-target arthropods), the eggs and/or larvae were relocated to a new plant. In the case of eggs, which are more sturdy and do not move, a destructive search was performed whereby every leaf was removed and individually inspected for eggs. Then the egg leaves were placed in an egg hammock on a fresh plant. In the case of first and second instar larvae, the old plant was clipped and placed with the new plant for several days, allowing the fragile and mobile larvae to move to the new plant on their own. After several days the old plant was removed and a destructive search was performed on the old plant material to find all eggs and any remaining larvae, which were relocated by hand to the new plant. Every egg plant manipulation/procedure was recorded on a spreadsheet log (Figure 9).



GEN NE: 1401 CONTAINER #: 3

Date	Current # Intact Eggs	Current # Partial Hatch	Total # Hatched Eggs	Current # Eggs MIA	Current # Eggs Inviable	Current Larvae Located	Current Larvae MIA	Total # DOA Larvae	Total # Pupae in Container	Total # Pupae Removed	Watered ✓	Notes	Initial
10/10	卌 卌 ||	0	0	0	0	0	0	0	0	0	✓	map est. 12 eggs found	JS NW
10/18	卌 卌 ||	0	0	0	0	0	0	0	0	0	✓	2nd check 12 eggs found.	LB ES
10/27	—	—	—	—	—	—	—	—	—	—	—	No nontargets found	TW/JS
11/12/14	卌 卌 ||	0	0	0	0	0	0	0	0	0	✓	No nontargets	JT
11/21	—	—	—	—	—	—	—	—	—		✓	watered	JT

Figure 9. Spreadsheet log of manipulations for a cohort of eggs/larvae

We only recorded the status of the eggs and leaves when the plant condition deteriorated and the eggs/larvae had to be moved onto a new plant. This was to reduce disturbance to the sensitive early instars, so it reduced the amount of data collected but increased survival. When relocated, we recorded the status of eggs as follows:

- Intact – still entire and not collapsed (we maintained a set for 2 years to check if they could diapause and hatch later to no success) (see Figure 10);
- Full hatch – larva ate a hole and left (Figure 11);
- Partial hatch – larva ate a small hole and then died without leaving the egg (Figure 11);
- Collapsed – egg structure collapsed (appears like deflated bubble wrap).

Figure 10. Intact Lange's metalmark butterfly eggs.

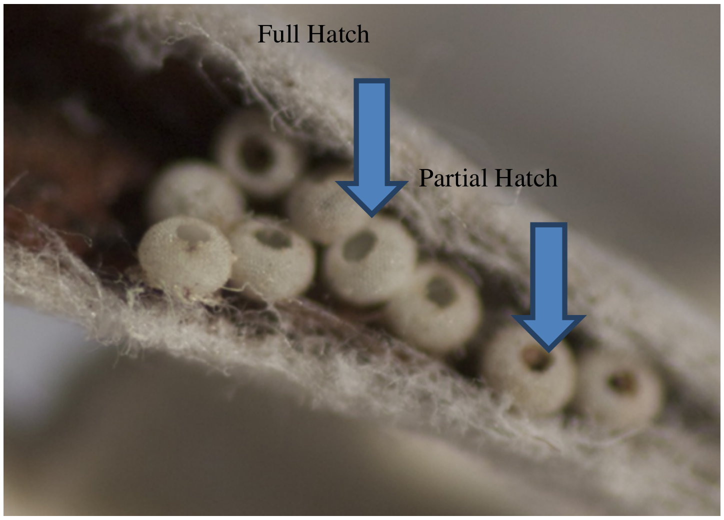


Figure 11. Full and partial hatch of Lange's metalmark eggs. The remains of the larva can be seen in the partially hatched egg, while only the shell remains of the fully hatched egg.

Larval Maintenance

Larvae were maintained in plastic containers fitted over the leaves of foodplants (Figure 12), which were then kept in multi-plant containers outside. These "multis" had their feet placed in soapy water to dissuade ants from entering. The larvae were, with a few exceptions, kept there until they pupated or released. The "multis" were moved each day with the sun (both within each day and also between seasons) to maximize afternoon sun exposure.

Figure 12. Example of enclosure for larvae on buckwheat foodplant during early instars.

As the larval foodplants were eaten, we periodically moved larvae onto new foodplants. This was done when the plant was not healthy, was being overgrazed by the larvae, or to pre-emptively split up batches of larvae that would consume more than the plant could produce. When being moved, larvae were placed, along with their substrate from the old plant, on a location between the petiole and stem of the new plant to ensure that they could easily reach the new foodplant and continue grazing. In each instance, the move was recorded on datasheets, and as the larvae matured and were moved, we were able to collect more data on the later instars.

There were some changes in the protocol for handling late instars over the course of the project. Early in the project, we moved some of the late instar larvae to Styrofoam containers with cut leaves as substrate (Figure 13). We did this when the larvae were large and getting close to pupation. The Styrofoam containers made it easy to find the pupae. Fresh food was inserted into the container from the top, with the stem extending down through a hole in the bottom into a water reservoir below. Multiple mesh-covered windows provided ventilation. The containers were exposed to dappled sun outside for a minimum of four hours per day. However, the Styrofoam container rearing of larvae was significantly less successful than the mondos, so by 2014 we kept the larvae in mondos until they pupated, then the pupae were relocated to a Styrofoam eclosion container.

Starting in 2013 a tarp to block night lighting was put up at closing at 4 P.M. and pulled back at opening at 8 A.M. to avoid disrupting the natural circadian rhythm of the larvae.

Figure 13. Sytrofoam containers for Lange's metalmark larvae nearing pupation.

Eclosion and Breeding

If not released, adult butterflies were placed in mating containers with live food plant and an unrelated butterfly of the opposite sex (Figure 14). Our goal was to produce dappled shade for the adult butterflies. A tarp was moved to create shade occasionally when the butterflies were overly active. The lighting was adjusted every five minutes to provide dappled sunlight. Butterfly pairs were hand-fed twice (in short succession) before being placed in the mating box. Mating stimulation and oviposition stimulation processes were supervised by an experienced technician for two weeks whenever the zoo was open (8 A.M.–4:30 P.M.). Plentiful fresh leaves, lantana flowers (which are a good nectar source), and fresh feeding stations were provided daily to keep butterflies alive and stimulated. We attempted breeding in various sizes and shapes of containers, but the successes always occurred in these small breeding containers.

Figure 14. Left: Lange's metalmark butterflies mating, August 2013, at America's Teaching Zoo/The Butterfly Project at Moorpark College. Center: Location of mating boxes. Right: Example of lantana flowers and buckwheat leaves in mating container

Habitat Assessment and Releases

At various points during the project period, we measured key features of the habitat within pre-defined management units at Antioch Dunes. The habitat assessment was undertaken first in 2008 before the project period, repeated in 2012, and then again in 2015 (all by Ken Osborne). In intervening years, a rapid assessment combined with the advice of USFWS biologists guided the release of captive butterflies.

Data collected for each of the management units at ADNWR were as follows:

Number of plants – estimate of number of *Eriogonum nudum* plants in the management unit.

Size of patches – estimate of size of foodplant patches if not the same as whole management unit.

% large plants – estimate of percentage of plants with mature rosettes > 4 inches across, and floral stems > 1.5 feet.

Plants per 4 m² – estimate of plant density within whole management unit or within area defined in "size of patches."

% oaks / % exotic – estimate of percent cover of oaks and exotic species within whole management unit or within area defined in "size of patches." Oak trees represent encroaching woody cover that is not consistent with typical Lange's metalmark habitat.

The following criteria were proposed as necessary conditions for release of larvae or adults into a management area (Longcore et al. 2008). These conditions can be assessed with the data collected combined with maps of the site.

- Number of host plants greater than 100.
- Density of host plants greater than 1 per 4 m^2.
- Proportion of host plants that is greater than 1.5 feet tall with 4" basal rosette is greater than 75%.
- Cover of exotic plant species is less than 50%.
- Cover of oaks is less than 10%.
- Separated from area occupied in 2007 by > 25 m.
- Area of patch > 400 m^2.

Results

Collection of Wild Females

2011

On September 5, 2011, we made a field visit and were joined by USFWS representatives Louis Terrazas, Susan Euing and Dave Kelly. Osborne was present to net and handle the butterflies under his research permit. We captured 7 adult Lange's metalmarks (5 females, 2 males; Table 1). Location of each capture was recorded with a Garmin eTrex Venture HC unit (datum: WGS84; Figure 15). Two captive-bred larvae were released according to approved protocols. All of these actions took place at the Sardis Unit, between 10:50 A.M. and 12:50 P.M. At least 10 individual adult male Lange's metalmarks were also observed on the Sardis Unit during this period. All individuals were treated according to established protocols (Johnson et al. 2007, Johnson and Longcore 2008, Johnson et al. 2011) and transported to Moorpark College.

Table 1. Location of collection of adult female and release of larval Lange's metalmarks at ADNWR, September 5, 2011.

Butterfly ID	Time Captured	Latitude	Longitude	GPS Error
Female 11001	10:53 A.M.	38.01388	-121.78288	±20 feet
Female 11002	11:08 A.M.	38.01375	-121.78275	±22 feet
Female 11003	11:10 A.M.	38.01385	-121.78284	±19 feet
Female 11004	11:43 A.M.	38.01311	-121.78430	±21 feet
Female 11005	12–12:45 P.M.	38.01389	-121.78298	±18 feet
Male 11001	11:57 A.M.	38.01323	-121.78436	±20 feet
Male 11002	12:00 P.M.	38.01313	-121.78439	±21 feet
Larvae Released	11:02 A.M.	38.01379	-121.78473	±23 feet

Figure 15. Location of males and females captured at the Sardis Unit of the Antioch Dunes National Wildlife Refuge.

2012

Three gravid females (12001, 12002, and 12003) were collected from the Sardis Unit of the ADNWR between 10:00 A.M. and 12:00 P.M. Wild females were collected on Labor Day, 2012, under Ken Osborne's permit. The females were fed artificial nectar and then put on cut plants in Styrofoam oviposition containers (Figure 16). We adjusted lighting conditions to try to maximize probing and oviposition without inducing heat stress. Females were transported back to the zoo in Moorpark after the day had begun to cool off and the peak oviposition window had passed.

Figure 16. Female Lange's metalmarks housed in Styrofoam containers during collection in 2012.

2013

Three wild females (1301, 1302, and 1303) were collected on September 2, 2013, by Ken Osborne at ADNWR.

They were brought back to Moorpark on September 3, 2013. Multiplant boxes were set up for them with a swamp cooler blowing to help keep them cool. The top of the box was set up to receive dappled light so that it was possible to use the swamp cooler to make a breeze while the butterflies were in dappled sun.


Figure 17. Left: Representatives from USFWS, Moorpark College, The Butterfly Project/The Urban Wildlands Group, and Osborne Biological Consulting at ADNWR. Right: Wild Lange's metalmarks at ADNWR on September 2, 2013. Three females were identified and taken into the captive breeding program.

Figure 18. Wild-caught Lange's metalmark probing and/or ovipositing on buckwheat leaf within an organza enclosure.

Figure 19. Lange's metalmark eggs being mapped once the female has died.

2014

Six wild females (14001, 14002, 14003, 14004, 14005, and 14006) were collected on September 1, 2014 by Ken Osborne at Antioch Dunes National Wildlife Refuge. The females were fed artificial nectar (honey/water solution) and then put on potted plants in oviposition containers. Lighting was adjusted to maximize probing and oviposition without inducing heat stress. However, females 14002 and 14003 were behaving in a manner that suggested that they would not be productive in captivity, so after a day of observation in the field, those two were released where they were captured. The four other females were transported back to the zoo in Moorpark after the day had begun to cool off and the peak oviposition window had passed.

Multiplant boxes were set up for them with a dappled light cloth suspended above them. The foundresses were observed and cared for from 8 A.M. to 5 P.M. daily. They were moved to maximize their activity (including oviposition) without allowing them to become heat-stressed. As necessary they were moved into the greenhouse with the swamp cooler blowing to help keep them cool. The females were put on fresh food plants every day to provide new flowers for nectar source and were fed with a honey solution a minimum of twice a day.

2015

On August 21, 2015, two females were collected from the wild (15001 and 15002). On September 7, 2015, three females were collected from the wild (15003, 15004, and 15005). All females were netted by Ken Osborne, treated according to established protocols, and transported to Moorpark College.

Maintenance of Females and Oviposition

2011

Following our established protocols (Johnson et al. 2007, Johnson and Longcore 2008, Johnson et al. 2011), the five wild-caught females that were collected prior to the start of the current HRP grant were confined in containers with foodplants and switched to a new container each day. Over the course of three weeks, the females laid 621 eggs (Figure 20). The females were of unknown age when captured and lived 9–23 days in captivity. We also confined captive-bred females with foodplants and were able to obtain 81 eggs. Four captive-bred females were in the ovioposition container, but the eggs came from an unknown subset of these females. Ultimately, the eggs did not hatch and were considered inviable.

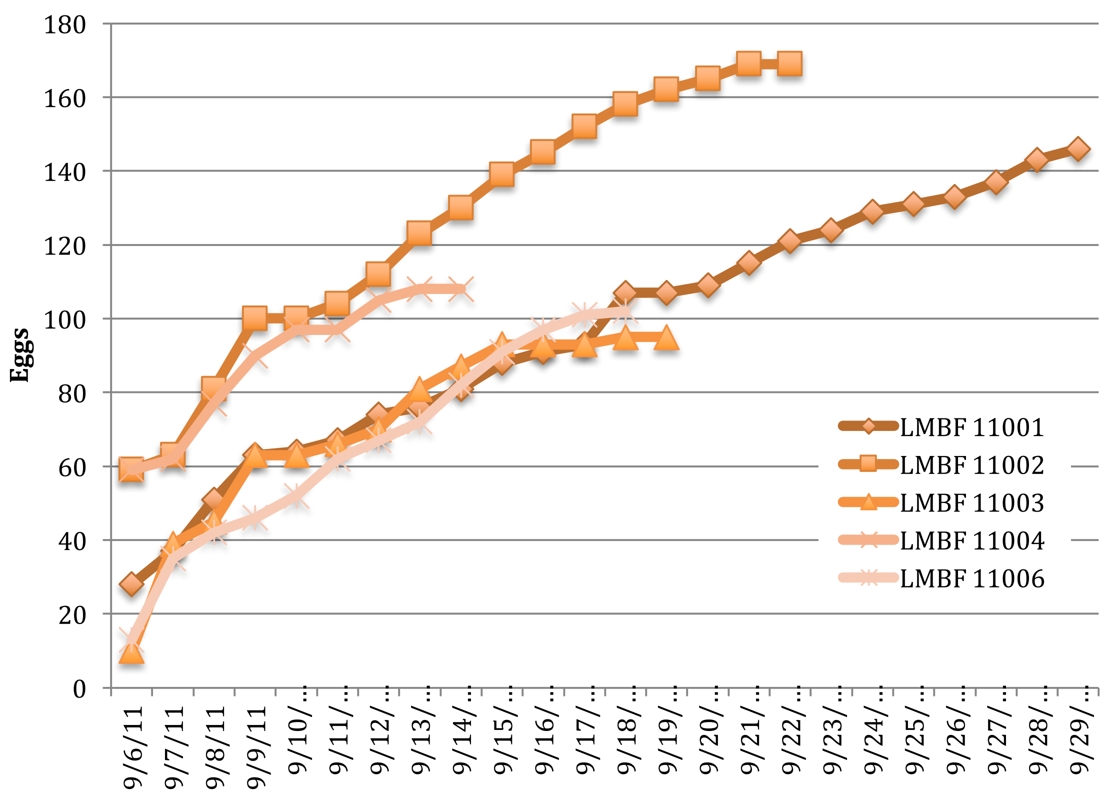


Figure 20. Accumulation of eggs from five wild-caught Lange's metalmark females in September 2011.

2012

From the three wild-caught females obtained in 2012 (12001, 12002, and 12003), egg production ranged from the low 50s to the high 80s (Figure 21).

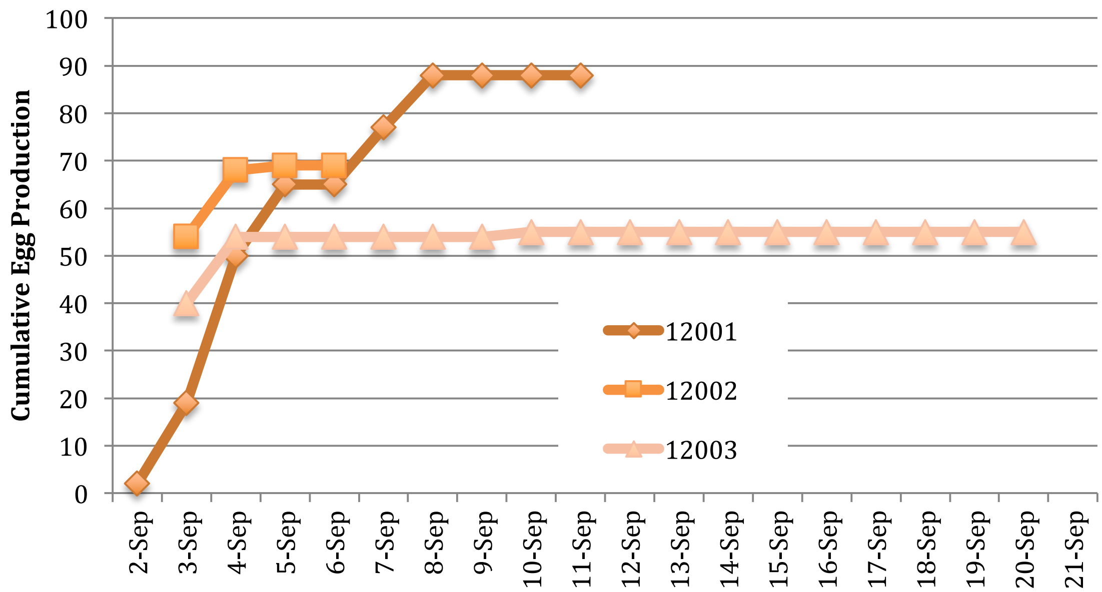


Figure 21. Cumulative egg production from female Lange's Metalmarks at ADNWR, September 2, 2012.

Average longevity for adult females was 11 days and was widely variable from 4 days to 18 days after capture. The shortest longevity belonged to our highest single day producer (54 eggs in a single day). Egg production ranged from 55 to 90 eggs per female and averaged 71 eggs per female. All eggs were mapped and placed in oviposition containers with living foodplant.

2013

In 2013, we minimized repeated searches for eggs because opening of containers is associated with lower overall survival. The three wild-caught females produced 137 eggs, which were set up in 16 separate containers.

2014

Again in 2014 we minimized repeated searches for eggs because opening of containers is associated with lower overall survival. No eggs were obtained from two of the six females. The number of eggs ranged from 20 to 47.

2015

The results of collection of wild-caught female oviposition will be reported in the final report; it has not yet occurred.

Summary

The number of total eggs obtained from wild caught females was relatively high in 2011, but much lower in 2012 through 2014 (Figure 22). Furthermore, the number of eggs per female was lower in 2014 than in some earlier years. Even though fewer searches were performed while eggs were being laid in the latter years, complete searches were performed at the end of the season so all numbers are comparable. The total number of eggs obtained ranged from a low of 135 in 2014 to a maximum of 630 in 2011.

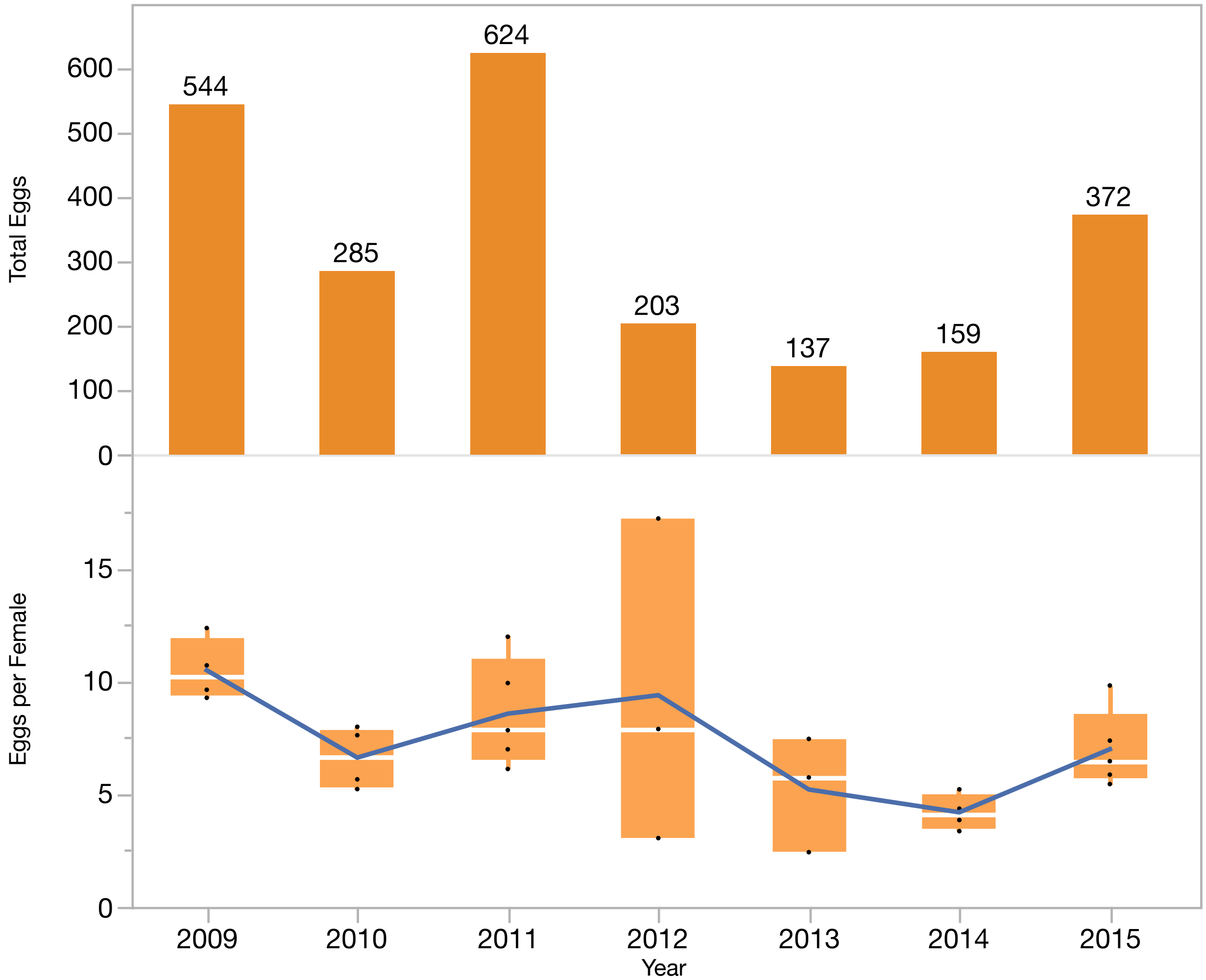


Figure 22. Summary of mean of eggs obtained from each collected female (mean connected by black line) and total eggs obtain from wild-caught females from 2009 to 2015.

Egg Maintenance and Hatch

2012

Of the 630 eggs counted from the five females collected in 2011, 362 (57%) hatched. Another 182 eggs were intact but failed to hatch (29%).

Table 2. Hatch statistics for eggs laid by Lange's metalmark females collected in summer 2011.

Foundress	Eggs Laid	Intact (failed)	Partial hatch	Missing	Collapsed	Full Hatch	% Hatch
11001	146	48	14	3	9	72	**49.3**
11002	173	21	5	14	1	132	**76.3**
11003	101	18	5	8	3	67	**66.3**
11004	108	55	1	0	0	52	**48.1**
11006	102	40	2	10	11	39	**38.2**
Total	**630**	**182**	**27**	**35**	**24**	**362**	**57.5**

2013

Larvae from all of the wild-caught females hatched in April and May. The crosses attempted in captivity in 2012 were not successful. By June we disposed of all eggs, whether or not they had hatched. We have previously looked for survival of eggs longer than a year in this species and found none. Detailed egg fate was not recorded because we minimized disturbance in the interest of increasing survival of eggs and larvae.

2014

Eggs began to hatch in January, with the majority of the hatch in February. We observed hatched eggs from females 13001 and 13002 and directly observed live larvae from female 1302 while relocating eggs and larvae.

Most of the captive-bred eggs that could be observed during this period were in various states of collapse and discoloration, suggesting that the captive matings did not result in viable eggs. In January, we assessed all the eggs as we switched the eggs to organza larval containers instead of mesh. In February and March, no captive-bred eggs required relocation, so none were assessed.

We maintained and did non-destructive counts of eggs from both wild-caught females and captive-bred butterflies. These totaled 137 eggs from females collected in 2013 at ADNWR and 194 from females that were mated in captivity from breeding the previous season's offspring.

2015

In January, intrusion into containers to count eggs and hatching larvae was minimal so statistics are sparse. Egg containers were switched from mesh to organza, to prevent the extremely small newly hatched larvae from leaving the containers. Hatch was confirmed by the presence of the first instar larvae and observation of hatched eggs. The first instar larvae received minimal interaction, because we wanted to prevent the loss of this fragile stage from over-manipulating plants. Plant health was monitored closely, due to the reduced airflow of the organza. We undertook four relocations with extreme care, with the old plants cut and placed in with the new plants to allow the larvae to move themselves to the new live plant.

Switching to organza was finished, and we kept the organza containers in place because the larvae were not yet large enough for mesh containers. We undertook seven relocations of early instars with extreme care, with the old plants cut and placed in with the new plants to allow the larvae to move themselves to the new live plants. After a few days the old material was removed and thoroughly searched. If the plant was aphid-infested, a thorough search was done to find and remove larvae and place them on the new plant. Exact numbers of larvae are still unknown because the counting process causes too much disturbance.

Larval Maintenance through Pupation

2012

First instars are tiny (Figure 23) and were maintained within the plastic containers affixed to plants. By the end of February, we observed some second instar larvae (Figure 24).

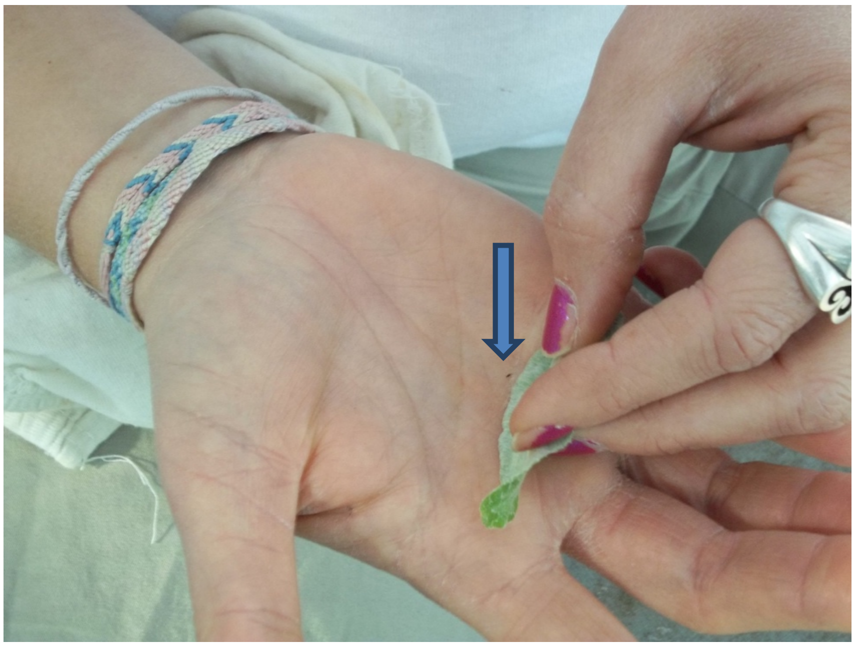


Figure 23. First instar larva of Lange's metalmark (blue arrow).

Figure 24. Second instar larva of Lange's metalmark on underside of *Eriogonum* leaf.

Of the 630 eggs counted from the females collected in 2011, 362 (57%) hatched. Another 182 eggs were intact but failed to hatch (29%). Because we did not open containers as much to track eggs from day to day, we lost track of 35 eggs during the hatch season. In April, we relocated 154 larvae, most of which were third instar.

Table 3. Relocation of Lange's metalmark larvae to new foodplant in April 2012.

Foundress	Total # of Active Larval Containers	Number of Larval Container Surveys in April	Total Larvae (Located in April)
11001	23	8	19
11002	17	11	44
11004	12	8	42
11004	8	7	32
11006	13	4	17
Total	**46**	**38**	**154**

In May, we relocated the remainder of the active larval containers and located 185 larvae, most of which were fourth instar. The total number of larval containers surveyed exceeded the number of active containers because some containers were surveyed more than once as foodplant was consumed. We located nine second instar larvae that were dead. All previous losses had been first instar larvae earlier in the season. The larger larvae consumed foodplant quickly so we contacted Susan Euing, a Wildlife Biologist with the USFWS, to arrange for more foodplant to be shipped.

Table 4. Relocation of Lange's metalmark larvae to new foodplant in May 2012.

Foundress	Total # of Active Larval Containers	Number of Larval Container Surveys in May	Number of Larvae Located in May	Total Larvae (Located in April and May)
11001	21	26	23	23
11002	17	24	67	77
11003	11	17	36	36
11004	9	11	32	32
11006	13	14	17	17
Total	**71**	**92**	**175**	**185**

All larval containers were surveyed in either May or June, with 165 larvae left alive at the end of June. These were all fourth and fifth instar. Near the end of June, 20 larvae died. We quarantined these containers in case there was an infection. We then relocated all of the stock to mesh-screened containers (as opposed to screening with organza). This provides less protection against potential parasites and parasitoids, but more ventilation and less heat retention. No further larvae died after being moved to containers with the mesh screening.

Foodplant was obtained from Susan Euing in June and an order placed with North Coast Native Nursery in Petaluma, California, for additional foodplant before Labor Day and collection of additional wild female metalmarks.

Table 5. Relocation of Lange's metalmark larvae to new foodplant in June 2012.

Foundress	Total # of Active Larval Containers	Number of Larval Container Surveys in June	Number of Larvae Located in June	Total Larvae (Located in May and June)
11001	11	21	16	18
11002	19	51	64	67
11003	10	26	33	34
11004	6	17	30	30
11006	7	9	15	16
Total	**53**	**124**	**158**	**165**

2013

The first instars were seen in January, and the second and third instars were seen by the end of March.

In April, we located 38 larvae during surveys of a subset of 7 containers (only those that required relocation due to ill health of foodplant). These were mostly second and third instars.

In May, we located 60 live larvae located in surveys of a subset of 19 containers that had to be switched out because of foodplant health. These were mostly fourth instars.

In June, all Lange's metalmark butterflies in organza containers were switched to mesh containers.

All larval containers were surveyed in June, with 62 live larvae located, as a mixture of fourth, fifth, and sixth instars. Twenty-eight larvae were found that had died. Four larvae were isolated as quarantine due to abnormal behaviors (such as producing sticky frass and going on frequent walkabout when fresh food was provided) in case it was an infection.

In June, we located one pre-pupa from female 12001. We initiated a Lange's metalmark butterfly rotational status check every day at the end of June, extending through July, August and September.

All containers were surveyed for larvae in July, and 30 were found. We minimized the number of times containers were opened and larvae were counted in the interest of the survival of the larvae. In July the larvae were a mixture of fourth, fifth, and sixth instars and pupae. Some difficulties with larvae earlier in the summer were resolved with better ventilation in the containers. The larvae were consuming foodplant quickly, but we had adequate plants on site to move larvae to new plants when necessary. Some of the rearing containers were closed out in July after a thorough search yielded no larvae. Final status of all eggs and larvae were recorded on data sheets for later analysis. After a final thorough search, all larval containers were closed at the end of August, with all larvae pupated.

In July, 27 pupae were located in rearing containers and transferred to eclosion cups. In August, the total pupae number increased to 58. Pupae were left on the material they were bound to by their own silk and placed into the eclosion containers.

Table 6. Number of Lange's metalmark pupae recorded in July and August 2013.

Geneline	July	August	Total
12001	18	2	20
12002	7	23	30
12003	2	6	8
Total	27	31	58

Pupae were stored in an eclosion cup with a dead/dry buckwheat leaf on top to shelter it from light. This is because disturbance causes the pupae to twitch, which is energetically expensive and therefore should be minimized. Eclosion cups were stored outside in an enclosing container protected from insects and under dappled sunlight to full shade. Notes were kept on all pupae and organized by container.

By August, 58 live Lange's metalmark pupae were removed from the larval mesh containers and transferred to eclosion cups, where they were placed on top of crushed walnut shells, which provide support without adversely impacting moisture, and covered with a leaf (Figure 25). The ventilated eclosion cups are sorted by geneline.

Figure 25. Lange's metalmark pupae in eclosion container with buckwheat leaves protecting each pupa from light and unnecessary disturbance.

2014

In April, larvae were mostly second and third instars. In May, larvae were mostly fourth instars. All larval containers were surveyed in June, with 18 live larvae located, as a mixture of fourth, fifth, and sixth instars. Eight larvae were found dead. Seventeen pupae were produced, left on the material that they were bound to by their own silk, and placed into the eclosion containers.

2015

In April, egg containers were switched from organza to mesh, to allow greater air flow through the container. The larvae are all much larger and right around fourth instar, which is large enough so that there is no real risk of larvae going through the mesh. The increased airflow is beneficial to the larvae and the plant, with healthier plants the larvae can feed on one plant for longer.

In May, switching to mesh was finished, and all of the multiboxes were sealed with the silicone caulking. Due to the greater heat and the greater airflow through the containers, the plants dry out more quickly and get watered more frequently. The larvae are also relocated to new plant more frequently because they eat more food the larger they get, and by the end of the month some larvae are observed as being fifth instars.

Starting in June, as all of the larvae reach fifth instar, a regular pupa check was started. No pupae were seen yet, but several larvae have reached sixth instar, the stage just prior to pupation.

In July, the larvae were all much larger (all fifth and sixth instar). In the sixth instar the larvae are preparing to pupate. Fifth instar larvae are still eating a lot of food, but sixth instar larvae slow down in eating, and spend more time looking for shelter places where they might pupate. In July, containers were opened more often on a rotation schedule; this was to check on the larvae for pupation. Some larvae did pupate, and one eclosed on July 29, 2015.

At beginning of August all remaining larvae were sixth instars, and some have pupated. On August 12, 2015 the LMB stock was relocated to Dr. Johnson's office on Moorpark College's campus. The temporary relocation was due to the zoo spraying for ants at one of the buildings on August 13, 2015.

Eclosion and Breeding

2011

Before the start of the current HRP grant, we had 19 captive pupae that eclosed between August 14 and September 9, 2011. The eclosion rate was 79% (15 of 19) and exhibited protandry, with males emerging a week before females (Figure 26). This segregation was probably enhanced by the small sample size.

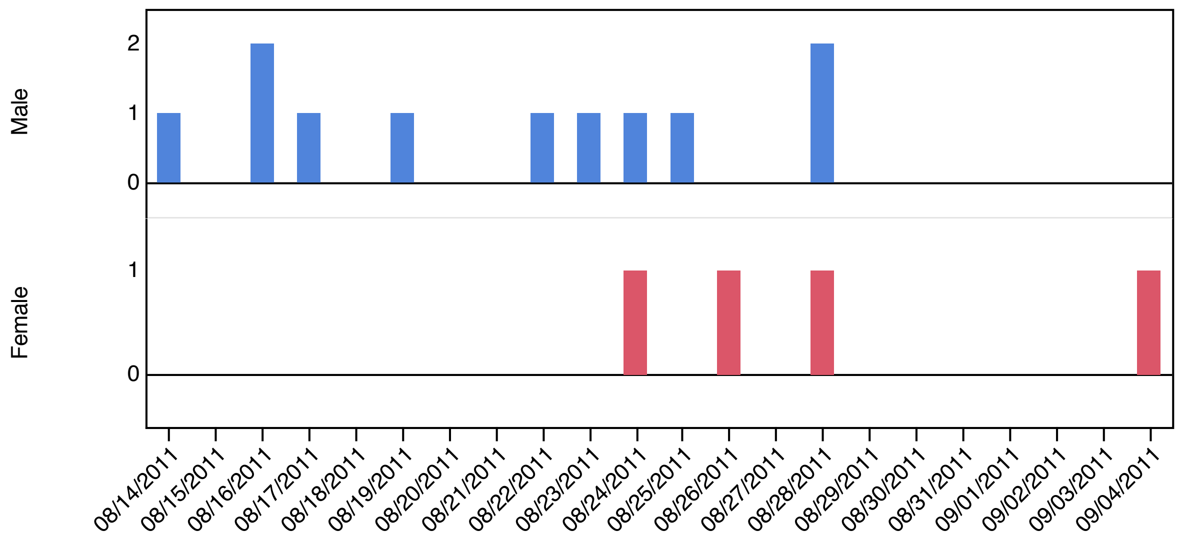


Figure 26. Distribution of eclosion dates for male and female captive-bred Lange's metalmarks in 2011.

The timing of the flight of the captive butterflies was reasonably well synchronized with that at ADNWR. There the flight started on August 25 and peaked shortly thereafter, so the timing at our southern California site was only slightly advanced in comparison.

2012

In July and August, the late instar larvae were fed and processed to prepare for eclosion. This involved weighing each pupa and relocating it to an eclosion cup. The pupae were fragile and could not be handled directly, so they were weighed with the substrate they pupated on. Then the substrate and pupal casing were reweighed after the butterfly eclosed to find the weight of the butterfly. Pupae released to the wild were not included in the data because no weight was taken after.

Imagoes eclosed throughout August and into early September (Figure 27), which coincided with the flight season in the wild and demonstrated the importance of rearing with exposure to natural temperature and lighting to stay synchronized with the wild population. The sex ratio was 30 males: 19 females for the 49 imagoes that eclosed in the lab. We released 79 pupae and 23 imagoes to the wild. The total annual production was 151 individuals.

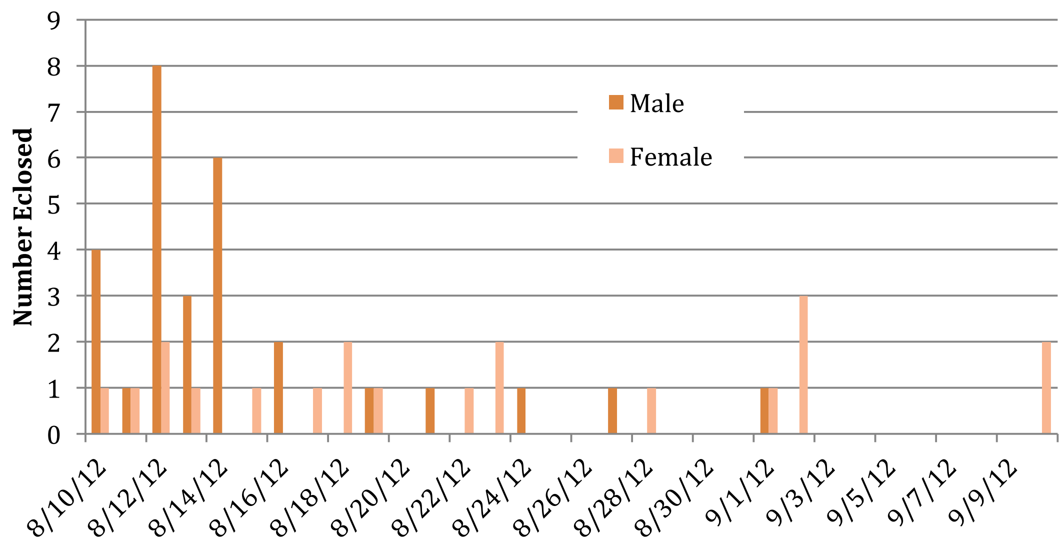


Figure 27. Eclosion of Lange's metalmark butterflies in captivity, summer 2012.

Table 7. Geneline crosses for attempting captive mating of Lange's metalmark, summer 2012.

Male	X	Female	Geneline	Mating Container	Container Number	Eggs
11003	X	11004	12LMB1	1	1	15
11002	X	11003	12LMB2	2	1	88
11001	X	11004	12LMB3	3	1	33
11001	X	11004	12LMB3	5	2	9
11002	X	11004	12LMB4	4	1	10
11002	X	11004	12LMB4/UNK*	6	2	10
11004	X	11002	12LMB5	Released Styro	1	UNK
11004	X	11002	12LMB5	7	2	16
11006	X	11002	12LMB6	Released Styro	1	UNK

*Female mated with 11001 Male in Mating Container 5 and then she was moved to Mating Container 6 to mate with 11002 Male after the males in Container 5 died.

Courtship was observed, but not mating. Although the imagoes had periods when they were not under observation, that time was limited. We later learned the matings were not successful. Matings were attempted with "aged" males and newly eclosed females. Several different techniques to facilitate mating were tried, including one-on-one hand pairing, different sized mating containers, chilling the female, and heating the males prior to placing them together.

Captive matings are rare and no one technique was singled out as working very well. In 2008 the first successful captive mating occurred in a Styrofoam container with newly eclosed females being placed one by one into the container with multiple "brother" imagos under dappled sunlight, with a slight breeze on a hot day with lots of noise and activity in the vicinity. Mating attempts since then have tried to recreate those exact conditions with limited success.

In 2015 for the first time a captive bred female was brought to the dunes and observed mating with a wild male. The female was then recollected and brought back to The Butterfly Project, and was treated as a gravid foundress. A second female was brought to the dunes on the second collection trip without a successful mating and was not returned to The Butterfly Project. Timing may be critical as well as the need for numerous males being present.

2013

Elosion began on July 15, 2013. With a daily check for eclosed butterflies, adults were processed on the same day they eclose and were transferred to a Styrofoam cup sorted by genelines and sex.

Table 8. Eclosion and release of pupae by geneline (m: male, f: female) .

Geneline (N)	July	August	September	Totals
12001 (20)	5 m; 1 f	1 f; 10 released	1 m; 1 released	6 m, 2 f; 11 released; 1 failed
12002 (30)	none	5 m, 10 f; 9 released	4 released	5 m, 10 f; 13 released; 2 failed
12003 (8)	1 m	4 f; 3 released	none	1 m, 4 f; 3 released 1 failed

By the end of September 29, all live pupae successfully eclosed into adult butterflies from larval mesh containers to eclosion cups on top of walnut shells. In addition, 28 live Lange's metalmark butterfly pupae were released during two releases at ADNWR. Eclosions were done by the end of September 2013.

Multiple pupae eclosed in August and September, so we placed captive butterflies in breeding containers and succeeded in inducing mating in the captive population. We observed mating and photographed it on August 25, 2013. Four crosses of females from one geneline with males from different genelines were set up and mating was observed in one of these boxes. The resulting output was 22 eggs from the box where mating was observed (Table 9).

Table 9. Captive mating of Lange's metalmark in summer 2013. Seats designate a location within the eclosion container.

Captive Cross	Geneline Crossed (Males)	Geneline Crossed (Females)	Initial count of eggs
13LMB1	12002 E8 Seat 2, Male	12003 E7 Seat 1, Female	22 eggs
(Mating observed)	12002 E8 Seat 3, Male	12003 E7 Seat 2, Female	
	12003 E8 Seat 4, Male	12003 E7 Seat 3, Female	
13LMB2	12003 E7 Seat1, Male	12002 E8 Seat 1 & 5, Females	144 eggs

		12002 E8 Seat 6, Female 12002 E11 Seat 2 or 4, Female 12002 E11 Seat 1 & 3, Females	
13LMB3	12002 Males	12001 E10 Seat 1, Female	23 eggs
13LMB4	Same 3 Males from 13LMB1	12003 E7 Seat 4, Female	0 eggs

2014

Eclosion occurred from July 14 through August 15, 2014. With a daily check for eclosed butterflies, adults were processed at the same time they eclosed and were transferred to a Styrofoam cup sorted by genelines and sex. Fresh *Eriogonum nudum* leaf, *Eriogonum nudum* flowers, and feeding stations (Q-tips soaked in nectar) were set up inside the Styrofoam cup. We hand-fed adult butterflies a minimum of twice a day to ensure adequate nourishment.

Of the three females collected in 2013, only two were fertile. Therefore, we could only try crossing 1302 with 1303. We did attempt this cross, but did not observe mating, however observations were only for 8 hours out of every 24, so it is possible that mating occurred when the butterflies were not under observation.

2015

One male imago eclosed on July 29, 2015 and kept in captivity for the remainder of his life. A female eclosed on August 20, 2015. In consultation with the Fish and Wildlife Service, we attempted to mate this captive female with a wild male. The female imago was placed on foodplant in at ADNWR, in an area active with male butterflies. The female stayed in place. After several hours a male approached the female at high speed and the two tumbled into the denser more basal growth of the buckwheat. With careful observation mating was observed and noted with photography. The female was later found, and identifiable by her mark on the right forewing and Osborne was able to recapture the now mated female imago. She was placed in an ovipostion container to be transported back to Moorpark College. The mated captive reared, wild-bred imago was designated 15LMB1. Another female eclosed on September 7, 2015 and was marked and placed on foodplant at ADNWR, but no successful mating occurred and was released to the wild.

No captive breeding attempts were made in 2015 this was part of the field discussion on the pupal release with Susan Euing and Louis Terrazas.

Summary

For butterflies where a weight was obtained, the average for a female imago was 74.9 ± 17.3 S.D. mg (n=15), for a male 65.7 ± 15.7 S.D. mg (n=28). Weight did not differ significantly by sex (Figure 28). Females were heavier, but not significantly so. Lighter males are often seen in protandrous species because development time and mass are related (Zonneveld 1996).

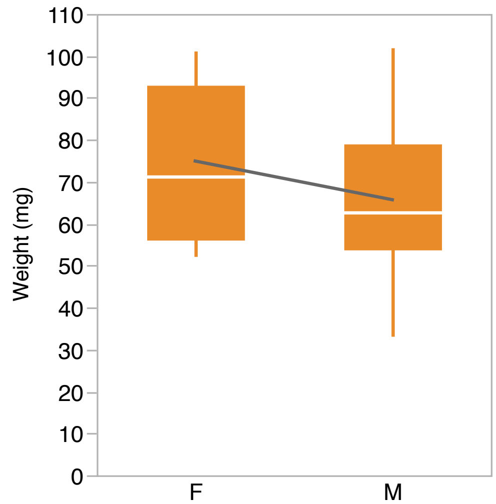


Figure 28. Weight (mg) of Lange's metalmark pupae that eclosed during 2011 and 2012. Mean weight did not differ significantly by sex.

Habitat Assessment

Ken Osborne conducted a habitat survey for Lange's metalmark butterflies at ADNWR in 2008 (previous funding), 2012, and 2015 (this funding agreement). The purpose was to identify the major habitat elements for the butterfly in the Stamm and Sardis management units already established for the refuge. These data included the total number of host plants, the size of host plant patches, the age distribution of host plants, the density of host plants, and other relevant characteristics, such as the percent tree cover. Raw data are provided in the appendix.

The total area of foodplant at both refuge units declined from 2008 to 2012 and 2012 to 2015, totaling approximately 1000 square meters less foodplant cover in each unit from 2008 to 2015 (Figure 29). In contrast, the total number of foodplant individuals increased from 2008 to 2012 in both units, and declined slightly in 2015 (Figure 31). This is consistent with restoration activities increasing the number of small, newly established foodplants, while the number of older, larger plants declines.

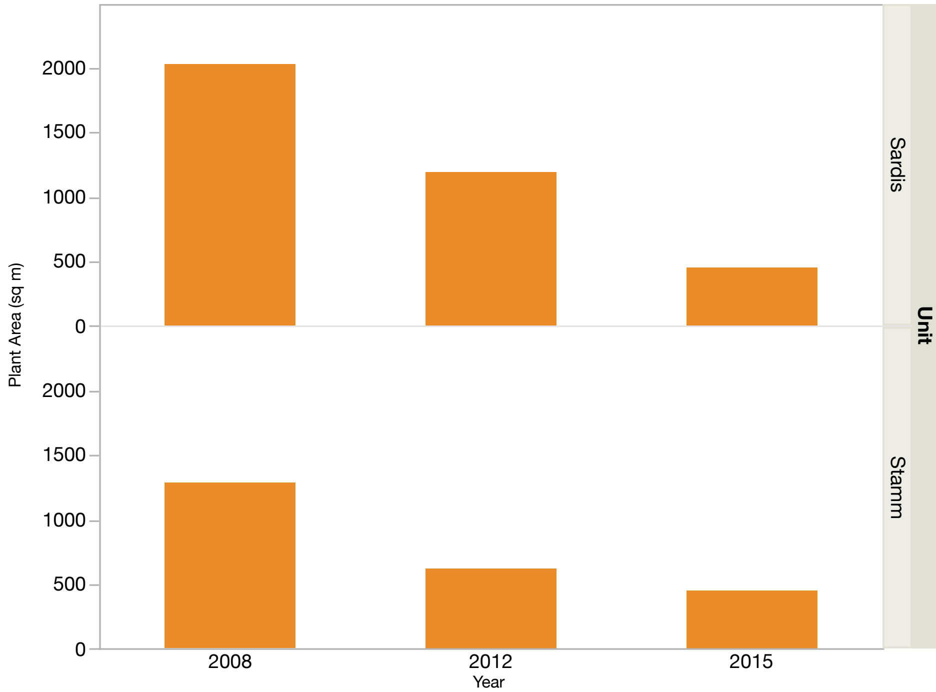


Figure 29. Total area of foodplant (*Eriogonum nudum*) has decreased in both management units from 2008 to 2015.

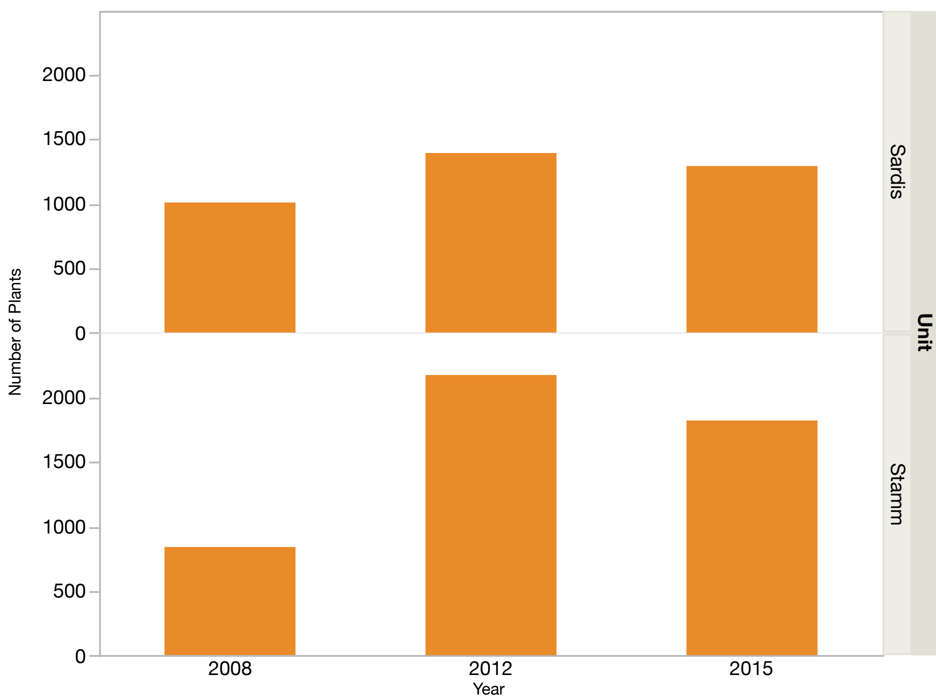


Figure 30. The number of foodplants (*Eriogonum nudum*) in each management unit has increased since 2008, indicating intensive restoration activities with many young plants.

Figure 31. Foodplant patch area for Lange's metalmarks found in each subunit of the Antioch Dunes National Wildlife Refuge in 2008, 2012, and 2015.

Figure 32. Number of foodplants for Lange's metalmarks found in each subunit of the Antioch Dunes National Wildlife Refuge in 2008, 2012, and 2015.

The declines in foodplant cover were spread throughout the two management units and the many mapping subunits (Figure 31). The increased plant numbers from 2008 to 2012 are concentrated in specific subunits where restoration actions have been underway, even as the total cover of foodplant has not recovered.

Release of Captive Stock

Releases of pupae and imagoes to the Sardis Unit occurred from late July through early August 2012 (Table 10). The July release was for the first 11 pupae that would be allowed better odds to achieve breeding in the wild since they were the "early" outliers for the captive stock. Louis Terrazas, a Wildlife Resource Specialist with the USFWS, helped with this process. Releases involved USFWS staff, volunteers for surveying the wild population, The Urban Wildlands Group, and Moorpark College volunteers and interns (Figure 33).

Figure 33. Release of captive-bred Lange's metalmarks at ADNWR, July 2012.

Table 10. Releases of Lange's Metalmarks at ADNWR, July–September, 2012.

Release Date	Location	Geneline	E-cup	Pupae	Imagoes
7/27/12	N/A	11002	1	6	0
7/27/12	N/A	11001	2	1	0
7/27/12	N/A	11003	3	4	0
8/14/12	PG&E EAST	11004	5	5	1M
8/14/12	PG&E EAST	11006	6	2	4M
8/14/12	93 Dunes	11001	7	2	1M
8/14/12	PG&E EAST	11003	8	5	1M
8/14/12	93 Dunes	11002	9	2	2M, 3F
8/14/12	93 Dunes SOUTHWEST	11004	10	2	2M, 1F
8/14/12	93 Dunes SOUTHWEST	11006	11	6	0
8/14/12	93 Dunes	11002	12	6	0
8/14/12	PG&E EAST	11002	13	5	0
8/14/12	93 Dunes	11002	14	7	0
8/14/12	93 Dunes SOUTHWEST	N/A	15	2	0
8/14/12	93 Dunes	11003	E16	6	0
8/14/12	93 Dunes	11004	E18	2	0
8/14/12	93 Dunes	11003	E19	2	0
8/14/12	93 Dunes	11006	E20	2	0
8/14/12	93 Dunes SOUTHWEST	11002	E21	5	0
9/3/12	PIT	11002	E23	0	2M, 3F
9/3/12	PIT	11004	E24	1	3F
9/3/12	PIT	11001	E26	5	0
9/3/12	PIT	11004	28	1	0
Totals				**79**	**23**

2013

Two releases occurred in 2013. The first was on August 2, 2013, when 21 pupae and 4 adults were released at ADNWR. The second release occurred on August 31, 2013, when 5 pupae and 11 adults were released at ADNWR.

2014

In 2014, we released 1 larva, 7 pupae, and 1 adult, on August 14, 2014, at the Sardis Unit of ADNWR.

2015

The first release involved 10 pupae, on August 6, 2015 at ADNWR. The substrate supporting the pupae was bundled together in groups of two and three. The bundles were held together with a piece of string using either a clove hitch or slipknot, and a length of the string was left as an extra piece. The extra string was then used during the release to tie the substrate bundles to the buckwheat host plant. Another release occurred on August 21, 2015. Five pupae were released to ADNWR end of access road East, five pupae were released to the '92 Dunes, and five pupae were released to the '95 Dunes. Also on the second release a captive raised female that had eclosed the day before was marked on the right fore wing on the ventral side and placed on live plant on the dunes. After several hours a mating was observed. The female was then recollected and brought back to The Butterfly Project.

Table 11. Status of captive breeding and release efforts for Lange's metalmark butterfly.

Year	Females Collected	Larvae Released	Pupae Released	Adults Released	Females Mated in Captivity
2007	5	n/a		n/a	n/a
2008	0	30		30	13
2009	4	88		0	n/a
2010	5	119		0	2
2011	5	2		0	4
2012	5	0	79	23	9
2013	3	0	28	15	4
2014	4	1	7	2	3
2015	5	0	30	1	0*
Total	***36***	***240***	***144***	***71***	***35***

*One captive female was bred with a wild male in the field and then re-captured.

Public Outreach

Public outreach activities were done on a volunteer basis and not paid for by the HRP grant. Outreach to the general public is ongoing at The Moorpark College rearing site for the Lange's metalmark rearing project and for the Palos Verdes blue butterfly rearing project (which has adjacent but separate facilities). A tri-fold educational brochure is always available to the public along the external fence. We also provide Lange's metalmark and Palos Verdes blue butterfly "wings" along the fence that children can stand in front of to have their picture taken (some adults too). A shadow box with specimens and a description of the life history for each butterfly is also attached to the fence (Figure 34). A staffed table with free crafts was set up for the Zoo's "Spring Spectacular" event each year.

Figure 34. Public outreach materials along the fence outside the rearing area for Lange's metalmark at America's Teaching Zoo at Moorpark College.

In 2012, we cooperated with the reporter for an article on Lange's metalmark in Bay Nature magazine (April–June, 2012, p. 57), which provided good exposure for the project and the conservation efforts being undertaken. We also hosted a visit at Moorpark College by interns from the Tatzoo project, which selected Lange's metalmark as one of the species for its summer 2012 program. More information is available at www.tatzoo.org. In addition, Dr. Johnson and students from the project gave public lectures that resulted in coverage in the local media. See links at:

http://www.topangamessenger.com/story_detail.php?ArticleID=5609

http://www.mpacorn.com/news/2012-09-28/Schools/Bringing_back_the_butterflies.html

In 2013, we were invited to run a booth at the Bug Fair at the Natural History Museum of Los Angeles County on May 18th and 19th. Volunteering student interns presented exhibit pamphlets and an educational shadow box with life history and pinned specimens of endangered butterflies. They interacted with adults and children and staffed a table with free crafts.

Project personnel were interviewed previously for a new book that has a large section on Lange's metalmark, and which has received significant critical acclaim: *Wild Ones: A Sometimes Dismaying, Weirdly Reassuring Story About Looking at People Looking at Animals in America*. This book generated numerous news stories that told the story of the butterfly and the captive rearing program, including in *The Atlantic* (http://www.theatlantic.com/technology/archive/2013/08/how-the-polar-bear-lost-its-power-and-other-animal-tales/278281/).

We ran crafts tables at two events at America's Teaching Zoo where the rearing site is located. The "Boo At the Zoo" event was on October 27, 2013, and the "Arctic Lights" event was on December 27–28, 2013. At each event we distributed educational pamphlets for children of different age groups, and displayed an information board about the rearing project. Project volunteers interacted with children and adults and manned a table with free butterfly crafts (Figure 35).

Figure 35. Children make butterfly sculptures out of tissue paper at an outreach event.

In 2014, a camera crew from the University of California Los Angeles (UCLA) interviewed Dr. Jana Johnson as a distinguished alumni and this video was posted at the UCLA Daily Bruin website under the headline *UCLA Alumna Saves Butterflies from Extinction*.

Educational Exchange

The project proposed setting up an educational exchange with California State Polytechnic University, San Louis Obispo. Difficulties were encountered in setting up this exchange and so in consultation with the project officer, a similar outreach effort was undertaken with the University of Southern California (USC) and with UCLA. The objective of the educational exchange was to increase capacity for rearing efforts for endangered butterflies and to attempt to rear a surrogate butterfly species with similar needs as Lange's metalmark.

The USC exchange was with the Landscape Architecture program. Three students were recruited and reviewed all of the written training materials for The Butterfly Project. To better document the materials, equipment, and setup for the facilities at The Butterfly Project, the students developed technical diagrams that document the specialized equipment used by the project (See Appendix). The students have visited the Moorpark College on several occasions and developed site plans for the rearing site to be able to then develop alternative designs and specifications for establishing a new rearing site.

Building on the plans and documentation developed by the USC landscape architecture students, a UCLA environmental science student was recruited to undertake the surrogate species rearing in summer 2014. The student has designed the enclosures, purchased the materials and constructed the rearing facilities entirely from the plans provided.

We identified a surrogate metalmark species (*Apodemia mormo arenaria* Emmel & Emmel), which is endemic to the El Segundo dunes on the coast of the Santa Monica Bay in Los Angeles. This species uses the coast buckwheat (*Eriogonum parvifolium*) as the larval foodplant.

Locally sourced food plant was obtained from the SEA Lab in Redondo Beach, which grows plants for local restorations at a nursery that was previously established in a cooperative project with The Urban Wildlands Group. The student surveyed the coastal dunes and located a site on City of Los Angeles property where the surrogate metalmark species could be taken without the need for any permits and obtained several butterflies. It was late in the flight period – perhaps shortened because of the drought – but a few remaining individuals were obtained. These individuals were then housed with the foodplants, provided nectar, and monitored until they died. Unfortunately, it does not appear that oviposition was stimulated in this attempt, but the feasibility of establishing a second site and a suitable surrogate species and source was shown.

Figure 36. ***Apodemia mormo arenaria*** **at Dockweiler State Beach (Photograph: John Eggers).**

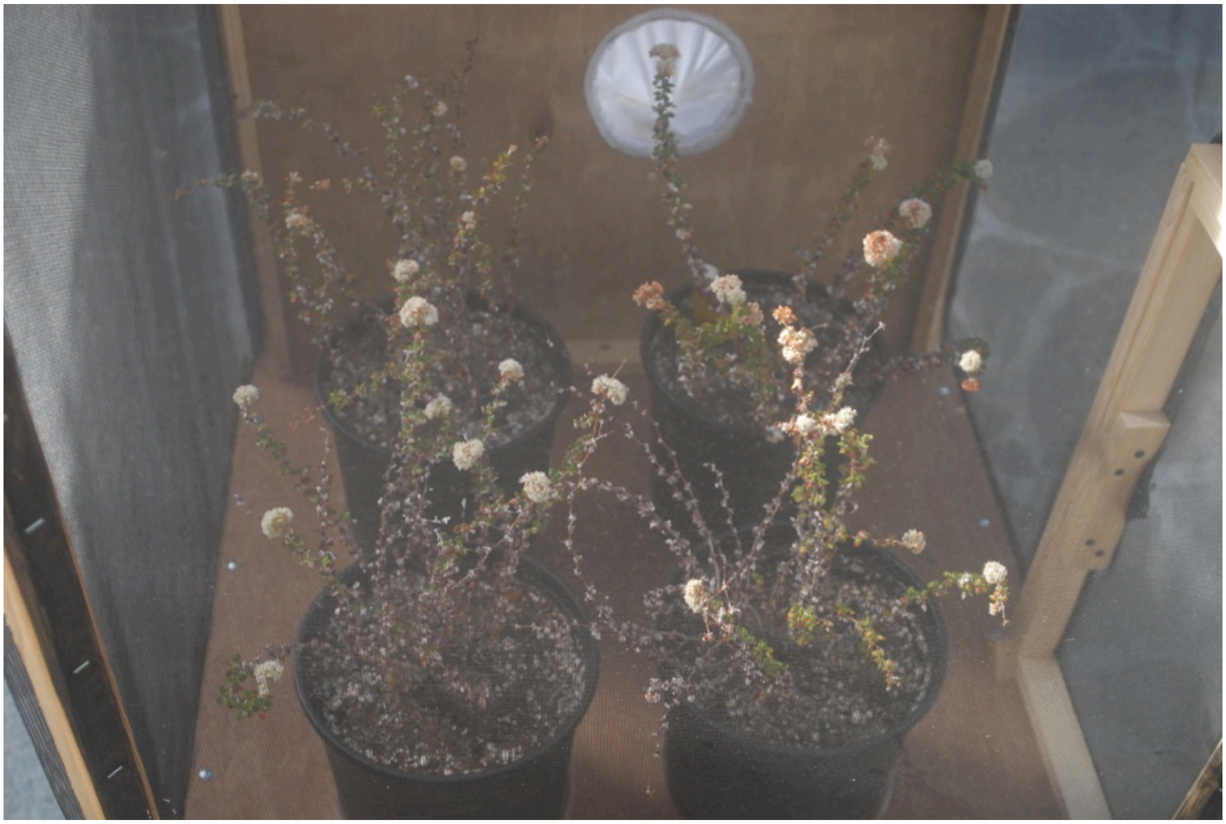


Figure 37. Captive rearing setup for surrogate metalmark species with appropriate buckwheat hostplant.

Discussion

Collection

Collections have run very smoothly. We typically collected and kept 5 foundresses or 10% of the peak flight. We have found that about 1 in 5 females collected will be infertile, suggesting that there may be some Allee effects occurring in the population (Calabrese and Fagan 2004).

The females collected in 2013 and 2014 were worn and probably farther into their lifespans than would be optimal to produce the maximum number of eggs. This may be the result of a shifting flight period in response to the drought. We have collected on the same weekend (Labor Day) in 2012, 2013, and 2014 and have moved that date earlier for 2015.

Oviposition and Adult Maintenance

We have been successful in stimulating oviposition in captivity and extending the longevity of the adult butterflies (est. 3–5 days in the field). We have been successful in keeping wild-caught adults alive in captivity. The data do not allow calculation of lifespan of wild-caught adults because age at capture is unknown. But given the short lifespan in the wild (est. 3–5 days), longevity of adults in captivity well exceeds this number.

Eggs

The system that we developed for mapping and counting eggs is an excellent one that has resulted in the building of a database that will be revisited for future research. The counts and maps were quadruple-checked during the September–December months. The maps suggest that the current literature stating that oviposition is at the base of the petiole of buckwheat (Black and Vaughan 2005) is inaccurate. Females do oviposit there, but they also oviposit on the top of the leaf, bottom of the leaf, along the edge of the blade, next to the midvein of the blade, all along the petiole and on the stem itself. Females appear to prefer blades with more topographic relief on them, giving plenty of indentations to house their eggs.

Successful egg hatching has been a challenge. Consequently, due to issues with partial hatches (where the first instar starts to hatch and then fails to complete the hatch, dying inside of the egg), when eggs are stored apart from living foodplant, we store our eggs on/with living foodplant. This does produce conditions where some eggs are covered by mold/fungus. It should be noted that mold/fungus does not necessarily mean the death of the egg; we have seen these eggs hatch successfully. The treatment of mold/fungus with bleach water is ill-advised because using a concentration that is high enough to kill the mold/fungus may also kill the egg, and using a concentration that is dilute enough to preserve the egg usually promotes fungal/mold growth due to the addition of water and the bleach being too dilute to kill it.

A very few eggs will hatch during the September–January time period, and the larvae that hatch early have a high mortality rate. With our protocols and in our location, the main hatch is in February, when the weather begins to warm up.

In past years containers with eggs and larvae were opened more frequently to obtain more data points, such as hatch date and instar progression. In a concern for eggs and larvae being lost due to the extra manipulation, especially first and second instar larvae that are easily knocked from the plant and difficult to recover if they are knocked from the plant (they perform a ventral flexion into a tight circle and refuse to grab onto anything once they fall), a protocol of limited manipulation was established in 2013.

During winter, it is a concern that eggs will get too cold being housed outside, and when expected temperature is lower than 50° F blanket covers are placed on top of the boxes to reduce temperature loss inside the multiboxes. In the past when expected temperatures are lower than 45° F egg plants are moved into a greenhouse with a heater set to 50° F, but for the 2015–2016 winter season plants were not brought into greenhouse. The reasoning behind this change in protocol is that low temperatures experienced in Moorpark are not significantly different from the low temperatures in Antioch. Matching of the captive stock's environmental temperature and humidity to that experienced in the wild habitat is vital for timing and success in the rearing. Also during the afternoon the greenhouse can become considerably warmer than surrounding temperatures, and risks stimulating an early hatch that will lead to losses. The greenhouses also have a much lower humidity than the outside surroundings and we wanted to avoid desiccating the eggs.

A set of eggs from a fertile Lange's metalmark female that did not hatch in the February following their oviposition were maintained for two and one half years to check for multi-year egg diapause. They eventually collapsed.

A few eggs were lost during their care. Their attachment to the leaf's trichomes can be tenuous (especially if the female was old at the time of oviposition) and may fall free. The intensive data that we collected in 2011 and 2012 increased our understanding of the species, but seems to have reduced the survival rate of eggs and larvae.

Larvae

Larvae are similar to eggs in that they survive better with fewer disturbances, but this precludes recording data. Losses of larvae in other studies are reported to be high (Arnold 1981). Losses are highest during the first two instars (the first three months). Larvae do not like to be disturbed at all and if accidentally disturbed tend to let go of the foodplant and fall to the substrate, wrapping themselves into a small ball (reminiscent of pillbugs). Because of this behavior, we use soil covers to insure that the instars do not fall into the soil where they cannot be recovered. It is very difficult to recover the smaller instars that fall to the soil cover due to vibration/disturbance because they are the size of half an eyelash. To return them to the plant requires pulling a piece of tissue paper apart and using the fibers to ensnare the first/second instar, and then wedging the tissue between the stem and the petiole of a leaf and leaving the instar to crawl free and back onto the plant.

Pupae

Care for pupae has improved drastically over the past four seasons. They are extremely delicate and in laboratory conditions prefer to pupate in communal shelters. They need to be protected from light to avoid expending energy during pupation. The data sheet and the weighing system for the pupae worked very well in 2015 and will provide further insight into the details of this step in the rearing process.

Breeding

It is incredibly difficult to stimulate metalmarks to mate in captivity. Arnold (1981) did not attempt breeding, and we are the only facility that has successfully bred Lange's metalmark in captivity (Fall 2007). This has been a very difficult feat to replicate. We continue to try to get the correct cues to create a reliable breeding approach.

Recommendations

The end of this cooperative agreement provides an opportunity to reflect back on the captive breeding program and its integration with the recovery efforts for the butterfly in general. The captive rearing program is a cornerstone of any recovery strategy (Richmond et al. 2015).

As we have articulated in the past, a captive program should be maintained as insurance against catastrophic loss of the wild population until the wild population exceeds 500 on the peak survey date.

The multi-year view of the success of the rearing program has led to an adjustment of the timing of the collection of the wild females. An earlier capture date (before Labor Day) for females is advisable during drought years because the timing of the flight during those years tends to be earlier than during wetter years, when rains generally extend later into the spring.

As the habitat quality at Antioch Dunes continues to improve, it may be time to revisit ideas surrounding the use of flight cages at the dunes. These can be used to confine a gravid female on a foodplant at a site were reintroduction is desired. The female will lay eggs on the plant and then can be moved to another location or kept in the captive breeding program.

The temporary release and recapture of a captive female to allow her to mate with a wild male proved successful in 2015. This approach should be repeated, given the ongoing difficulty of reliably inducing mating activity in captivity.

If and when funds are available for an intensive research program, the most productive efforts might be in assessing survivorship of larvae in the wild. Although labor-intensive, such an effort might yield insights on the role of soil in the life cycle of larvae, food preferences (green leaves, old leaves, young leaves, moist leaves, turgid leaves), and, importantly, mortality rates at each instar for comparison with the captive breeding program. Such a study might best be undertaken by a postdoctoral researcher focusing solely on the species.

Finally, the habitat management and enhancement programs being undertaken at Antioch Dunes NWR must be continued for the captive breeding program to be anything other than a temporary conservation measure. The innovative restoration efforts currently being undertaken to build new, dynamic dune habitat are the best hope for long-term recovery of the species.

Summary of Expenditures

Total expenditures from 2011–2015 were as follows.

Project Management	$49,426
Employer Expenses	$11,369
Rearing Director	$54,500
Student Stipends	$77,424
Supplies (inc. plants)	$6,608
Travel	$6,692
Educational Exchange	$7,500
Entomologist	$7,751
Rent	$4,800
Insurance	$1,317
Indirect Expenses	$21,145
Total	***$248,531***

No major property was obtained during the project.

Data Collected

All quantified data collected in this project are represented in this report. Laboratory notebooks contain day-to-day notes collected in implementing the project and will be kept on file at The Butterfly Project.

Analytical Tools

No special tools are necessary to process the data collected in this project.

Problems, Delays, or Adverse Conditions

We did not reach an agreement to work with California Polytechnic University, San Luis Obispo. Instead we undertook educational exchanges with USC and UCLA. This delayed the exchange somewhat but still met the intent of the exchange, which was to increase capacity for captive butterfly rearing. The USC students documented the equipment and physical layout of the rearing efforts in a manner that will make it far easier for a new rearing site to be established. The UCLA student showed that the equipment could be built from scratch using the plans only and identified a suitable surrogate species that was obtained and maintained in the new equipment. The combination of these experiences and outputs demonstrated that launching a new program is feasible and improved the technical documentation for the practices and physical plant at the current rearing facility.

Literature Cited

Arnold, R. A. 1981. Captive breeding of the endangered Lange's metalmark butterfly. Final report for the period 1 Sept. 1980 - 30 Sept. 1981. University of California, Berkeley.

Arnold, R. A. 1983. Ecological studies of six endangered butterflies (Lepidoptera, Lycaenidae): island biogeography, patch dynamics, and the design of habitat preserves. University of California Publications in Entomology 99: 1–161.

Bettelheim, M. 2005. Taking refuge: the endemic nature of the Antioch Dunes. Bay Nature 2005: 8–11.

Black, S. H., and D. M. Vaughan. 2005. Species profile: *Apodemia mormo langei*. *In* M. D. Shepherd, D. M. Vaughan and S. H. Black (eds.), Red List of Pollinator Insects of North America, CD-ROM Version 1 (May 2005) ed. The Xerces Society for Invertebrate Conservation, Portland, Oregon.

Calabrese, J. M., and W. F. Fagan. 2004. Lost in time, lonely, and single: reproductive asynchrony and the Allee effect. American Naturalist 164: 25–37.

Comstock, J. A. 1938. A new *Apodemia* from California. Bulletin of the Southern California Academy of Sciences 37: 129–131.

Davenport, K. E., R. E. Stanford, and R. L. Langston. 2011. Flight periods of California butterflies for "resident species", subspecies and most strays to the state. The International Lepidoptera Survey Newsletter 11: 1–67.

Feininger, A., R. Vishniac, and E. S. Ross. 1955. A communal life on the dunes [Painting by Walter Linsemaier]. Life 39: 50–55.

Johnson, J. J., and T. Longcore. 2008. Protocol to protect genetic integrity of captive Lange's metalmark butterfly at The Butterfly Project, Moorpark College, pp. 1–4. The Urban Wildlands Group, Los Angeles.

Johnson, J. J., T. Longcore, A. Clause, G. Pratt, J. Dunn, and K. Osborne. 2007. Propagation handbook for Lange's metalmark butterfly, *Apodemia mormo langei*. First edition, pp. 1–18. The Urban Wildlands Group, Los Angeles.

Johnson, J. J., J. Jones, M. Baudour, M. Wagner, D. Flannery, D. Werner, C. Holden, K. Virun, T. Wilson, J. Delijani, J. Delijani, B. Newton, D. Gundell, B. Thummar, C.

Blakey, K. Walsh, Q. Sweeny, T. Ware, A. Adams, C. Taylor, and T. Longcore. 2011. Captive rearing of Lange's metalmark butterfly, 2010–2011, pp. 1–24. The Urban Wildlands Group, Los Angeles.

Longcore, T., J. J. Johnson, and K. Osborne. 2008. Lange's Metalmark butterfly augmentation plan, pp. 1–9. The Urban Wildlands Group, Los Angeles.

Proshek, B., J. R. Dupuis, A. Engberg, K. Davenport, P. A. Opler, J. A. Powell, and F. A. H. Sperling. 2015. Genetic evaluation of the evolutionary distinctness of a federally endangered butterfly, Lange's Metalmark. BMC Evolutionary Biology 15: 73.

Rabinowitz, D. 1981. Seven forms of rarity, pp. 205–217. *In* H. Synge (ed.), The biological aspects of rare plant conservation. Wiley, New York.

Reveal, J. L. 2007. A new variety of *Eriogonum nudum* (Polygonaceae) from California. Phytologia 89: 287–289.

Rey Benayas, J. M., S. M. Scheiner, M. G. Sánchez-Colomer, and C. Levassor. 1999. Commonness and rarity: theory and application of a new model to Mediterranean montane grasslands. Conservation Ecology 3: 5.

Richmond, O.M.W., Kelly, D. and Longcore, T. 2015. Lange's Metalmark Butterfly Threat Assessment and Ranking of Potential Management Alternatives: Final Report. U.S. Fish and Wildlife Service, Pacific Southwest Region. National Wildlife Refuge System Inventory and Monitoring Initiative. Sacramento, CA, USA.

Spencer, W., P. Fiedler, G. Geupel, M. Holyoak, P. Kelly, and G. Wylie. 2008. Bay Delta Conservation Plan: Independent Science Advisors Report Concerning Non-Aquatic Resources. Bay Delta Conservation Plan Steering Committee.

U.S. Fish and Wildlife Service. 1984. Recovery plan for three endangered species endemic to Antioch Dunes, California. U.S. Fish and Wildlife Service, Portland, Oregon.

Zonneveld, C. 1996. Being big or emerging early? Polyandry and the trade-off between size and emergence in male butterflies. American Naturalist 147: 946–965.

Appendices

Raw Data from Habitat Surveys of Antioch Dunes National Wildlife Refuge, 2008, 2012, and 2015

Unit	Site	Year	# plants	Plant Area (m^2)	% large plants	Plants per 4 m^2	% Tree Cover	% Exotic	Notes
Sardis	92 Dunes	2008	40	1200	75	5	10	95	
Sardis	92 Dunes	2012	75	300	95	5	25	95	
Sardis	93 Dunes	2008	150	5000	75	5	50	80	sinuous distribution
Sardis	93 Dunes	2012	200	5000	90	7.5	50	90	occupied
Sardis	95 Dune Additional	2008	100	600	80	10	10	75	
Sardis	95 Dune Additional	2012							
Sardis	95 Dunes North	2008	120	1200	95	10	10	90	
Sardis	95 Dunes North	2012	25	105	95	7.5	0	95	
Sardis	A	2015	250	160	90	3	30	95	main area
Sardis	B	2015	50	200	95	3	30	95	
Sardis	C	2015	50	150	100	3	5	99	
Sardis	D	2015	250	300	95	3	5	99	
Sardis	Domtar	2008	50	1800	100	2	5	80	
Sardis	Domtar	2012							
Sardis	E	2015	100	700	95	2	1	99	
Sardis	East of Corridor	2008	50	3200	80	2.5	0	95	
Sardis	East of Corridor	2012	100	350	100	5.5	10	95	
Sardis	Eucalyptus Hill	2012	170	1400	100	3	5	90	
Sardis	Flat	2015	50	150	95	6.5	5	95	
Sardis	Main Woods	2015	0				75	99	main wooded area
Sardis	PG&E New Dunes	2008	60	2400	80	2.5	5	95	
Sardis	PG&E New Dunes	2012	100	1250	90	7.5	10	95	
Sardis	PG&E North End/River Crest	2008	10	400	50	1	50	100	

Sardis	PG&E North End/River Crest	2012						100	
Sardis	PG&E West	2008	50	600	75	6	10	90	
Sardis	PG&E West	2012	55	200	100	7.5	10	90	clearing n of tower
Sardis	PG&E West	2015	0				5	95	
Sardis	PG&E West new Dunes	2015	100	2400	95	5.5	5	95	
Sardis	River Crest Triangle	2015	150	125	66	8.5	50	100	
Sardis	River Flat	2012	50	200	97.5	7.5	25	90	
Sardis	S	2015	0				10	100	south end
Sardis	Sardis 95 Dunes	2008	300	4900	85	10	25	95	LMB in 2007
Sardis	Sardis 95 Dunes	2012	375	4900	95	10	25	95	
Sardis	Small Inner Hill	2012	70	200	95	7.5	0	90	
Sardis	South Corner Slope	2012	30	200	95	5	25	100	one worn LMB
Sardis	South of Path	2008	50	1600	75	5	0	100	
Sardis	South of Path	2012							
Sardis	South of Tower	2015	35	200	95	3.5	10	95	heavy gypsum deposits
Sardis	South Plateau	2012	15	100	100	4	0	95	
Sardis	SW Corner Slope	2015	30	60	100	3	10	100	
Sardis	West of Corridor	2008	30	1500	75	10	0	95	
Sardis	West of Corridor	2012	130	1300	99	7.5	5	95	
Sardis	West Slope	2015	0				10	100	
Sardis	X	2015	90	200	90	6	10	100	SW bole
Sardis	Z	2015	140	750	90	3	30	75	
Stamm	95 Dunes	2015	60	100	100	5.5	5	99	
Stamm	Blowout	2008	120	2800	95	2.5	0	50	
Stamm	Blowout	2012	180	500	95	7.5	0	90	vetch
Stamm	Blowout	2015	150	1200	95	5.5	0	75	dense, vetch-brome

Stamm	Car Body	2012	25	75	100	5	0	100	
Stamm	Car Body	2015	20	15	100	3	5	95	
Stamm	Car Body + Vinyard	2008	50	1500	95	5	0	95	
Stamm	East Plateau	2008	50	300	5	4	0	95	planted
Stamm	East Plateau	2012	50	200	98	5	0	95	plants in north
Stamm	East Plateau	2015	0				0.1	100	mostly brome
Stamm	Hardpan 2/River Plateau	2008	10	300	100	2.5	0	95	
Stamm	Hardpan 2/River Plateau	2012	140	850	99	5	1	95	all on eastern end
Stamm	Hardpan 2/River Plateau	2015	80	100	95	10	10	95	eastern remnant
Stamm	Hardpan 3	2008	40	400	100	5	0	75	
Stamm	Hardpan 3	2012	220	300	99	3.5	0	95	
Stamm	Hardpan 3	2015	150	400	100	6.5	1	75	
Stamm	Hardpan 4	2008	35	1800	100	1.5	0	95	
Stamm	Hardpan 4	2012	250	1000	95	5.5	0	95	
Stamm	Hardpan 4	2015	300	300	100	6.5	0	75	
Stamm	New	2015	5		95	1	0	0	new dune
Stamm	Northeast	2008	100	2500	95	10	0	75	
Stamm	Northeast	2012	160	300	98	7.5	0	90	
Stamm	Northeast	2015	70	100	95	3	0	95	
Stamm	Restored Vineyard	2015	400	625	95	10.5	0.1	75	
Stamm	Restored Vinyard	2008	200	2500	90	10	0	50	grazed
Stamm	Scarified	2008	75	1600	99	4.5	0	95	hardpan
Stamm	Scarified	2012	300	1400	95	5.5	0	95	
Stamm	Scarified	2015	330	2000	100	10	0	95	
Stamm	South of Path	2008	60	550	95	7.5			
Stamm	South of Path	2012	180	1200	95	7.5	1	95	
Stamm	South of Path	2015	150	104	99	5.5	1	99	
Stamm	Stamm 95 Dunes	2008	90	800	95	7.5	0	50	
Stamm	Stamm 95 Dunes	2012	70	1200	99	7.5	5	95	
Stamm	Triangle	2015	0				20	90	
Stamm	Triangle to Oak	2008	10	400	100	1	0	50	partially grazed
Stamm	Triangle to Oak	2012	20	25	95	10	10	75	grazed
Stamm	Triangle to Oak	2015	10	15	100	10	10	100	

Stamm	Vineyard Plateau	2015	100	425	95	5.5	1	75
Stamm	Vinyard East	2012	150	120	75	8	5	75
Stamm	Vinyard Plateau	2012	50	500	95	5	0	100
Stamm	Vinyard West	2012	385	1025	95	10	1	95