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**FINAL REPORT
FOR
PALOS VERDES BLUE BUTTERFLY YEAR 2009 CAPTIVE REARING
ON
DEFENSE FUEL SUPPORT POINT
SAN PEDRO, CALIFORNIA
AND
THE BUTTERFLY PROJECT
MOORPARK, CALIFORNIA**

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EXECUTIVE SUMMARY

In 2009 the captive population of Palos Verdes blue butterfly (PVB) was reared at the Defense Fuel Support Point (DFSP) and The Butterfly Project at Moorpark College. Sufficient numbers of larvae and adults were available to conduct release onto managed habitat areas. Key findings and outcomes are as follows:

- The November and February partner meetings were key to this season progressing smoothly and successfully.
- 512 gravid females and an estimated 3,000–5,000 third and fourth instar larvae were released to the wild in compliance with existing U.S. Fish and Wildlife Service permits.
- At the close of the season 1,834 pupae were in captive stock from 2009 and previous years.
- The focus of the rearing program is on reintroductions to the wild establishing new populations, research and a refugium population.

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1. Introduction

The Palos Verdes blue butterfly, *Glaucopsyche lygdamus palosverdesensis* (Lepidoptera: Lycaenidae: Polyommatainae) (Figure 1), was thought extinct in 1983 when the last known population of the time was bulldozed to make way for a baseball field (Mattoni 1993). The subspecies was subsequently discovered on the Defense Fuel Support Point (DFSP) in San Pedro in 1994 (Mattoni 1994). Palos Verdes blue butterflies at DFSP were found to feed on both *Lotus scoparius* and *Astragalus trichopodus lonchus* (both Fabaceae) as larvae, which occurred there naturally and is now found in revegetated coastal sage scrub (Mattoni 1994).

Before 1994, larvae of the Palos Verdes blue butterfly had been observed to feed solely on *Astragalus trichopodus lonchus*, discovery of its use of *Lotus scoparius* and *Astragalus* expanded the known larval foodplants for the subspecies (Mattoni 1994). In captivity, Palos Verdes blue adults (imagos) seem to prefer to oviposit on the *Astragalus*, while the larvae show a strong feeding preference for the *Lotus*. The preferential oviposition may be an artifact of blooms being present primarily on the *Astragalus* in nursery-reared foodplant. Egg production rates are highly impacted by exposure to direct sunlight. In developing our secondary level of containment for the 2009 season, it was noted that the egg production was diminished (presumably from the decreased light exposure due to the secondary level of containment). Egg production in Moorpark was similar to San Pedro, where the marine layer frequently leads to less light exposure for the females.

In 1994, a captive propagation program was established to guard against extinction (Mattoni et al. 2003). The number of pupae in captivity at the end of each season has varied from 93 to 4,513. The maximum production came from the 2008 season and represents unprecedented success in comparison with other lycaenid rearing reports (Herms et al. 1996). This report outlines the 2009 captive rearing season.

The rearing project meets in part the conditions of the United States Fish and Wildlife Service's (USFWS) Biological Opinion on the Formal Section 7 Consultation for the Chevron 1-8" Pipeline and Associated Government Pipelines Project, Defense Fuel Support Point, San Pedro, Los Angeles County, California (1-6-96-F-09). The current captive propagation program utilizes methods developed by Johnson, Pratt, and Mattoni in line with recommendations by the USFWS (Johnson et al. 2008; Mattoni et al. 2003; Mattoni 1988; Pratt & Stouthamer 2002).

Rearing for the 2009 season was conducted by Dr. Jana Johnson as permitted under USFWS Biological Opinion 1-6-96-F-09. Additional care was provided by the subpermittees on List C of the Fourth Amendment to the Biological Opinion 1-6-96-F-09. Subpermittees received extensive training prior to handling the captive stock.

Captive rearing was conducted at two locations in 2009. The laboratory facilities at DFSP were used for small portion of the stock. The remaining stock were reared at The Butterfly Project, which is a collaborative effort between The Urban Wildlands Group and Moorpark College, including America's Teaching Zoo and the Department of Biology, where Dr. Johnson is employed. Since 2006, the PVB population has significantly increased by implementing a dynamic rearing approach with labor intensive methods performed by subpermittees. The new methods

require increased labor and the majority of the production occurs at the Moorpark College rearing site, because of the availability of skilled student labor.



Figure 1. Captive reared, gravid Palos Verdes blue butterfly females released to the wild (Linden H. Chandler Preserve). Photo by Shanna Foster.

2. Methods

2.1. Pupae and Eclosion Chambers

Pupae from the 2008 rearing season that remained in diapause from previous seasons had been placed in refrigeration at the beginning of winter 2008, with the exception of a group of pupae that had been part of an experiment that had been unrefrigerated since 2007, and continued to be unrefrigerated, stored in the DFSP lab on the counter. The lab window is screened and barred, and was therefore left open to allow the lab to equalize with ambient outdoor temperature.

The DFSP pupae were removed from refrigeration on February 16, 2009 and the Moorpark pupae were divided into two “pulls” to spread the labor requirements out and increase the likelihood of having males later in the season (males are resistant to feeding and burn energy patrolling for females. This creates a shorter life span for the males and can be problematic for later eclosing females.) Three quarters were pulled on February 14 and 15 and the remaining quarter was pulled on March 4.



Figure 2. Subpermitees weighing the pupae after they have been sorted according to geneline. The pupae were established in individual assigned seats of geneline specific eclosion cups at that time. (photos by: Jana Johnson).

The pupae were subsequently sorted according to geneline and then weighed using an electronic scale to the nearest mg and recorded in a spreadsheet (Figure 2). Handling of the pupae was with Bioquip featherweight forceps to prevent damage when placing them into the eclosure chambers with crushed walnut shells for substrate. The subpermitees worked in pairs to insure the accuracy of the data record. The weighed pupae were transferred into a specific individually assigned seat of a geneline specific eclosion cup at the same time.

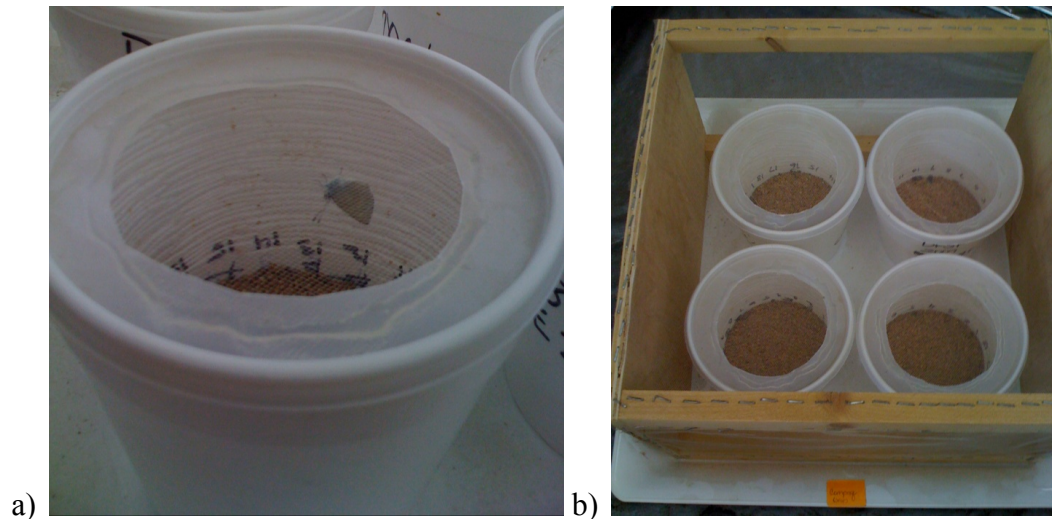


Figure 3. a) New mesh lids that contain eclosing butterflies within their eclosion cup. The substrate inside is crushed walnut shells, the inside walls were scored with a bobby pin to provide traction for the new imagoes. The numbers inside of the cup are the assigned seats for the individual pupae housed in the cup (see appendix for example datasheet for collection of this information). The geneline information is recorded on the outside of the cup. The eclosion cups are stored four to a tray (b) with an eclosion box as the secondary level of containment and the greenhouse as tertiary containment. The stock of individuals descended from an aberrant miniature female was maintained separately.

The eclosion cups at DFSP were the same as used in 2007 (Johnson et al. 2008). Both rearing locations maintained one geneline per eclosion cup, which was noted on the outside of the cup. The eclosion cups at Moorpark College were the same as used in 2008 (Johnson et al. 2009), but with one improvement: a meshed lid that secured containment of the eclosing butterflies to the eclosion cup (Figure 3). These were stored four cups to a tray with an eclosion box over them for secondary containment. The greenhouse served as tertiary containment.

Eclosion is associated with moisture, heat, light exposure, and possibly pheromones. We controlled the timing of eclosion for DFSP stock by blacking out the window of the lab and setting heatlamps on switch timers. The pupae were misted sporadically until blue began to show through the shell of the pupa, but not after. There was no further misting after “bluing” began.

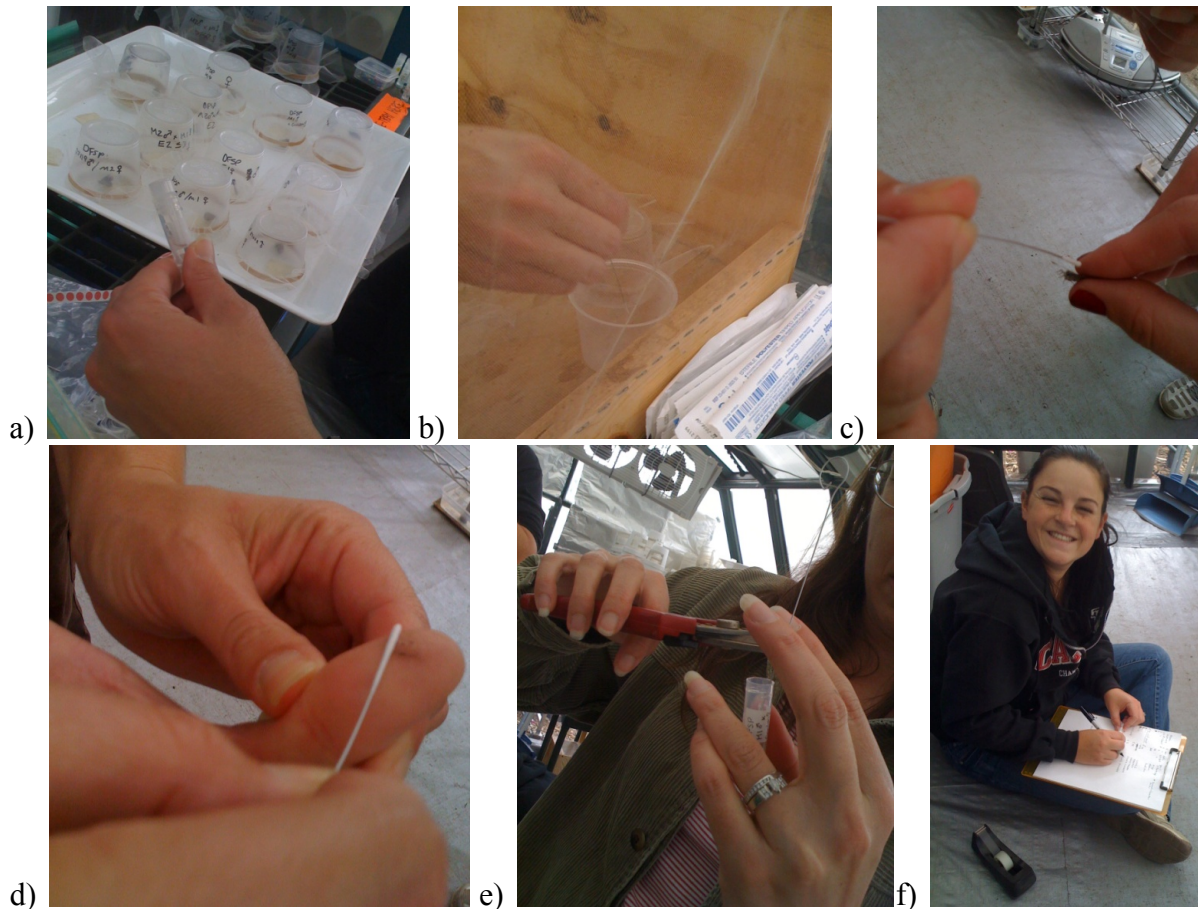


Figure 4. Late eclosion from the sex-specific, unbred holding area (a) were used to test a noninvasive DNA collecting techniques. Cloacal swabs designed for birds were used for the collection. Techniques included: trying to swab them without restraint inside of the handling box (b), “winging” the butterflies for restraint and then swabbing either the ventral side of their abdomen or the dorsal side of their thorax and abdomen (c) or swabbing the scales that remained on the sampler’s fingers (d–e) from having restrained them for the technique. Data were recorded (f) for all samples, which were individually packaged, labeled, and transferred to UCLA for analysis.

We exposed stock at The Butterfly Project to sunlight through the greenhouse walls. No heat-lamps or humidifiers/swamp coolers were utilized. The pupae were misted daily until bluing began. One day misting occurred due to a miscommunication and there was an increased rate of failure to expand with the following day's eclosions.

Boxes were checked for eclosion twice a day from the removal of the pupae through the end of the eclosion period. Daily eclosion checks were performed throughout the summer. Late eclosions were not bred, and were used to investigate non-invasive DNA sampling techniques (Figure 4).

Upon eclosure, the eclosion cup containing imagoes was transferred into a handling box. This is a new method that allows for multiple subpermitees to process emerging imagoes in the same greenhouse without mixing the genelines. The handling boxes had previously been used for manipulation of endangered stock in the field, the application to the lab has been one of the innovations for safety and control of the butterflies, efficiency in usage of lab space, and has decreased stress for the individuals involved in processing.

The handling boxes are constructed out of plywood and mesh with an entry sleeve similar to a multibox container (Figure 5).

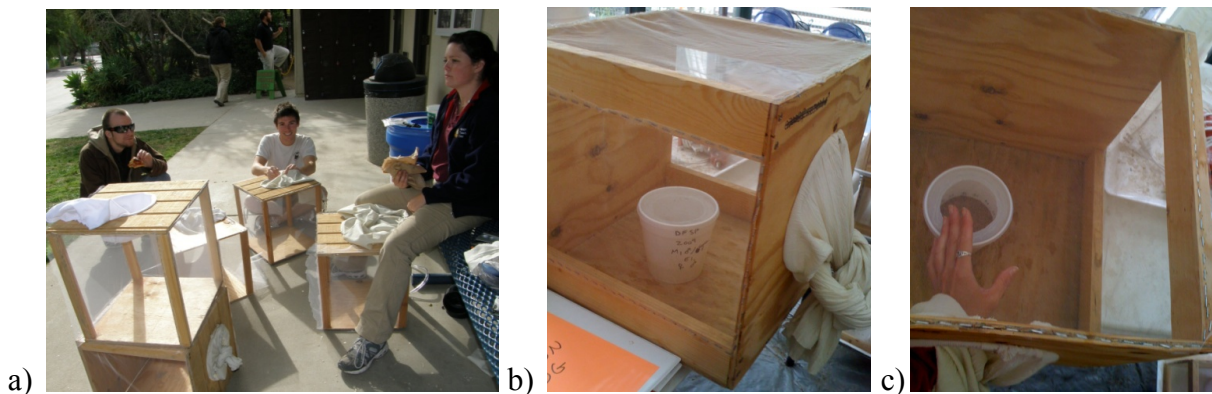


Figure 5. a) Subpermitees constructing the handling boxes. b) A handling box with an eclosion cup inside and then c) demonstration of access to the eclosion cup through the sleeve entry.

This new system for processing allows the handling container to serve as the first level of containment once the eclosion cup is opened and the greenhouse serves as the second level of containment. This is the first season that these two levels of containment were possible at all times.

Once the eclosion cup was open inside of the handling box, the newly emerged imagoes could be processed into holding containers. These holding containers have been standardized to a plastic container that we formerly used for other purposes, but has proven itself valuable as a holding/sorting container (Figure 6). The holding/sorting container is genotype and gender specific and properly labeled. It is secured on the open side with mesh and a thick rubber band, then removed from the handling box and placed into the sorting area of the greenhouse.

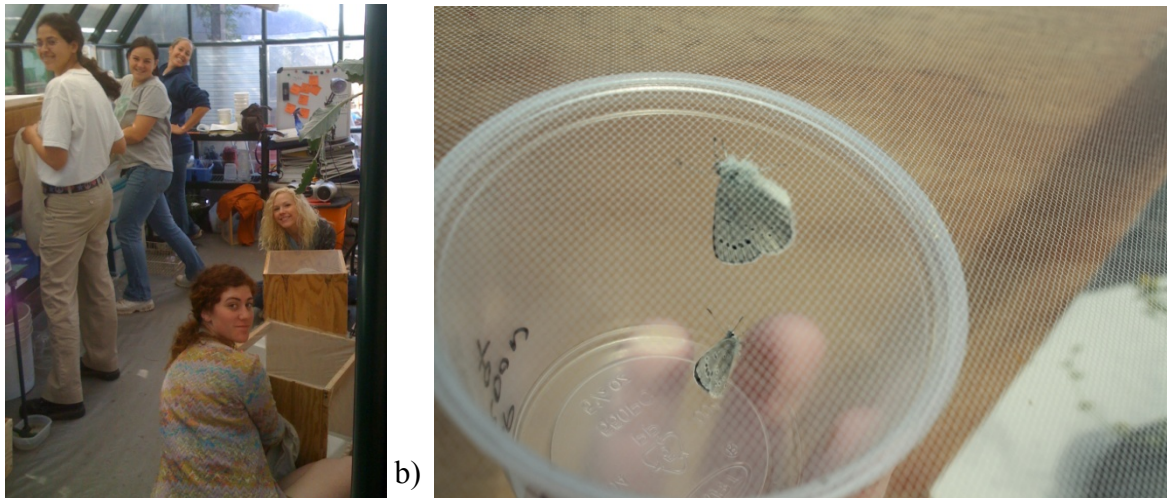


Figure 6. a) Five handling boxes in use by five subpermites in the new greenhouse. b) Two Palos Verdes blue butterfly “sister” females being transferred from an eclosion cup to a holding/sorting container inside of a handling box.



Figure 7. a) Holding containers in the holding area of the new greenhouse. They are oriented upside down to allow for a DFSP honey-water solution saturated feeding station. b) This picture of the multibox inside of a tent is not from the Palos Verdes blue season, but demonstrates the arrangement.

The eclosed imagoes were sorted by geneline and sex and placed in the holding area of the new greenhouse. Butterflies were fed daily while in the holding area while held in sex and geneline specific containers. Based on the distribution of individuals available from each geneline, crosses were established in multiplant boxes (same mass breeding and oviposition containers as the previous two seasons). The brothers from one geneline (preferably a couple of days old) were crossed with sisters from an unrelated geneline (preferably the same day of eclosion). The multi-

plant boxes were maintained ant free within a mesh tent with a vinyl roof (the second level of containment).

Eclosions were recorded in the spreadsheet file for each individual with the date of eclosion, and, when possible, the sex of the individual. If there were multiple imagoes with both sexes present in a single cup, the sex ratio present was recorded, but sex was not assigned to individual seat numbers.

Individuals from the parasitism group of pupae and those with eclosion anomalies (failure to expand, etc) were placed into sex-specific multiplant boxes. They were not bred and were used for educational purposes with zoo patrons and academic classes. They were identified to sex following the same procedures reported in 2007 (Johnson et al. 2008).

2.1. Breeding

Because the captive population is now large, mass rearing techniques were employed. Per the various parties involved and in consultation with the USFWS, not all butterflies were bred. Genelines with limited individuals were maintained in sex specific holding containers in the holding area. All individuals were fed daily and maintained until they died of natural causes.

The butterflies that were bred were housed in multiplant boxes. Multiplant boxes consist of a larger box with three or more potted foodplants inserted inside the box and kept above the ground by legs on the bottom of the box. The box has two sides of plywood with “sleeve” tunnels to allow access and two sides of mesh. The roof is solid clear plastic to eliminate threat from rain and allow sun. The legs are kept in soapy water containers to exclude predators (especially ants, which will harvest adult butterflies). There was the added level of containment by the mesh tent with the vinyl roof with between 4 and 6 multiboxes per mesh tent.

“Sisters” from one lineage would be combined with “brothers” from a separate lineage in each multibox to mate. Crosses were determined daily depending on which individuals eclosed that day. The crosses were designed to maximize diversity of nucleic DNA by mating the butterflies available on a particular day that were least related to each other. With one wild population left and the main concern being to establish robust and self-sufficient new populations, we focused on overall diversity rather than inbreeding specific maternal lines. Releases (except the publicized release at DFSP) were selected from these multiplant boxes. Gravid females were released to Linden H. Chandler Preserve after ovipositing in the multiplant boxes (Figure 8). This insured that we would not lose the genelines if the female were to be predated prior to ovipositing in the wild. We also released eggs and larvae after harvesting eggs from each cross into larval containers. This release protocol was observed at both rearing locations.

We ran two crosses per multiplant box at each location. At the end of the first cross, the gravid females were released and the males were retired to a field cage on the base. It was noted that the males in the field cage were deceased within 48 hours and there were an abundance of ants that gathered in the field cage.



Figure 8. Releases at Linden H. Chandler Preserve including various subpermitees and author Dr. Peter Laufer who featured *The Butterfly Project* in the final chapter of *The Dangerous World of Butterflies* (Lyons Press, 2009; photos by Jana Johnson, Damien Renner, and Tyler Wilson).

On March 20, 2009 there was a press event associated with a release of 60 unbred females and 20 males from the holding area of the new greenhouse to the wild at DFSP.

2.2. Adult Maintenance

All adults were hand fed daily as previously described (Johnson et al. 2008). Captive adults were fed with specialty honey from the hives maintained by Commander Ramer at DFSP, thereby providing artificial nectar similar to nectar sources available on DFSP. Honey was used as a nutrition source following research in 2007 that showed adults fed honey lived on average 4.5 days longer than those fed with “Fierce Melon” Gatorade (Johnson et al. 2008). By physically placing butterflies on the provided nectar, instead of just providing them access to it, has increased longevity of individually caged adult butterflies from 14 days (2005) to a maximum thus far of 38 days (2007). Adults were fed in their multibox containers. Holding containers were fed in the holding area of the new greenhouse.

2.3. Larval Rearing

For DFSP, plants with rearing chambers were stored on an ant free table in the miniature greenhouse attached to the side of the nursery trailer, on tables in the main trailer and on the desktop near the window in the lab within the trailer. This divided the stock to protect against losing all of the stock to a single stochastic event. It also maximized the use of the few windows present in the trailer. The Butterfly Project housed egg and larval stock in rearing containers in the greenhouse and multiplant boxes outside the greenhouse.

All locations were protected from rain and defense against predators while allowing exposure to sunlight. Predator exclusion included but was not limited to placing the legs of tables and multiplant boxes in containers of soapy water, vigilant elimination of any substance that would attract predators, fine cloth that allowed ventilation while excluding pests, and the buildings themselves.



Figure 9. a) Albert Owen (NAVFAC SW Div) and Michele Desrochers viewing an oviposition container (similar to a larval container). b) Larval containers being recapped in the old greenhouse (Photos by: Kim Ramseyer).

Rearing chambers on the potted plants were checked daily for egg development and any signs of aphids or earwigs. Aphids and earwigs were removed by hand when discovered.

First instar Palos Verdes blue butterfly larvae were able to remain in their larval containers on the potted foodplant because organza cloth (reduced gauge material) effectively trapped them on the live foodplant. They were also reared in the multiplant boxes.

Upon reaching 4th instar, they were transferred into the individual rearing containers to prevent cannibalism. The smaller instars experience high mortality in these small, limited ventilation individual containers, therefore the cannibalism is a tolerated risk for the smaller instars.



Figure 10. Storage of late-instar larvae in stacked “condos” of creamer cups.

When the larvae pupated, their container was emptied and left open to allow proper ventilation for pupal skin hardening. After complete hardening of the pupae, their containers were closed and were stored at room temperature.



Figure 11. Pupation sequence. When the larva is prepupal (left) the condo is left open for ventilation to stimulate pupation. Once the pupae have hardened to the darker brown coloration, the majority of the vegetation may be removed from the cup and the cup is resealed.

The pupae were placed into refrigeration at the beginning of October to simulate winter, guarantee no premature eclosions, and aid in synchronizing the 2010 eclosion period.

On, November 2 and 3, construction for a new building, entrance, and alligator enclosure resulted in possible electrical interruption to the PVB refrigerator. America's Teaching Zoo, Moorpark College facilities, and the contractors all worked together and had additional meetings in order to address the need for not interrupting electricity to the PVB refrigerator. Meanwhile, we prepped, tested, and put in place a gas-powered generator as an option to maintain the refrigerator. On the morning of November 2, the foreman kindly arranged for the refrigerator to have continuous power supply. It was the only continuous power on zoo grounds during the two-day period.

Data from the digital thermometer reflects that the refrigerator temperature regime was not disturbed. The digital thermometer is monitored hourly during zoo operating hours by zoo students on "rounds." In addition, a graduate student with UCD has loaned the project iButtons that log temperature automatically and one iButton was placed in the fridge (just after the pupae went into the refrigerator) and one iButton was placed just outside the refrigerator. We will be able to provide the hourly data from these iButtons in the 2010 report.

3. Results

3.1. Subpermittee Labor

The labor data was compiled from the compliance notebook where all subpermittees record their hours and activities. It does not include Johnson's hours. From January to the end of May, 2560 subpermittee hours were logged on PVB labor at Moorpark College. Peak hours were for weigh in, when the pupae are pulled from the refrigerator, individually weighed and established in eclosion chambers and the PR event on DFSP grounds.

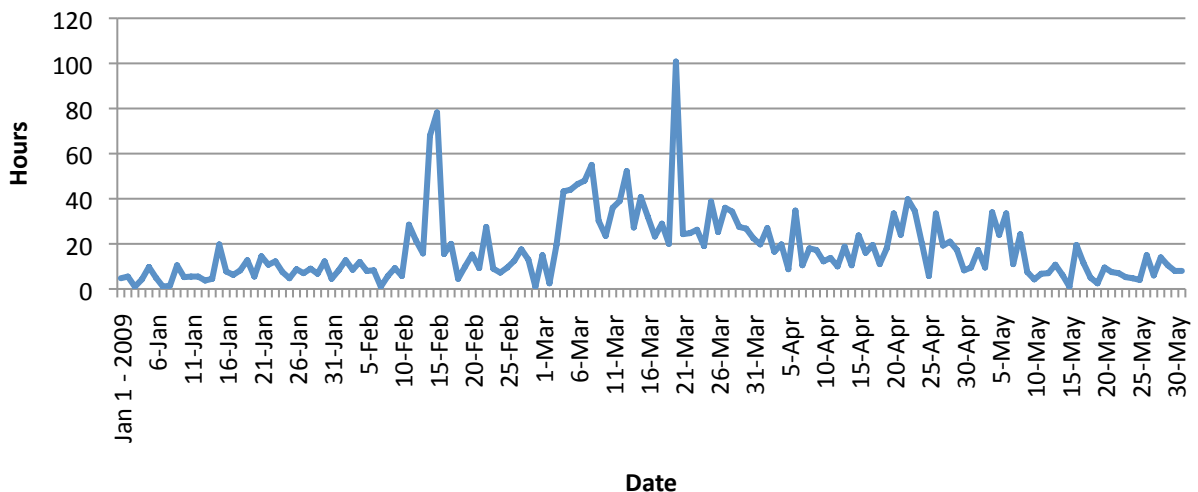


Figure 12. Daily total hours logged by subpermittees during the most active part of the season.

3.2. Pupae and Eclosion

In 2009, 2,165 butterflies eclosed with a sex ratio of 982 male : 964 female : 217 unknown. This even sex ratio suggests that the species is not infected by *Wolbachia*, a parasite that skews sex ratio toward males that has been identified as a concern for other endangered lycaenids (Nice et al. 2009).

DFSP captive stock had a 201: 260 male-female ratio with 37 unknown (sex not recorded in raw data) and one possible gyandromorph, but there were eclosion anomalies with the side of the body that appeared male and threw doubt on the phenotypic determination. Peak eclosion was 21 days after the pull on 3/9 with 59 eclosions on that day.

Captive stock at Moorpark College had a 781: 704 male-female ratio with 80 unknown (sex not recorded in the raw data). Peak eclosion was 26 days after the first pull on March 12 with 157 eclosions on that day. Our ability to spread the distribution of eclosion over several weeks was a huge improvement over the previous year. Figure 13 contains information from 2002–2009 (including 2008 data, which was missing from 2008 report).

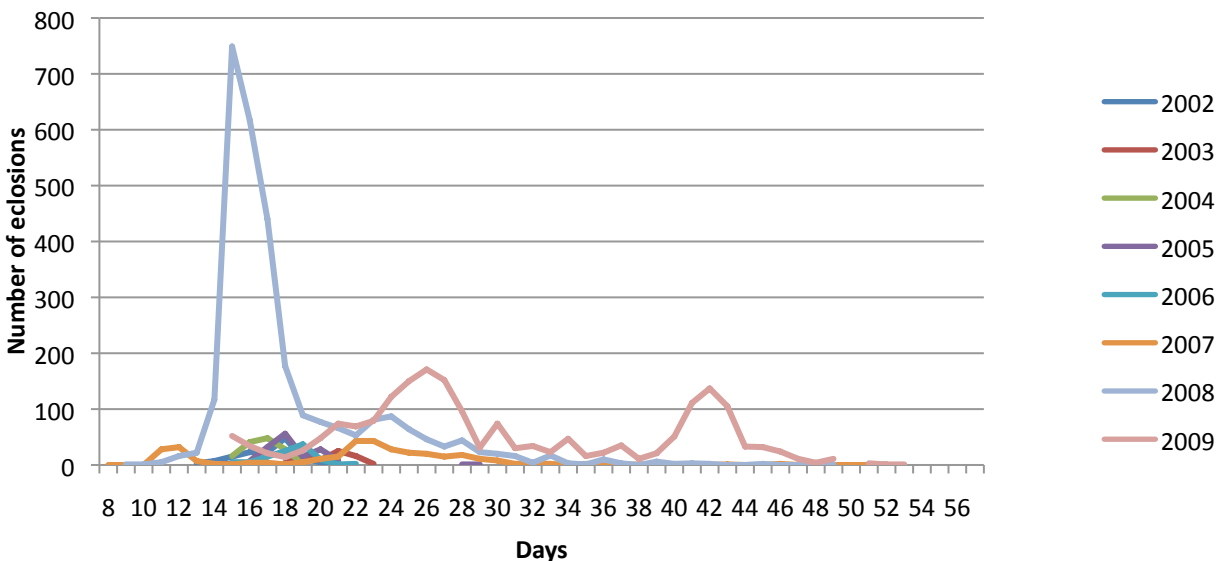


Figure 13. Comparison of eclosion curves for imagos 2002–2009. The bimodal distribution of emergence in 2007 results from pupae at DFSP being removed from refrigeration and eclosing before the pupae at The Butterfly Project. The labor required to maintain butterflies in 2008 was orders of magnitude greater than in any previous year. The two peaks that are apparent in 2007 represent butterflies at DFSP (first peak) and The Butterfly Project (second peak). The 2009 bimodal distribution reflects the two different pulls at The Butterfly Project.

In 2008, the unrefrigerated pupae at DFSP for the most part did not eclose and those that did so eclosed in an unsynchronized manner. These previously unrefrigerated pupae were returned to refrigeration in fall 2008 to try to trigger synchronized eclosion for them in spring 2009. The parasitism pupae from an aborted experiment by an outside collaborator were left at room temperature to examine eclosion of pupae without refrigeration (that is, they have never been refrig-

erated). These pupae were in their second year and experienced a high eclosion rate of 79% (34 eclosions in 2009 of 43 total pupae from the beginning). These eclosions were synchronized with the eclosion of the refrigerated pupae at DFSP (Figure 14). There are just 3 of the original parasitism pupae left, as one pupae was too light and 5 eclosed in 2008.

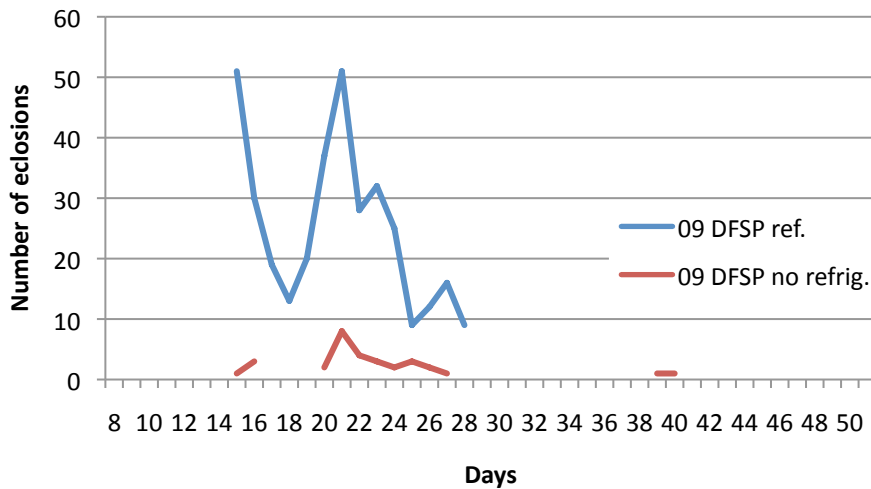


Figure 14. Eclosion curves for refrigerated and unrefrigerated pupae at DFSP in 2009.

This triggered an examination of the data from the yearly pupal tallies. The refrigerated pupae show high rates of eclosion in their first year and lower rates in their second year (rate defined as percent of original number of pupae). Pupae allowed to remain at ambient temperatures at all times show the opposite pattern (Figure 15). A statistical test of ratio of year 1 to year 2 eclosions is highly significant (ANOVA, $F_{1,6} = 389.32$, $p < 0.001$).

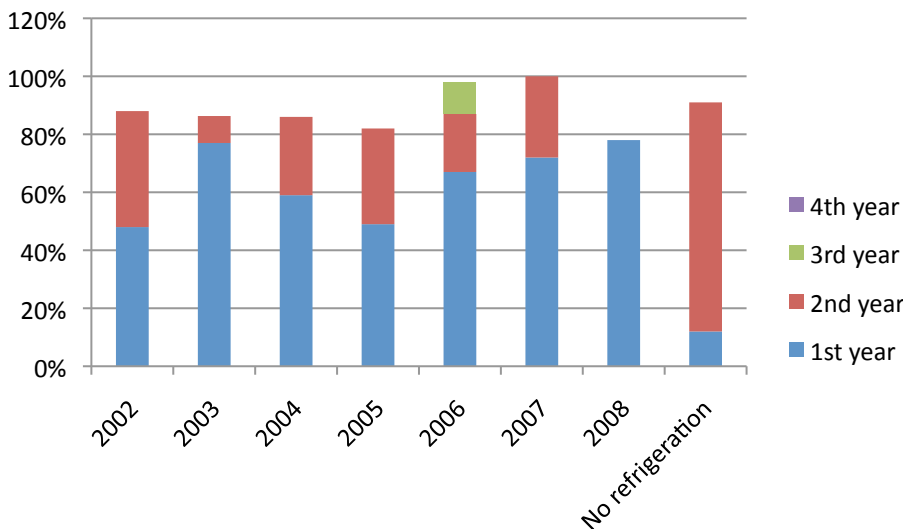


Figure 15. Percent eclosion by year for 2002-2008 compared with pupae not refrigerated.

3.3. Adults

The eclosion rate was 81%. The pupae that were not retained and did not eclose were those that weighed less than 40 mg. Those were then placed in a Ziploc baggy, crushed, checked for fluid (there was none) and then disposed of. We take these precautions to insure that we do not dispose of a viable pupa that could then eclose and become an introduced species in another location.

Table 1. Number of pupae and eclosion rates for 2009 season.

	Number at Start of Season	Number Eclosed	Percent Eclosed	Number Did Not Eclose (# viable)	Percent Did Not Eclose
2006 Stock	86	60	70%	26 (14)	30%
2007 Stock	1,218	1,102	90%	116 (71)	10%
2008 Stock	1,360	1,003	74%	357 (300)	26%
Total	2,664	2,165	81%	499 (385)	19%

The adult butterflies exhibited surprisingly few aberrations. Two primary issues arose, failure to expand properly (these were maintained in gender specific multiplant boxes, cared for daily and used for educational purposes) and miniature stature (these were maintained separately).

A small number (22, 1%) of the butterflies failed to expand properly. The peak was 5 on March 13th with 2 on the 12th, 2 on the 14th, and 1 on the 15th. A misunderstanding led to misting the pupae on March 12, after the blue of the imagoes began to show through the pupal shell, and a spike of failures to expand was associated with the event.

Miniature stature arose in several gene lines and were placed into the gender specific box with the eclosions issues and not bred.



Figure 16. A pair of Palos Verdes blue butterflies that commenced copulation just minutes after they were released at DFSP.

The descendants of the inbred “mini” miniature line were placed into a breeding multiplant box, but failed to mate or oviposit. This is consistent with Miami blue experiments with inbreeding where after just 3 generations the offspring would fail to court/mate/oviposit (Jaret Daniels, pers. comm.)

A total of 512 gravid females were released to the Linden H. Chandler Preserve in consultation with Palos Verdes Peninsula Land Conservancy and US FWS. 60 virgin females and 20 males were released on DFSP, just outside of the trailer area. There were concerns that after so many generations in captivity that the imagoes would be unable to breed in the wild. Within a half hour of the release four breeding pairs were confirmed at the release site (Figure 16). This prompted us to move away from the release site and allow the butterflies to acclimate without interference.

3.4. Larvae

To avoid an issue with food supply for larvae and maximize the number of geneline crosses, several thousand (at least 2000 – 5000) eggs through fourth instar larvae were released to Linden H. Chandler Preserve. The potted foodplants in the multiplant boxes were harvested for up to 6 dozen larvae (relocated to larval rearing containers) and then the foodplants were clipped and bagged and transported to the Preserve. The foodplant with eggs and larvae were then interwoven with living in situ plants at the Preserve. Larval relocation was possible without direct contact of the larvae by transferring the plant they were located on (whether into a larval container for further rearing or into a bag for release).

The PVPLC nursery suffered setbacks with the foodplant grown for the rearing program this past spring. Lily Verdone (PVPLC) and Johnson met several times and Longcore contacted Arthur Bonner for hints on plant propagation. There have been several changes instituted by PVPLC and we are hopeful that 2010 will be a season with plenty of foodplants for the stock. The observations of the need for young growth from the 2008 rearing season are consistent with the Miami blue’s use of only the terminal meristematic tissue (Jaret Daniels, pers.comm.)

3.5. Pupae

This disposition of pupae processed in 2009 is summarized in Table 2. Close to 2,000 pupae were retained in storage at the end of the season, which will provide an ample stock for breeding and release activities planned for 2010.

Table 2. Summary of pupae in storage and disposition of adults and larvae in 2009.

	Number
2006 Pupae (did not eclose)	14
2007 Pupae (did not eclose)	71
2008 Pupae (did not eclose)	300
2009 Pupae (new)	1,449
<i>Total Pupae in Storage</i>	<i>1,834</i>
Adults Released in 2009	592
Eggs and Larvae Released in 2009	3,000–5,000

3.6. Deceased

Deceased adults (and larvae) are housed at Moorpark College, DFSP, UCLA (for genetic research), and at Dr. Johnson's office. A list of institutions is being created for the U.S. Fish and Wildlife Service (USFWS) approval to distribute these deceased individuals for educational purposes. University of California, Riverside is no longer a repository for specimens.

3.7. Risk Management

The location of the Moorpark College near undeveloped open space results in a level of risk from wildfire. We have a protocol for evacuation that is coordinated with America's Teaching Zoo to manage the risk posed by this natural hazard. On September 22, 2009 it was necessary to implement this protocol and evacuate the pupae. On this day, Santa Ana winds were gusting and we had already secured the site against the wind. The sequence of events that unfolded were as follows.

10:33 A.M. A fire was reported in Filmore burning towards Moorpark.

11:20 A.M. The Zoo convened an evacuation meeting and began prepping for evacuation.

11:24 A.M. Johnson was notified of the situation and authorized Amanda Lansing to mobilize subpermites to evacuate both the Palos Verdes blue stock and our other endangered butterfly, Lange's Metalmark.

11:30 A.M. Johnson dismissed her Introductory Biology class, notified Longcore and Dr. Hoffmans (Dean). Longcore then informed USFWS offices responsible for the two species.

11:45 A.M. Johnson joined the evacuation efforts already underway. The pupae were evacuated per our evacuation protocol in the same manner they were evacuated previously (2007). The only deviation was that all the PVB pupae were in Johnson's car due to half of the stock being safely located on DFSP. We exited the zoo gates just as the vans for the vertebrate animals entered the gates.

2:15 p.m. All the stock was safely relocated to the secondary location. The fire proceeded to burn within a ½ mile of campus that Tuesday evening, triggering the evacuation of all birds from the zoo.

By Wednesday morning, the fire had shifted to the west of the zoo. The pupae were returned to the zoo the following Saturday by Johnson.



Figure 17. Evacuation of the PVB pupae. a) The fire approaching Moorpark College on September 22, 2009. b) The packaged PVB pupae. c) The pupae in Johnson's car.

4. Discussion

We continue to be in the fortunate position of being able to produce more offspring than we are capable of rearing in captivity; therefore breeding continues to be dictated by the availability of reintroduction sites.

4.1. Eclosion

The distribution of the eclosion was easier to manage this year with the pull occurring on three separate days, thus spreading out the peak eclosion onto three separate days. We will utilize this technique again to guard against having a spike in eclosions on a single day.

The decision to pull DFSP significantly earlier than The Butterfly Project and the use of heat lamps to set eclosion to 6 P.M. at DFSP also allowed for easier management of distribution of labor. This will be utilized again to facilitate labor at the two sites that are located 90 miles apart.

During eclosion at The Butterfly Project, there was a miscommunication that resulted in misting of the pupae after eclosion had begun (March 12). Perhaps 10 individuals were affected by the misting. This is significantly less than the hundreds that were purposely not allowed to breed due to our overages and therefore did not have an impact on the project as a whole. Still, the mistake

will be guarded again in the future by making signs and hanging them to remind everyone not to mist after eclosion has begun.

The high rate of unknown sex in the data was a source of irritation, so several steps were taken to improve data collection. First, the new propagation technicians (students) were brought onto the project in Fall 2009 for Spring 2010 and spent a full semester training them on all techniques including data collection. Second, there is a new data form for recording eclosions (See Appendix). This data sheet includes a diagram of the eclosion cup and all information regarding individual id of the pupae, weight, sex and date of eclosion for individuals in a cup will be recorded directly onto this form (previously it was an eclosion log that had entries for all of the information, but all the cups were recorded mixed together on the single eclosion log).

There are also a few issues with either cups being accidentally bumped and causing the pupae in the cup to roll into different seats (at which point they become unknown) and of eclosing butterflies disturbing the remaining pupae in the cup making it difficult to determine the seat assignments. To minimize this, we will be inserting short dividers (cut flashcards) to prevent the pupae from shifting to a neighboring seat. This will also facilitate collecting data to further test Adam Clause's technique of identifying butterfly sex prior to eclosion. He developed this technique while working at The Butterfly Project and with sufficient data we are confident that this will provide a method to identify sex of pupae before eclosion.

4.2. DFSP unrefrigerated pupae

The unrefrigerated pupae at DFSP eclosed synchronously with the refrigerated DFSP pupae and in large numbers (this was their second season since pupating). This was exciting for several reasons. If we can move away from refrigerating the DFSP stock, then we lower the risk of loss due to a double power failure and triggering eclosion inside of an inactive refrigerator (resulting in death). The refrigerator at The Butterfly Project is monitored hourly by zoo students, this labor pool is not available at DFSP and that increases the risk of a power failure at DFSP going undetected. This also makes it easier to evacuate the pupae in an emergency. Evacuating refrigerated pupae is much more difficult and results in the possibility of triggering their eclosion due to the temperature fluctuations during the evacuation.

The results showed that the unrefrigerated pupae were more likely to eclose in their second season rather than their first season. This suggests that the refrigerated pupae are artificially triggered to eclose faster than the built in eclosion mechanism. For releases, this is vitally important and could explain the lower than expected adult sightings from 2008 releases. If the natural cycle is for eclosion in the second year after pupation, then the 2008 releases should be seen in 2010. That also suggests that releases should be performed two years in a row at each site to establish a population that has some members eclosing every year. Thus we should return to the 2008 release sites in 2011 and release in the "bust" year to even out the population size oscillations. We will be releasing at the 2009 release sites in 2010 to ensure that this two-year cycle is established in both even and odd years.

Both NAVFAC and USFWS have agreed to leaving the DFSP stock unrefrigerated for now to see if a larger sample size reflects the same results. We will also be examining the impact of lack

of refrigeration on overall eclosion rates. It appears that there may be minimal increase in pupal losses due to the additional year spent in diapause.

4.3. Mating

We noted a decreased rate of mating and oviposition overall. This may be due to the new “two layers of containment” protocol which decreased the exposure to direct sunlight. The second layer of containment for the multiplant boxes were mesh tents with vinyl roofs. The vinyl roof decreased the exposure to direct sunlight. In 2010, we will use new pop-up mesh boxes as the secondary layer of containment that will have mesh roofs to allow direct sunlight exposure with minimal interference.

We will continue to have a “holding” group that are not bred. The individuals that are not used for breeding purposes will be used for research, education, and outreach. They will be cared and maintained. There will be no culling.

The geneline denoted “mini” did not breed this year, despite being provided with the same treatment as other breeding boxes. This is in line with Jaret Daniels (pers. comm.) observation that within 3 generations of inbreeding a geneline of Miami blue, the adults will fail to mate. This further supports our decision to breed for heterozygosity rather than inbreed specific maternal lines.

The matings observed in the field between released “virgin” females and released males at the DFSP media event also suggests that our breeding for heterozygosity has not eliminated the behaviors necessary for successful breeding in the wild. This will be submitted for journal publication shortly.

4.4. Releases

The release of gravid females and larvae in 2008 resulted in confirmed sightings of adults at those locations in 2009.

Release of 512 gravid females, 60 “virgin” females, 20 males, and an estimated 3,000–5,000 larvae in 2009 went smoothly. The only issue was increased foot traffic and foodplant damage after the publicity surrounding the release. Releases will now occur in back years in order to guarantee that the reintroduction sites do not experience a “boom – back to bust” cycle (as suggested by the unrefrigerated pupae). Survival in the field has not been studied on this species, however, release of Taylor’s Checkerspot eggs to the wild were studied with a 0.8% survival rate (Mary Linders, pers. comm.). Studying the survival rate of a contingent in the field would be fascinating.

4.5. 2010 Overview

At the Imperiled Butterfly Conservation and Management symposium at the Maguire Center in Gainesville, Florida (October 27–30, 2009) Johnson and other participants discussed the drawbacks of describing the ecology of species in peril from remnant populations. These may not be representative of the species as a whole in optimal conditions. An analogy would be describing the human population by studying a refugee camp. Many of the published natural history articles

on the Palos Verdes blue contain vague claims that need to be addressed in more detail, and notations that do not agree with the current state of data.

Preparations for the 2010 season are ahead of schedule. We recruited students at the beginning of fall semester this year, so their training has been extended by 6 months prior to helping with PVB. New more intensive training documents have been developed and the team has already been exposed to caring for endangered foundresses, eggs and first instar larvae with Lange's Metalmark butterfly (the other species present at the project).

There have been several changes (noted earlier) to our care and rearing protocol, which we will continue to adjust for the benefit of the species

We will be attempting to focus on research into color morphs, pheromones, vibrational communication, predicting sex of pupae as they start to blue, water loss in the captive pupae, and moving forward with DNA analysis of captive, historic, and wild Palos Verdes blue butterflies (with proper approvals from USFWS).

It has been two years since any wild stock has been brought into the captive stock, and we will be requesting permission to bring 5–10 wild larvae into the captive stock to provide wild stock breeders for the 2011 breeding season. Another option would be to capture wild females, swab them to collect their DNA, contain them for 24–48 hours (collecting their egg production), and then release them.

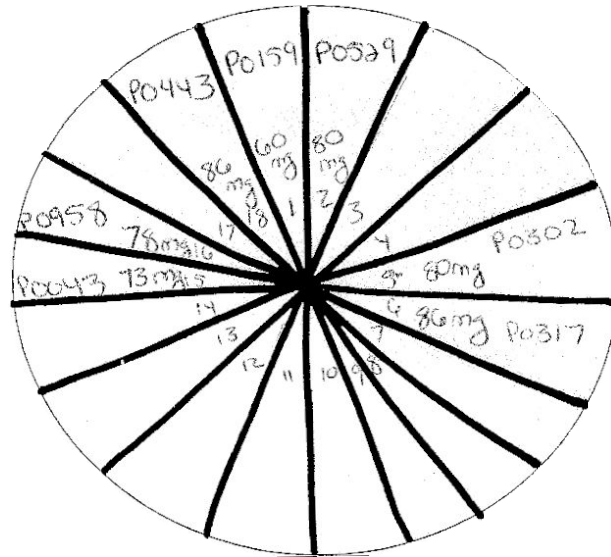
5. Literature Cited

- Hermes, C. P., D. G. McCullough, D. L. Miller, L. S. Bauer, and R. A. Haack. 1996. Laboratory rearing of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae), an endangered butterfly in Michigan. *Great Lakes Entomologist* **29**:63–75.
- Johnson, J. J., A. Clause, C. Behringer, A. Lansing, and T. Longcore. 2008. Final Report for Palos Verdes blue butterfly year 2007 captive rearing at Defense Fuel Support Point, San Pedro, California and The Butterfly Project, Moorpark, California. Pages 1–28. The Urban Wildlands Group (NAVFAC SW Cooperative Agreement # N6871-04-LT-A0017), Los Angeles.
- Johnson, J. J., A. Lansing, A. Clause, D. Renner, C. Behringer, R. Lewis, and T. Longcore. 2009. Final Report for Palos Verdes blue butterfly year 2008 captive rearing at Defense Fuel Support Point, San Pedro, California and The Butterfly Project, Moorpark, California. Pages 1–18. The Urban Wildlands Group (Defense Logistics Agency Agreement # N662473-LT-R0011), Los Angeles.
- Longcore, T. 2008. Final report for 2008 Palos Verdes blue butterfly adult surveys on Defense Fuel Support Point, San Pedro, California. Defense Logistics Agency Agreement #62473-08-LT-R0011. The Urban Wildlands Group, Los Angeles.
- Mattoni, R., T. Longcore, Z. Krenova, and A. Lipman. 2003. Mass rearing of the endangered Palos Verdes blue butterfly (*Glaucopsyche lygdamus palosverdesensis*: Lycaenidae). *Journal of Research on the Lepidoptera* **37**:55–67.
- Mattoni, R. H. T. 1988. Captive propagation of California endangered butterflies. California Department of Fish and Game, Sacramento, CA.

- Mattoni, R. H. T. 1993. The Palos Verdes blue, *Glaucopsyche lygdamus palosverdesensis* Perkins and Emmel. Pages 135–136 in T. R. New, editor. Occasional Paper of the IUCN Species Survival Commission, No. 8. Conservation Biology of Lycaenidae (Butterflies). IUCN, Gland, Switzerland.
- Mattoni, R. H. T. 1994. Rediscovery of the endangered Palos Verdes blue butterfly, *Glaucopsyche lygdamus palosverdesensis* Perkins and Emmel (Lycaenidae). *Journal of Research on the Lepidoptera* **31**:180–194.
- Nice, C. C., Z. Gompert, M. L. Forister, and J. A. Fordyce. 2009. An unseen foe in arthropod conservation efforts: the case of *Wolbachia* infections in the Karner blue butterfly. *Biological Conservation* **142**: 3137–3146.
- Pratt, G. F., and R. Stouthamer. 2002. Quino checkerspot 2002 report: captive breeding.

Appendix: Data Sheet for Recording Eclosion Position of Pupae

6/9/09



Geneline: M2 ♀ × 07028 ♂ - 2008

Eclosion Cup Number: E2

Gender Tally:

♂ 	♀
-------	-------

Eclosion Date/ Gender

- | | |
|-------------------|-------------------|
| 1) <u>NY</u> | 2) <u>NY</u> |
| 3) <u>3/22 ♀</u> | 4) <u>3/26 ♀</u> |
| 5) <u>NY</u> | 6) <u>NY</u> |
| 7) <u>3/16</u> | 8) <u>6/1 ♀</u> |
| 9) <u>3/13 ♂</u> | 10) <u>3/14 ♂</u> |
| 11) <u>3/18 ♀</u> | 12) <u>3/19 ♂</u> |
| 13) <u>3/16</u> | 14) <u>3/18 ♀</u> |
| 15) <u>NY</u> | 16) <u>NY</u> |
| 17) <u>3/15 ♂</u> | 18) <u>NY</u> |