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FINAL REPORT
FOR
PALOS VERDES BLUE BUTTERFLY YEAR 2010 CAPTIVE REARING
ON
DEFENSE FUEL SUPPORT POINT
SAN PEDRO, CALIFORNIA
AND
THE BUTTERFLY PROJECT
MOORPARK, CALIFORNIA

COOPERATIVE AGREEMENT NUMBER:
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EXECUTIVE SUMMARY

In 2010 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 releases onto managed habitat areas. Key findings and outcomes are as follows:

- The focus of the rearing program continues to be on 1) reintroductions to the wild to establish new populations, 2) research, and 3) a refugium population.

- Meetings with all partners in November provided key coordination that allowed the season to progress smoothly and successfully.

- One hundred seventeen female and 87 male butterflies were released to the wild in compliance with existing U.S. Fish and Wildlife Service permits.

- At the close of the season 829 pupae were in captive stock from 2010 and 815 from previous years.

- Technical innovation in the rearing program was made in the form of "seat dividers" that held to track individual pupae at the time of eclosion. These dividers were, however, difficult to work with and prone to failure (becoming dislodged). New seat divider systems have been developed for the 2011 season.

- Research on the captive population resulted in significant results, including:

    - Gut content is associated with the integument color in fourth instar larvae.

    - Fourth instar larvae provided with a diet that includes *Lotus scoparius* flowers are significantly heavier pupation weight compared with all other diets. Weight at pupation is associated with fecundity as adults.

    - Duration of pupation (either one year or more) may vary by sex. Therefore, although in general breeding across generations is inadvisable, it may actually be built into the autecology of this species and, therefore, unavoidable.

- National exposure for the efforts was achieved with the publicized released at Friendship Park & Chandler. Also, through a week of guest postings on the microblogging website Twitter for Indianapolis Zoo and being featured on US FWS website during National Pollinator Week.

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1. Introduction

The Palos Verdes blue butterfly, *Glaucopsyche lygdamus palosverdesensis* (Lepidoptera: Lycaenidae: Polyommatinae) (Figure 1), was thought extinct in 1983 when the last known population of the time was bulldozed for a baseball field (Mattoni 1993). The subspecies was subsequently discovered on the Defense Fuel Support Point (DFSP) in San Pedro in 1994 (Mattoni 1995). Palos Verdes blue butterflies at DFSP were found to feed on both *Lotus scoparius* and *Astragalus trichopodus lonchus* (both in the family Fabaceae) as larvae, which occurred there naturally and is found in revegetated coastal sage scrub (Mattoni 1995).

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 2010 captive rearing season.

Figure 1. Captive reared, male Palos Verdes blue butterfly released to the wild (Chandler) in 2010. Photo by Ann Dalkey.

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 (Mattoni 1988, Pratt and Stouthamer 2002, Mattoni et al. 2003, Johnson et al. 2008).

Rearing for the 2010 season was conducted under the authority of Dr. Jana Johnson as permitted under USFWS Biological Opinion 1-6-96-F-09. Additional care was provided by the subpermitees on List C of the Fourth Amendment to the Biological Opinion 1-6-96-F-10. Subpermitees received extensive training prior to handling the captive stock.

Captive rearing was conducted at two locations in 2010. 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 subpermitees. 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.

2. Captive Breeding Methods

2.1. Pupae and Eclosion Chambers

Pupae from the 2009 rearing season that remained in diapause from previous seasons had been placed in refrigeration at the beginning of winter 2009, with the exception of the stock held at DFSP. At DFSP, the "parasitism pupae" (from a previous experiment on parasitism) have been unrefrigerated since 2007, and continued to be unrefrigerated, stored in the DFSP lab on the counter. The stock from 2008 was left unrefrigerated to increase the scope of this experiment, with permission from USFWS and NAVFAC. The lab window is screened and barred, and was therefore left open to allow the lab to equalize with ambient outdoor temperature.

The pupae at Moorpark College were removed from refrigeration in two groups to spread the required labor over a longer period. The first half was pulled on January 30 and the second half was pulled on January 31, 2010.

The pupae were subsequently sorted according to geneline and then weighed using an electronic scale to the nearest mg and recorded in an excel spreadsheet. Handling of the pupae was with Bioquip featherweight forceps or the pads of our fingers. The subpermitees worked in pairs to insure the accuracy of the data record. The weighed pupae were transferred into an individually assigned seat of a geneline-specific eclosion cup. "Seat dividers" were used for the first time to reduce the movement of the pupae from jostling or the eclosion of neighboring pupae (Figure 2).

Figure 2. a) Students and Dr. Johnson who processed the pupae upon removal from refrigeration, b) scale with pupae on it and eclosion chamber with "seat dividers," c-d) detail of eclosion chambers and numbering system, e) data sheet for eclosion chambers, and f) students holding trays of small cups, each containing a Palos Verdes blue butterfly pupa.

Figure 3. Left: an eclosion chamber with ventilated lid inside an eclosion box that provides a second layer of containment. Right: The two layers of containment allow pupae to be transported between the zoo office facilities used for the sorting and weighing and the greenhouses for stimulation for the season while maintaining two levels of containment at all times (photos by: Jana Johnson).

The eclosion cups at DFSP were switched out to the new Styrofoam cups with seat dividers and lids (see Figure 2). Both rearing locations maintained one gene-line per eclosion cup, which was noted on the outside of the cup. The pupae were handled with Bioquip featherweight forceps or the pads of our fingers to prevent injury when placing them into the eclosure chambers with crushed walnut shells for substrate.

The eclosion cups at Moorpark College were the same as used in 2009, but with the introduction of seat dividers. These are plastic notebook dividers cut into rectangles and inserted into the walnut shells between seats. 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. Because the DFSP stock was not refrigerated, we weighed all pupae, recorded the data and moved them into the improved eclosion cups. We then controlled the timing of eclosion for DFSP stock by blacking out the window of the lab and setting heatlamps on switch timers. This allows us to work on two eclosion events per day, one at Moorpark College and one at DFSP. The pupae were misted sporadically only until the blue of the wings began to show through the pupal casing, which is referred to as “bluing.”

Figure 4. Setup for eclosion of Palos Verdes blue butterfly at DFSP laboratory. Clockwise: windows blacked out to allow control of timing, student workers and eclosion cups with lights on, data sheet, timer for lighting system, humidifier.

We exposed stock at The Butterfly Project to sunlight through the greenhouse walls. No heat-lamps or humidifiers/swamp coolers were used. The pupae were misted daily until bluing began. No misting occurred after bluing was observed.

Eclosion check was performed twice each day from the removal of the pupae through the end of the eclosion period. Eclosion check was performed with the help of a penlight to insure adequate light on each and every pupa as it was examined for bluing and eclosion. Daily eclosion checks were performed throughout the summer. Late eclosions were not bred, and were utilized for public education.

Upon eclosure, the eclosion cup containing imagoes was transferred into a handling box. This is a method developed in 2009, which 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 major advances 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 multiplant container (Figure 5).

Figure 5. Left: Eclosion check with a penlight. Center: A handling box with an eclosion cup inside. Right: demonstration of access to the eclosion cup through the sleeve entry.

This 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 used to use for other purposes, but has proven itself valuable as a holding/sorting container (Figure 6). The holding/sorting container is geneline and gender specific and properly labeled. It is secured on the open side with mesh and a thick rubberband, then removed from the handling box and placed into the sorting area of the greenhouse.

Figure 6. Five handling boxes in use by five student workers in the greenhouse. Two Palos Verdes blue butterfly "sister" females being transferred from an eclosion cup to a holding/sorting container inside of a handling box.

Eclosions were recorded in the excel file of individuals with the date of eclosion, and when possible the sex of the individual. If multiple imagoes of both sexes were present in a single cup, the sex ratio was recorded, but sex was not assigned to individual seat numbers. Imagoes were identified to sex following the same procedures reported in 2007 (Johnson et al. 2008).

One group of pupae had been involved in an experiment on parasitism in a past year and adults from this group, as well as any adults with eclosion anomalies (e.g., wings failed to expand) were not bred and were used for educational purposes with zoo patrons and academic classes.

Figure 7. Left: Sorting 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. Right: The new "Monarch Castles" employed as the secondary level of containment.

2.1. Adult Maintenance

Adults were maintained in multiplant and uniplant 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. Uniplant boxes have a single plant and allow for crosses of smaller numbers of PVB. 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 take 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.

The eclosed imagoes were sorted by geneline and sex and placed in the holding area of the greenhouse. Butterflies were fed daily while in the holding area while held in gender and geneline specific containers. Based on the distribution of individuals between genelines, 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 multiplant boxes were maintained ant free within a secondary containment system. The tent from 2009 had issues with the lack of a floor and with lack of direct sunlight exposure due to the roof. In 2010 we switched to the Monarch castle from Live Monarchs, Inc. It is a fully contained, collapsible cube with a zippered door (Figure 7). One wall of the cube is vinyl and the remainder, is a fine mesh. This vinyl wall position is problematic due to trapping heat. We found that positioning it as a side wall, parallel to wind current was best for minimizing its heat retaining properties. Our other issue has been "dry rot" due to exposure to the intense sun, low moisture conditions of southern California. These "Monarch castles" will be a recurring expense.

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 Lt.Col. Ramer (ret.), the former Commander 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, longevity of individually caged adult butterflies has increased 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.

2.2. Breeding

The captive population is now large, so mass rearing techniques were employed. Per the agreement with the partners, and in consultation with USFWS, only part of the stock was 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 and uniplant boxes.

"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 releases of unbred males and females from our "holding" stock at Friendship Park and the Chandler Reserve) were pulled from these multiplant boxes. Gravid females were released to the Chandler Reserve after ovipositing in the multiplant boxes. This insured that we would not lose the genelines if the female was predated prior to ovipositing in the wild.

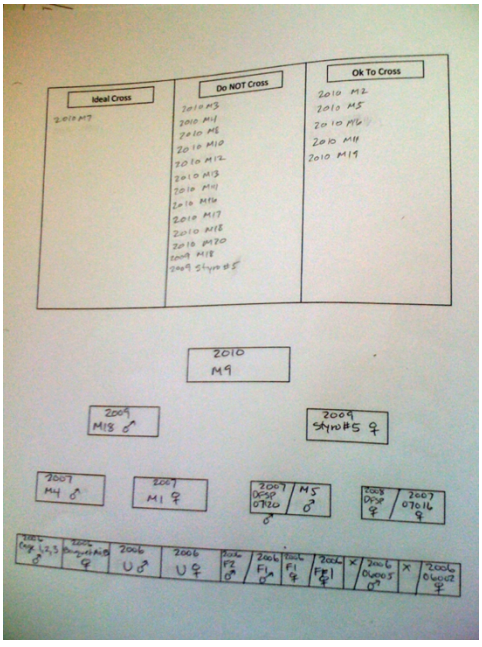

Figure 8. An example of a lab sheet planning for crosses to ensure as great genetic diversity as possible. "Family tree" for the present lineage is at the bottom, crosses (good, bad, and ok) are listed at top. This facilitates decisions based on which individuals eclose on a given day.

No breeding was performed at DFSP in 2010 because there were too few eclosions. The eclosing individuals were transported to Moorpark College and either bred or cared for in the "holding" stock.

Figure 9. Student workers inside greenhouse and Monarch castles.

2.3. Larval Rearing

There was no breeding and therefore no larval rearing on DFSP in 2010. The unrefrigerated pupae were monitored throughout the season and the few eclosions were moved to The Butterfly Project. We expect 2011 to be a busy season with the unrefrigerated stock at DFSP.

The Butterfly Project housed egg and larval stock in rearing containers in the greenhouse and multiplant boxes outside the greenhouse, inside the Monarch Castles (Figure 9).

All locations were protected from rain and defended 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. 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.

Figure 10. Process for providing live foodplant for ovipositioning butterflies. Top: preparing foodplant with barrier to use in oviposition container. Bottom left: "Doublestack" container developed for ease in managing butterflies, with increased height, meshed sides, and multiple sleeved entreies. Bottom right: Access to foodplant, butterflies, and larvae through sleeved entries.

Upon reaching 4th instar, larvae were transferred into individual rearing containers to prevent cannibalism (Figure 11). The smaller instars experience high mortality in these small, limited ventilation individual containers, therefore the cannibalism is a tolerated risk for the smaller in-stars.

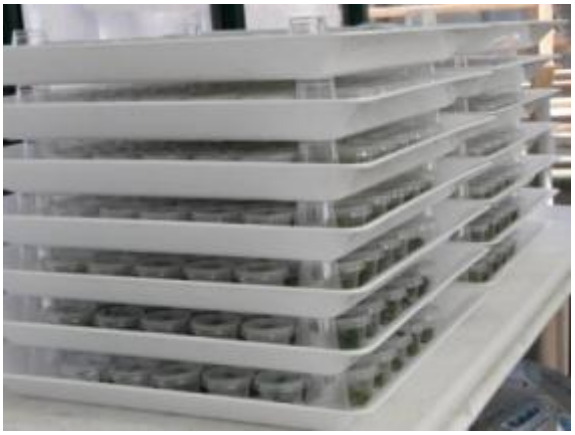

Figure 11. Storage of late-instar larvae in stacked "condos" of creamer cups.

When the larvae pupated, their container was emptied and left open to allow proper ventilation for pupal skin hardening (Figure 12). After complete hardening of the pupae, their containers were closed and were stored at room temperature.

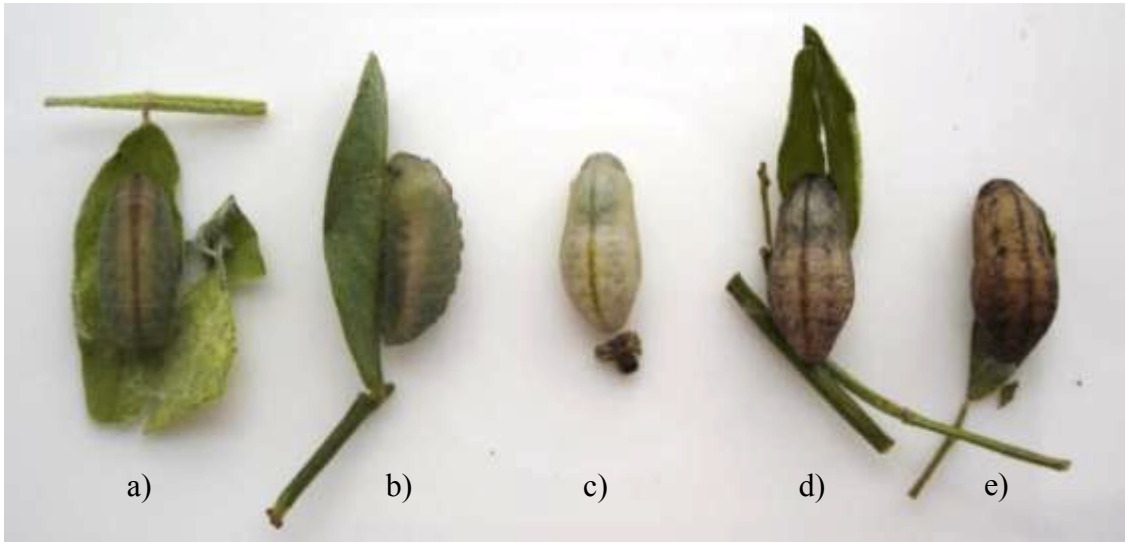

Figure 12. Pupation sequence. When the larva is prepupal (a, b) the condo is left open for ventilation to stimulate pupation. Once the pupae have hardened to the darker brown coloration (d, e), the majority of the vegetation may be removed from the cup and the cup is resealed.

The pupae at The Butterfly Project were placed into refrigeration on November 6, 2010, to simulate winter, prevent premature eclosions, and aid in synchronizing the 2011 eclosion period.

The refrigerator was held within the range of 40–50 ºF, as verified hourly by zoo staff during rounds. The ibuttons from last season did not function properly and were returned to the UC Davis student who had loaned them to the project.

3. Results of Captive Breeding

3.1. Pupae and Eclosion

Pupae lose weight during the overwintering months. For pupae produced in the 2008 flight season, their 2009 average weight was 87.14±11.87 S.D. mg (n=263). In 2010, 9 of these pupae had fallen below 40 mg and were not viable. For the remaining viable pupae, mean weight was 78.68±11.02 S.D. mg. Viable pupae had lost 8.44±6.39 S.D. mg (n=254) during the year, while those that did not survive lost 63.7±9.29 S.D. mg (n=9).

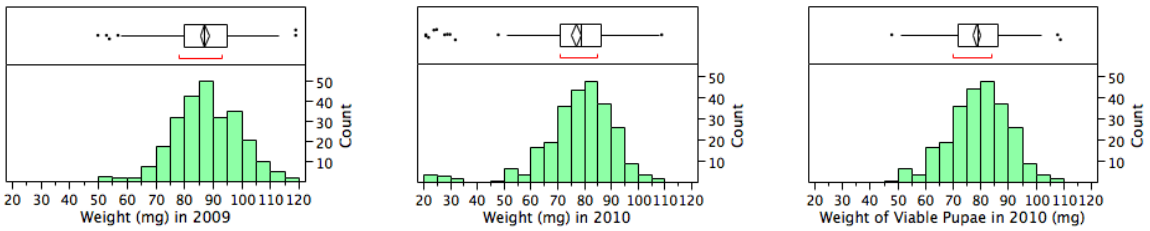

Figure 13. Histograms of pupal weights in 2009 and 2010 for pupae from 2008 season. The box plots above each graph show the median (vertical line), mean with confidence intervals (diamond), quartiles (box indicates middle 50% of data), whiskers (1.5 times the interquartile range), possible outliers (dots outside the whiskers) and densest 50% of observations (red bracket). Left: Weights in 2009. Center: Weights in 2010, note outliers below 40 mg that pull the mean value below the median. Right: Weights and 2010 excluding dead pupae, note mean and median are now closer.

3.1. Adults

Overall, 998 butterflies eclosed with a sex ratio of 447 males: 460 females (1:1.03). Written records were ambiguous for 91 butterflies and these are excluded from further analysis. DFSP captive stock had a 5 male: 16 female ratio (1:3.2) (Table 1). The Butterfly Project captive stock had a 442 male: 444 female ratio (1:1) (Table 1). Peak eclosion was 31 days after removal from refrigeration with 116 eclosions on that day (Figure 14). This is later than usual, probably due to the cooler and wetter spring weather in 2010.

Table 1. Sex ratio of Palos Verdes blue butterflies eclosing in 2010 by year of pupation. "Unk" indicates butterflies for which written notation was ambiguous.

	The Butterfly Project					DFSP				
Year	Male	Female	Unk	Total	Ratio	Male	Female	Unk	Total	Ratio
2009	350	270	59	731	1.29:1	3	1		4	3:1
2008	90	172	30	244	0.46:1	-	-		-	-
2007	2					2	15	2	19	0.13:1
2006		2		2						
Total	***442***	***444***	***89***	***975***	***1:1***	***5***	***16***	***2***	***23***	***0.31:1***

Our new seat dividers and data entry sheets were a significant improvement over the previous year. During the 2009 season we were not able to record the sex of each butterfly as it eclosed. Multiple butterflies (of the same geneline) would eclose in the same container and we would

know the sex of each but not with which pupa it was associated (n=217). In 2010 we were able to note the sex of the butterfly emerged from each pupa using the new system. Some student workers, however, produced an ambiguous notation, an error that will be corrected in the 2011 season by writing out "Male" and "Female" on the data sheets.

The eclosion rate was 55.0% (Table 2). This low eclosion rate can be attributed to two factors. First, the one year old, unrefrigerated pupae at DFSP had a very low eclosion rate (compare with 74% in 2009 and 72% in 2008 for first year pupae). Second, the unusually cool, overcast, and wet spring likely depressed eclosion rates.

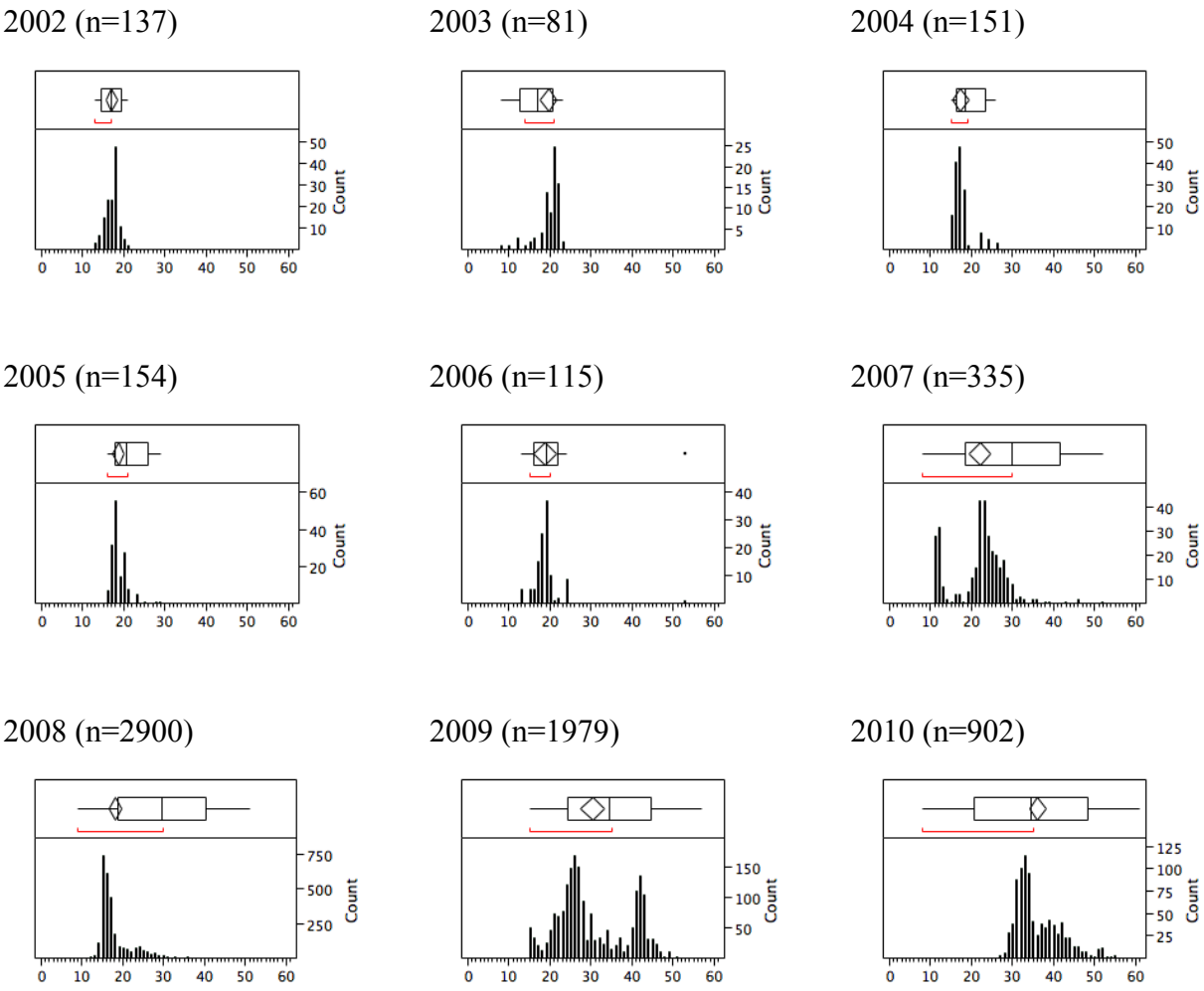

Figure 14. Comparison of eclosion curves for imagoes 2002–2010 by days after removal of first pupae from refrigeration. Note that the count axis varies between graphs. The bimodal distribution of emergence in 2007 resulted from pupae at DFSP being removed from refrigeration and eclosing before the pupae at The Butterfly Project. The 2009 bimodal distribution reflects the removal from refrigeration at two different times.

Table 2. Number of pupae and eclosion rates for 2010 season.

	Number at Start of Season	Number Eclosed	Percent Eclosed	Number Did Not Eclose
2006 Stock	14	2	14.3%	12
2007 Stock	66	21	31.8%	45
2008 Stock	303	292	96.4%	11
2009 Stock	1430	683	47.7%	747
Total	***1813***	***998***	***55.0%***	***815***

We examined the association between sex of the emerging butterfly and length of diapause and found that females are more prevalent in the multiyear diapausing pupae that successfully eclose (Table 1), although with relatively few data points, the relationship was not statistically significant.

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

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 (17) of the butterflies failed to expand properly. Miniature stature arose in several gene lines and were placed into the gender specific box with the eclosions issues and not bred.

We continue to cross the lineages in order to create the greatest nucleic heterozygosity possible in the captive stock. No wild butterflies were brought in to the program because of the limited flight at DFSP in 2010 (Longcore and Osborne 2010).

Figure 15. Released butterflies mating at Friendship Park.

Fifty-eight male and 23 female butterflies were released to Friendship Park and 29 male and 94 gravid female butterflies were released to Chandler Reserve in consultation with Los Angeles County Department of Parks & Recreation, Palos Verdes Peninsula Land Conservancy and US FWS. Release of unmated males and females on Chandler Park resulted in multiple confirmed matings within minutes (Figure 15).

3.2. Larvae

Due to the reduced eclosion rate, observed breeding, and oviposition rate (presumably due to the weather this 2010 flight season) no larvae were released this year.

Table 3. Summary of pupae in storage and disposition of adults and larvae in 2010.

	Number
2006 Pupae (did not eclose)	12
2007 Pupae (did not eclose)	45
2008 Pupae (did not eclose)	11
2009 Pupae (did not eclose)	747
2010 Pupae (new)	829
Total Pupae in Storage	*1644*

4. Research Projects

Several small research projects were undertaken as part of the captive breeding and by collaborators with material provided by the program. All research efforts were approved by USFWS.

4.1. Influence of Last Instar Diet on Pupal Weight

We had 45 green color morph individuals were placed into one of three diets. Yellow group ate *Lotus scoparius* leaves and flowers. White group ate *Astragulus* leaves and flowers. Green ate *Lotus scoparius* leaves only. They were monitored for their color daily until pupation. They were weighed once the pupae hardened.

To evalute the influence of diet on pupation weight, we devised an experiment with 800 fourth instar larvae. Up to the fourth instar, all had a diet consisting of *Lotus scoparius* and *Astragalus trichopodus* as influenced by the oviposition choice of the female and availability of foodplant during rearing operations. For the final instar we divided larvae into treatments; most larvae continued with a mix of food items (control group), while 34 were fed yellow material only (flowers and very few leaves from *Lotus scoparius*), 32 were fed green material only (leaves only from *Lotus scoparius*), and 27 were fed white material only (flowers from *Astragalus trichopodus*).

Figure 16. Food prepared for diet experiment, showing yellow (*Lotus* flowers), white (*Astragalus* flowers), and green (*Lotus* leaves) prepared for feeding to larvae.

Pupae from the control group weighed 82.3±11.1 S.D. mg, yellow treatment weighed 95.8±10.5 S.D. mg, white treatment weighed 84.7±12.6 S.D. mg, and green treatment weighed 87.3±11.4 S.D. mg (Figure 17). Because variances were equal but sample size was not we compared each pair with the Tukey-Kramer test for honest significant difference with unequal sample sizes. The yellow treatment was significantly ($p<0.05$) heavier at pupation than the other treatments and the control. The other treatments and control were not significantly different from each other.

The mode of days into the experiment until pupation was 11 days for all treatments, however, the mean for white (*Astragalus* flowers) was 10 days, for *Lotus* green vegetation only was 11 days, and for *Lotus* with vegetation and flowers was 12 days. This made a difference in their weight.

This experiment indicates that there are differences in time to pupation and weight of pupae that are influenced by diet. It does not disentangle whether weight is solely a function of time to pupation or of the nutritional content of the diet.

Many studies establish a link between greater larval mass and higher food quality (Blais 1953, Pullin 1987, Mevi-Schütz and Erhardt 2005), so the most likely explanation is that the *Lotus* flowers are of higher quality than *Astragalus* or the leaves alone.

The net result of using Lotus flowers and vegetation as the diet for fourth instar larvae is greater pupal weight, which is associated with greater fecundity of the imago, so using this diet is preferable from a rearing perspective.

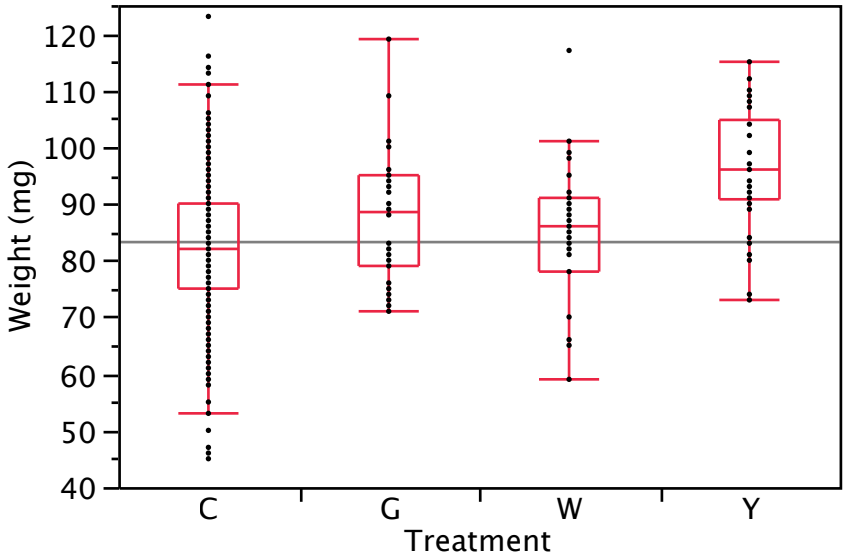

Figure 17. Weight of pupae following different diets during fourth instar with outlier box plots: C, control (n=701), G, green (*Lotus scoparius* without flowers; n=32), W, white (*Astragalus trichopodus* flowers; n=27), Y, yellow (*Lotus scoparius* flowers; n=34).

4.2. Pheremonal Communication of Pupae

Using the method we developed to determine the sex of the pupae of Palos Verdes blue butterflies (Johnson et al. 2008), we selected 8 bluing females and 6 bluing males. These pupae were terminated in 2 Wheaton conical bottom v-vials filled with methlyene chloride, HPLC grade. These samples were immediately shipped on ice to Dr. Mirian Hay-Roe of the University of Florida and USDA for pheromone testing. One vial held the males, the other the females.

Dr. Hay-Roe found quantitative differences in some compounds, which distinguish males from females. Some of these compounds are known to be involved in sex recognition or communication.

We would like to proceed with another shipment of samples during the 2011 rearing season and then some in situ testing with the compounds. It is possible that the eclosion hormone can be used as a pheromone to synchronize eclosion and this would be an avenue for further research.

4.3. Other External Collaborations

Some first instar larvae were provided to John Stark, Ph.D., of Washington State University for investigations into the impact of herbicides on larvae. These larvae were exposed to experimental treatments of the glyphosate-based herbicide Roundup and survived to pupation. Dr. Stark has refrigerated them will remove them for the spring 2011 season to attempt to break their diapause.

UCLA's Conservation Genetics Resource Center was able to extract DNA from a non-destructive method that only requires brief handling of each butterfly. Our previous collabora-

tions have developed a microsatellite library suitable to describe the population genetics of the species (Johnson et al. 2009).

5. 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.

5.1. Eclosion

DFSP eclosions were from unrefrigerated stock. Only 4 of the 22 eclosions were from 2009 pupae, 18 were from multiple year diapauses pupae (2008 or older). This meant that one-year-old unrefrigerated pupae had a 1 in 152 chance of eclosing, while multiyear pupae had a 1 in 4 chance of eclosing.

The adds further support to results from 2009 that suggested the unrefrigerated pupae are more likely to eclose in their second season rather than their first season (Johnson et al. 2010). This suggests that the refrigerated pupae are artificially triggered to eclose faster than the natural 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 individuals 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 insure that this two-year cycle is established in both even and odd years.

Both NAVFAC and FWS have agreed to leaving the DFSP stock unrefrigerated for now and seeing if a larger sample size reflects the same results. We will also be examining the impact of lack of refrigeration on overall eclosion rates. The two-year total eclosion rate is 91% for the unrefrigerated pupae, which is not significantly different from two-year rates for pupae from the period 2002–2008 that were refrigerated (88 ± 6% S.D.)

5.2. Mating

We noted but did not quantify a decreased rate of mating and oviposition overall. Primarily, this year it was probably due to the weather, which also delayed the eclosion peak by 10 days compard with past years (see Figure 14). This may also be due to the new "two layers of containment" protocol, which decreased the exposure to direct sunlight. The second layer of containment for multiplant boxes was a mesh cube, which did slightly decrease the exposure to direct sunlight.

We will proceed with mating between generations. With the skewed sex ratio in multiyear pupae (more females than males enter multiyear diapause) and the success of mating being higher when there are multiple males for each female, the primary way to mate multiyear females is with the next generation males.

The geneline denoted "mini" is dying out due to lack of breeding and oviposition. This is in line with Jaret Daniels' observation that within 3 generations of inbreeding a geneline of Miami blue, the adults will fail to mate (pers. comm.). 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 Chandler Park release event also suggests that our breeding for heterozygosity has not eliminated the behaviors necessary for successful breeding in the wild.

5.3. Oviposition

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 1995). 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 influenced 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. Despite switching in 2010 to a secondary containment system that was mesh on the roof in addition to the walls, egg production and mating was still observed as reduced compared with single level of containment. However, it was a cool, wet year and we are hopeful that with increased light and temperature the new secondary containment system will allow adequate ventilation and light exposure for elevated mating and oviposition in future seasons. Regardless, the importance of two layers of containment outweighs the decrease in productivity.

5.1. Releases

The release of gravid females and larvae at Friendship Park and Chandler Preserve in 2009 resulted in confirmed sightings of adults at those locations. Ann Dalkey, PVPLC, reports 19 progeny from 2009 releases and 24 individuals from 2010 releases (after the day of release event) for a total of 43 PVBs observed in March – May 2010 at the Chandler Reserve.

Releases at both sites went smoothly (Figure 18). The only issue was destruction of a hilltop on Chandler by an individual establishing a Native American ritual site without permission. Releases should now occur in back to back years 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 (*Euphydryas editha taylori*) eggs to the wild were studied with a 0.8% survival rate (Mary Linders, Washington Department of Fish and Wildlife, pers. comm.). Studying the survival rate of a contingent in the field would be fascinating.

Figure 18. Releases at Friendship & Chandler including various subpermitees. Boy Scouts investigating PVB adults set for release. (photos by Jana Johnson and Travis Longcore).

5.2. Public Outreach

On March 6, 2010 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 Friendship Park. March 8, 2010 NBC filmed release of captive-bred individuals to Chandler. On March 10, 2010, NBC filmed The Butterfly Project at America's Teaching Zoo, Moorpark College. The story aired March 21 across the nation and the releases were covered by other media outlets (e.g., *Daily Breeze*, *Los Angeles Times*).

5.3. 2011 Overview

We recruited 36 students at the beginning of fall semester this year, so their training will have been underway for 6 months prior to helping with PVB. 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 and we will continue to adjust it for the benefit of the species.

We will be attempting to focus on research into color morphs, pheromones, weight associated with diet, predicting gender of pupae as they start to blue, water loss in the captive pupae, and moving forward with DNA analysis of captive, historic, and wild PVB (with proper approvals from US FWS).

It has been three 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 for the 2012 season. Another option would be to capture wild females, swab them for genetic purposes, contain them for 24–48 hours (collecting their egg production) and then rerelease the females.

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