# Captive Rearing the Endangered Mardon Skipper (*Polites mardon*) and Taylor's Checkerspot (*Euphydryas editha taylori*) Butterflies: Initial Results (Lepidoptera, Nymphalidae)

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**Abstract:** Many of the butterfly species or subspecies that are largely restricted to lowland grasslands in the Willamette Valley-Puget Trough-Georgia Basin Ecoregion are known to be rare and declining. The mardon skipper (*Polites mardon*) is listed as Endangered in Washington. The Taylor's checkerspot (*Euphydryas editha taylori*) is a Species of Concern in Washington, and is red-listed and probably extirpated in Canada. Captive rearing and reintroduction, in conjunction with habitat restoration and enhancement, are essential components in the recovery of these butterflies. Initial results from captive rearing of the Taylor's checkerspot and mardon skipper are promising. Survival to diapause was over 80% for Taylor's checkerspot larvae that were reared from eggs laid by captive females. The larvae were fed two different known host plants, *Plantago lanceolata* and *Castilleja hispida*, prior to diapause. Both hosts were readily accepted, and prediapause weight did not differ between larvae fed the different hosts. Initial results from diapause survival for the Taylor's checkerspot indicate that it can survive diapause in captivity. Stimulation of egg laying, asynchrony of larval development, and pupal eclosion timing have proven to be difficulties in mardon skipper captive rearing.

**Key Words:** mardon skipper, *Polites mardon*, Taylor's checkerspot, *Euphydryas editha taylori*, captive rearing, reintroduction, host plant, endangered butterflies, Willamette Valley-Puget Trough-Georgia Basin Ecoregion, Washington

## Introduction

Throughout the Willamette Valley-Puget Trough-Georgia Basin Ecoregion, grasslands with a largely native plant community have declined in extent to about 2% of the area they occupied in 1850 (Crawford and Hall 1997). This is due primarily to agricultural development, urbanization, gravel mining, succession to forest, and invasion of nonnative plant species. Many species of grassland-dependent vertebrates have declined dramatically and are threatened with extinction or have been extirpated (Leonard and Hallock 1997; Rogers et al. 1997; Ryan 1997; Rogers 2000). Plants of the prairie have declined as well. The golden paintbrush (*Castilleja levisecta*), a Taylor's checkerspot (*Euphydryas editha taylori*, W.H. Edwards 1888) host plant, is federally listed as Threatened under the U.S. *Endangered Species Act*. Several other grassland plant species and plant communities are imperiled.

Work on insects, thus far mostly confined to butterflies, is beginning to show the same pattern of population decline and endangerment among prairie obligates. Butterflies are often considered to be both good indicator (Black et al. 2001) and umbrella (Launer and Murphy 1994; New 1997) taxa. Thirteen species of butterflies that use grasslands in the ecoregion are listed as Endangered, Threatened, Candidate, or Extirpated (or equivalents) by national or state/provincial governments. These imperiled butterflies, along with the dramatic reduction in extent of native grassland, indicate that the Willamette Valley-Puget Sound-Georgia Basin grassland ecosystems are in crisis.

The Taylor's checkerspot and mardon skipper (*Polites mardon*, W.H. Edwards 1881) butterflies were more widespread and abundant prior to the large-scale loss of open, fescue-dominated grassland habitat. The Taylor's checkerspot is a Species of Concern in Washington, and is considered critically imperiled by state and provincial natural heritage programs. The mardon skipper is listed as Endangered in Washington, and the once robust Puget Sound populations are especially vulnerable to extirpation (Potter et al. 1999). The Taylor's checkerspot and mardon skipper have been petitioned to the U.S. Fish and Wildlife Service and are candidates for emergency listing under the *Endangered Species Act* (Vaughan and Black 2002a, 2002b). Both butterflies require high quality native grassland vegetation to survive (Hays et al. 2000). The grassland, bald, and savanna landscapes upon which the mardon skipper and Taylor's checkerspot depend are threatened by such things as forest encroachment; invasion by nonnative plants; urban, suburban, and ranchette development; agricultural practices; and herbicides. Butterflies themselves are directly threatened by several activities as outlined in Potter et al. (1999) and Vaughan and Black (2002a, 2002b).

The Taylor's checkerspot is small and has wings that are spectacularly checkered with white and brick orange-red spots on a deep brown/black background. The historical distribution of the Taylor's checkerspot is the Willamette Valley-Puget Trough-Georgia Basin grasslands and grassy balds from southern Vancouver Island through the Willamette Valley in Oregon. Currently, there is one small population left in Oregon, and the subspecies apparently has been extirpated from British Columbia. As recently as 1996, several populations in the Puget Sound grasslands numbered in the thousands. By 2000, only three populations were located, and even in the largest of these, few checkerspots were found (Fleckenstein and Potter 1999; Remsberg 2000). Taylor's checkerspot numbers crashed dramatically leaving researchers with few butterflies to study (Hays et al. 2000). Surprisingly, in spring 2003, three new colonies were discovered on small lowland balds near Port Angeles, Washington.

There are no clear answers as to why the Taylor's checkerspot populations have crashed recently, even in protected areas. Obviously, habitat loss played the major role in earlier declines and may be driving recent extirpations by disrupting metapopulations. Many other populations of *E. editha* act as metapopulations and exhibit tremendous variability in abundance and local distribution (Ehrlich 1961; Singer and Ehrlich 1979; Harrison et al. 1988; Boughton 1999; McLaughlin et al. 2002). The fragmentation of prairies and the low vagility of this checkerspot

indicate that isolated populations can no longer act as metapopulations (Char and Boersma 1995). Isolation increases the chances that the populations will become permanently extirpated by making natural recolonization unlikely. Climate change may also make extirpations more likely (McLaughlin et al. 2002).

The Taylor's checkerspot is known to use *Castilleja hispida*, *C. levisecta*, and *Plantago* spp. (Guppy and Shepard 2001; Pyle 2002), and *Collinsia parviflora* and *Plectritis congesta* as host plants. The current distribution of *Castilleja hispida*, probably the most important host historically, is highly patchy, and many existing prairies contain few or very small numbers in isolated patches. *Plantago lanceolata* is widespread, but the Taylor's checkerspot may not be able to use it as a sole food source. Only the Oregon population appears to rely entirely on *P. lanceolata*.

The mardon skipper is a small brown/orange skipper with stubby wings, and is separable from other prairie-dwelling skippers by differences in color and pattern (Scott 1986; Pyle 2002). The extant Washington mardon skipper populations consist of a few hundred individuals at fewer than 30 geographically isolated sites. Three of these sites are in Puget Sound; the remainder are in the southern Cascades. The host plants of the mardon skipper are *Festuca roemeri*, *F. idahoensis*, and probably *F. rubra* (Potter et al. 1999). The species probably uses other grasses, but this has not been documented except in the lab where it has been reared on 'lawn grasses' (Newcomer 1966; recently by Nunnallee and Runquist, unpublished data). The mardon skipper seems to prefer areas of extensive *Festuca* in close proximity to nectar plants (Hays et al. 2000).

Thus far, management and recovery efforts for both butterflies have concentrated on control of invasive plants in existing populations, host plant enhancement, population monitoring, searches for new populations, research on nectar preferences, and initial development of captive-rearing techniques. Government and nonprofit conservation organizations have been actively working to conserve both butterflies in the region. The Xerces Society, the Washington Department of Fish and Wildlife, The Nature Conservancy, and Fort Lewis have been particularly active in the United States.

Butterfly captive rearing for reintroduction is a fairly new initiative in North America (Lipman et al. 1999), although it has been used in several cases in Great Britain (Duffy 1977; Oates and Warren 1990; Pullin 1997), and the potential for captive rearing to contribute to the conservation of wild populations has been recognized for some time (Pyle 1988). Captive rearing of vertebrates is well established as an emergency tool in conservation biology. Vertebrates are more forgiving in captive situations than butterflies because they tend to have significantly longer reproductive lives, but when captive-rearing methods are well developed, butterflies have the capacity to produce larger numbers of individuals quickly. In the case of these butterflies, there is only one opportunity, about two weeks long, for an individual to successfully reproduce. Mistakes at any point in the butterfly life cycle commonly are catastrophic for the captive population, and mistakes are highly likely during initial rearing. Because of a short life cycle, the probable negative genetic consequences associated with captive breeding (Bryant et al. 1999;

Saccheri et al. 1999; Nieminen et al. 2001; Brook et al. 2002), and the substantial long-term augmentation of wild populations with captive-bred animals (Heath et al. 2003), captive rearing using limited numbers of wild-mated females to produce eggs primarily for reintroduction is more conservative and more appropriate for butterflies than common vertebrate methods such as long- term captive breeding.

Butterfly captive rearing is often initially unsuccessful, and there undoubtedly will be surprises. The Taylor's checkerspot and mardon skipper are very much in danger of extinction, but there are still enough individuals and remaining habitat that taking a few individuals from the population for captive rearing is unlikely to have a significant negative effect in the near future. Waiting for an even more dire emergency with only one chance to place a butterfly species into captivity and breed it (i.e., the California condor strategy) would have been foolhardy given likely initial failures. Protection, restoration, and enhancement of habitat is far more important than captive rearing in the long run, but captive rearing can help bridge the gaps in fragmented habitats. Development of captive-rearing methods is happening concurrent with habitat restoration and enhancement, and invasive plant management. This will ensure that there are habitats available for reintroduction. Establishing reintroduced populations will significantly contribute to the recovery of the Taylor's checkerspot and mardon skipper.

Captive rearing will be necessary for propagating enough individuals for reintroduction into areas from which these butterflies have been extirpated. Both have been extirpated from habitat already thought to be suitable, and habitat restoration and enhancement is occurring at those sites. At one site, intensification of prescribed fire was the probable cause of mardon skipper extirpation. At three sites, Taylor's checkerspot, formerly abundant, vanished during an El Niño event even though there was little obvious change in habitat. These locations are out of the dispersal distance for butterflies in extant colonies. For reintroduction purposes, a reasonable number of founders must be used to avoid inbreeding depression. The reproductive potential of adults can produce at least a 10–100-fold increase in the captive population once techniques are refined. Reintroduction success is positively correlated with the number of released individuals (Oates and Warren 1990).

*E. editha* is known to be difficult to rear, although stimulation of oviposition in captivity is easy (Singer et al. 1992). Difficulties have centered on the inability to get caterpillars to either break or survive the lengthy diapause. Recent successes in captive rearing of other *E. editha* subspecies indicate that *taylori* captive rearing is possible. Outdoor rearing had not been successful for other *E. editha* subspecies, possibly due to excess shading created by netting enclosures. This may not be true for the Taylor's checkerspot given that cloudy spring weather often occurs throughout its range.

Mardon skippers are also difficult captive-rearing subjects. In an earlier pilot attempt, none of the larvae survived long enough to pupate. In a second pilot attempt, many larvae pupated, but they either began eclosing months early or failed to eclose at all (Nunnallee, unpublished data). All previous efforts used 'lawn grass' rather than a native host, as was used in this attempt. Low

egg production was thought to be due to using older butterflies, but this may not have been the case.

The development of captive-rearing methods is an iterative process, and to some extent this will always be true. Often one finds a new way to accidentally kill butterflies each year. Each year the stage at which the butterfly dies should get later, or the timing of eclosion should get closer to the wild population. In this project, a new approach that should speed up the rearing process somewhat is being tried. A relatively large number of eggs are needed for experimental rearing. Certainly not so many that wild populations will be endangered, but enough that there will be sufficient numbers of butterflies to test different rearing techniques. In this way, adaptive management techniques can be used to improve rearing. In the case of the Taylor's checkerspot, one female provided enough (126) eggs for initial testing of three different host plants and different group sizes, plus feasibility testing of outdoor rearing. Sixteen mardon skipper females did not provide enough (61) eggs to test as many things as were hoped for, but there were enough eggs to test the feasibility of outdoor rearing and indoor petri dish hand rearing. These initial results will inform captive-rearing attempts for these butterflies using eggs collected in spring 2004.

## Methods

Many of the hand-rearing methods used were common to both butterflies. This rearing project used a combination of methods developed by Dave Nunnallee (in 2002), Washington Butterfly Association, for mardon skippers; Gordon Pratt, University of California at Riverside, for Quino checkerspots (*Euphydryas editha quino*); and the Oregon Zoo for Oregon silverspots (*Speyeria zerene hippolyta*)[1] (Anderson et al. 2001). Techniques developed at the zoo are particularly applicable to largescale rearing. While some details of Oregon silverspot rearing are different, many of the same general hygiene methods are applicable to any large scale butterfly-rearing project. The standard techniques include control of cross contamination by pathogens by keeping larvae segregated; treating all surfaces that will come into contact with larvae by autoclave or with alcohol or chlorine disinfection; promoting frequent hand washing by workers, searching host plant material for predators; and using unsprayed host plants.

Larvae were raised in petri dishes and given appropriate food and space (see Table 1 and mardon skipper methods). The key to providing adequate host plant material was to be pay close attention and add host plant material if the larvae were eating more than expected.

[1]NatureServe Explorer (version 4.0, July 2004) lists this subspecies as the hippolyta fritillary.

**Table 1: Care of hand-reared Taylor's checkerspot larvae.**

| *Stage*[a] | *Action* |
|---|---|
| Egg | Eggs laid in captivity. Ninety-two of 94 eggs hatched. |
| Instar 1 | Placed in 5.5-cm petri dishes with moist, not wet, filter paper in bottom. Two groups of five, two groups of ten were placed on *P. lanceolata*. One group of three, five groups of five and two groups of ten were placed on *C. hispida*. One group of five was provided with both hosts. Moved to new dish/fresh plants every other day. Two medium (2.5 cm) *C. hispida* or one medium (6 cm) *P. lanceolata* leaf per five larvae |
| Instar 2 | Treatments and dishes as above. One medium *C. hispida* or half a *P. lanceolata* leaf per larvae |
| Instar 3 | 8.5-cm dish, otherwise as above. Two medium (2.5 cm) *C. hispida* or one medium (6 cm) *P. lanceolata* leaf per larvae |
| Instar 4 | 13.5-cm dish, otherwise as above. Four medium (2.5 cm) *C. hispida* or one medium (6 cm) *P. lanceolata* leaf per larvae. Began to treat as diapausing when they stopped feeding despite fresh host material added |
| Diapause | After all larvae began diapause, they were weighed. Petri dishes in a humidified chamber until 6 October. Moved to well plates and Toledo jars. Two groups of larvae were kept in refrigerator at 4°C through early March, one group in Toledo jars initially then transferred back to petri dishes on 12 November, the other group was in well plates. One group kept in insulated cooler hydration chamber outdoors. Larvae checked at least weekly |
| Instar 4[b] | Return to feeding in early March. Dish treatment as in instar 4. Extra fresh plants important during this period |
| Instar 5[b] | Treatments and dishes as above; moved daily to new clean dish with fresh plants daily |
| Pupae[b] | Pupae allowed to eclose in cages kept in sheltered area outdoors |
| Adults[b] | Will be returned to site of capture unless there are diseases evident in the captive population, in which case, they will be destroyed to eliminate the risk of disease transmission to their wild counterparts |

[a]There is some overlap with different individuals molting at different times. Sometimes larvae in a group were in different instars, but they generally stayed together.
[b]Future proposed care.

During the first instar to halfway through the third instar, larvae were moved by being picked up with a cleaned, water-moistened, size 0 paintbrush. This was done under a stereomicroscope at 8–16x magnification. In later instars, larvae were moved by being scooped up with a small flat spatula. The larvae were segregated into groups as they hatched to minimize the transmission of disease and to allow for testing of different rearing methods. The assignment of larvae to groups was random in the case of the Taylor's checkerspot. With mardon skippers, the assignment was close to random but was not perfect due to differences in hatching time. Larvae were never

allowed to come in contact with larvae outside their group after hatching. All surfaces that touched the larvae were disinfected to minimize pathogen transmission between groups. Petri dishes were washed and soaked in a 2% chlorine bleach solution between uses by larvae. The paintbrushes and spatulas used for handling larvae were washed in 95% ethanol, then washed twice in distilled water between handling each group of larvae.

Day length and average temperature were similar to those of outdoor conditions at a lowland south Puget Sound grassland. The oviposition and rearing area received indirect sunlight and was in a room with windows that were constantly open to the outdoors for all of the adult captivity and most of the larval feeding season. The amplitude of temperature change throughout the day was less than outdoors. With other butterfly species, temperature has proven to be an important factor in the success of egg laying; therefore, the temperature was warmed somewhat on cooler days in an attempt to optimize mardon skipper egg laying. Temperatures in the rearing/oviposition room were kept at a minimum of 24ºC during the day.

Diapausing Taylor's checkerspot larvae and mardon skipper pupae, both of which continued to be segregated, were placed in either an outdoor 'humidor' chamber in an insulated cooler that contained 4 L of water in the bottom, or in a humidor chamber in a non frost-free refrigerator at 4ºC. The larvae and pupae were individually checked weekly throughout diapause, and the containers were checked for mold and mildew.

Statistical analyses performed included Student's *t* test (using MS Excel 2002) and two proportions test (using MINITAB Statistical Software 2000).

### *Taylor's Checkerspot Methods*

A single adult female Taylor's checkerspot that was destined to be a voucher specimen was captured in a newly discovered colony on a grassy bald near Port Angeles, Washington. She was kept alive and laid 126 eggs on a *C. hispida* plant in the one-gallon (4.4 L) cage in which she was kept. Thirty-two of the eggs were given to a local volunteer captive-rearing expert. Those larvae subsequently died in the second instar after feeding on *Plantago major*, apparently not an acceptable host. The remaining 94 eggs were used for this captive-rearing study (Table 1). Ninety-two eggs hatched between 11 and 16 June, and the larvae were divided into 15 groups: 10 small groups of 3–5 larvae each, and 5 large groups of 9–10 larvae each. Two groups of five were raised outdoors on two enclosed and potted *C. hispida* plants, but these will not be discussed further because their status is uncertain.

The plant material provided to the caterpillars was cut from the host plants and immediately washed. Whole stems of *C. hispida* were cut from the plants. When leaves were removed from the stems of *C. hispida*, they dried out quickly and become unpalatable to the larvae. Whole leaves of *P. lanceolata* were cut from plants growing in an area that received some water from a nearby lawn sprinkler and that was 200 m from a recently extirpated *P. lanceolata*-feeding Taylor's checkerspot colony. Only leaves that appeared to be fairly new and tender and without

significant herbivore damage were selected. Whole stems or leaves of host plants were put in a salad spinner strainer and were thoroughly washed with a forceful but not bruising stream of water for at least 30 seconds. After washing, the plant material was spun in the salad spinner to remove most of the water. The plants were then examined under a stereomicroscope at 5x magnification, and all other insects and eggs were removed.

Except for the larvae that were reared outdoors on the potted plants, all groups were kept in a humidified environment in the same room where they were reared until 6 October. On that date, the caterpillars were put into cold diapause. Two sets of groups were placed in a refrigerator at 4ºC. One of the refrigerated group sets was placed in well plates in which the area in between the cells was filled with distilled water. The other refrigerated set of groups was placed in Toledo jars (i.e., canning jars), and the caterpillars were suspended in a silk organza cell above the water. Because of apparent dehydration, these caterpillars were given water to drink and were moved back into petri dishes in a hydration chamber in the refrigerator in mid-November. The third set of groups was placed in an insulated outdoor enclosure (i.e., a 48-L camping cooler), and was exposed to outdoor conditions. The two outdoor groups were placed, still on the potted *C. hispida*, along the south side of a building.

### *Mardon Skipper Methods*

In consultation with personnel from the U.S. Forest Service and U.S. Fish and Wildlife Service, the Pacific Crest Trail Meadow was selected as the site where female mardon skippers would be collected for captive rearing. A total of 10 females was captured on 11 and 18 July 2003. An additional six very worn females were captured at the Grape Fern Meadow location on 1 August 2003, but none of them laid eggs.

The risk to the mardon skipper population was managed by taking less than 5% of the adult population, roughly and conservatively estimated, by parallel simultaneous transects (unpublished data). Except for the three females that were captured at the Pacific Crest Trail Meadow site on 11 July, risk was further managed by keeping only females that showed some obvious wing wear. Restricting capture mainly to females with some wear ensured that the females had mated and had probably laid part of their eggs in the wild.

The skippers were captured using a fine-mesh net. They were examined to determine their sex (males were released) and age (state of wear), and were immediately transferred to small containers that were placed in a cooler maintained at approximately 5–10°C. Within six hours of capture, the female skippers were transferred to a cage for ovipositioning. Cages were 10 gallons (44 L), 5 gallons (22 L), and 2 gallons (8.8 L) in size. The 10- and 5-gallon models were glass aquaria with tight screen tops; the 2-gallon cages were made entirely of plastic. Each cage held a single butterfly. The cages had been used previously to hold other skippers to ensure the cages were escape-proof. These other skippers were used as surrogates for developing feeding techniques.

While in the cage, each butterfly was fed daily using a cotton swab soaked in a 10% solution of commercially available butterfly nectar. A new sterile cotton swab was used for each butterfly. Touching the nectar-soaked cotton swab gently to the butterfly's antennae sometimes stimulated them to begin searching with their proboscis. At that point, the cotton swab was rolled gently under the butterfly to allow feeding to begin, and then the swab was placed on the floor of the cage. If skippers failed to respond two days in a row to nectar presented in this manner, they were encouraged to feed by having their proboscis unrolled with the head end of a size 0 insect pin while being held by the wings just above the body. After quite a bit of struggling, they generally began feeding once their proboscis contacted the nectar. At that point, it was usually possible to gently release them onto the cotton swab and place them on the bottom of the cage. In order to prevent butterflies from escaping when the nectar was provided, a 100 watt lamp was shone on the back of the cage. This drew the butterfly away from the opening and to the back of the cage. Only one butterfly escaped for any length of time—two days.

Egg laying did not proceed at a very rapid rate, so several additional strategies were tried to encourage egg laying: potted host plants (*F. roemeri*) were placed in three cages; cages were placed in partial direct sunlight (it was difficult to keep temperatures from spiking above 30°C); corrugated surfaces (corrugated cardboard, corduroy cloth) for tactile stimulation were added to the cage; temperatures were varied; nectar concentrations were increased to 20% for three of the butterflies; and cut nectar source flowers (garden heliotrope [*Heliotropium arborescens*], often heavily used by other skippers) were added to two of the cages. Mardon skipper adults very clearly behaviorally thermoregulate. When they attempted to bask in the direction of the light from the window, a heat lamp and/or heater was used to increase the temperature and perceived insolation in an attempt to increase feeding or ovipositing activity.

Upon the butterfly's death or within seven days, whichever came first, the eggs were removed from the cage using a moistened paintbrush and placed on slightly moist filter paper in the first petri dish. The filter paper was kept slightly moist, and any hatched larvae were removed daily and placed in rearing dishes in groups of five. Due to difficulties in stimulating oviposition, only 61 eggs from 16 different females were produced. Except for the third capture group, mardon skipper females were not extremely worn when captured. This was important to ensure that they still contained viable eggs.

*F. roemeri* has been used as the sole food for mardon skipper larvae. Plants were grown in pots, and the leaves were clipped, inspected for predators or other insects, and wrapped in a dry paper towel in a tight bundle approximately 1cm in diameter and 12 cm long. The paper towel was wetted to hydrate the leaves and caterpillars, and to hold the bundle together. The leaves were provided with their base in the paper towel and their tips all facing one way. Hand-reared larvae were kept in 13.5-cm petri dishes; six groups had five larvae, one group had three. The larvae were moved to a new dish with fresh host plant material as soon as frass buildup became obvious or host plant material began to yellow in 1–3 days. This required gently pulling apart the

nests the larvae had constructed in the provided grass bundle. Host decay was variable apparently depending on ambient temperature and condition of the supplied host material.

The exception to these care guidelines were the 14 larvae that were reared on potted *F. roemeri* outdoors. There were two groups of five and two groups of two in netting enclosures over the pots. The plants grew well in the enclosures and did not allow for any monitoring of the larvae. Because there is no way to find out how this rearing method is working until the skippers either eclose or fail to eclose in the spring, these groups will not be discussed further.

The larvae were allowed to pupate and then were removed after two days (to allow the pupae to harden) to a 5.5-cm petri dish. One half of the pupae were placed in plain petri dishes in a hydration chamber in a refrigerator at 4ºC; the other half were placed in petri dishes in a hydration chamber outdoors in an insulated cooler. The Toledo jar method, which has proven successful in overwintering the Oregon silverspot and Karner blue (*Lycaeides melissa samuelis*), was used initially but was unsatisfactory, so the pupae were transferred back to petri dishes. All of the petri dishes containing pupae were in plastic humidor containers. The plastic container contained a bottle of sterilized water and a thin layer of water in the bottom to maintain high humidity. The pupae were individually checked weekly throughout their diapause, and the containers were checked for mold and mildew. When mold or mildew was detected, the pupae were moved to a new humidor and clean dishes. In mid-June 2004, the pupae will be removed from the refrigerator and placed into eclosion cages with fine screening to protect them from parasitoids. The cages will be kept in a shaded area outdoors, and the pupae will be misted daily with water. The cages will be checked daily, and emerged butterflies will be noted and released at their site of origin.

## Results

While these results are preliminary, it is clear that it is possible to rear Taylor's checkerspot larvae from egg through diapause and mardon skipper larvae from egg through adult. The remainder of the Taylor's checkerspot life cycle has yet to be completed, and the mardon skippers have yet to produce adults at the right time of year. Although there was significant mortality in both butterfly species, a substantial proportion of both made it through a large part of their life cycle—*E .e. taylori* through winter diapause and *P. mardon* to pupation. There were more Taylor's checkerspots than mardon skippers, and that made it possible to do additional tests involving various aspects of rearing.

Survival from egg through near diapause end for all hand-reared Taylor's checkerspot larvae was 84%. The difference between survival on the two hosts, *P. lanceolata* and *C. hispida*, was not statistically significant (85% for *C. hispida;* 90% for *P. lanceolata;* two proportions test, $P = 0.517$) (Fig. 1). Weight gain also seemed to be unaffected by host plant (mean weight = 2.62 mg when fed *C. hispida* and 2.59 mg when fed *P. lanceolata;* Student's *t* test, $P = 0.809$). One group of five larvae was fed both *P. lanceolata* and *C. hispida*. The larvae readily fed on

both hosts and generally chose which ever host they encountered. Their mean weight (2.42 mg) was not significantly different from the other larvae (Tukey's pairwise comparison, $P > 0.05$), although the small sample size gave little power to detect differences between this group and the others.

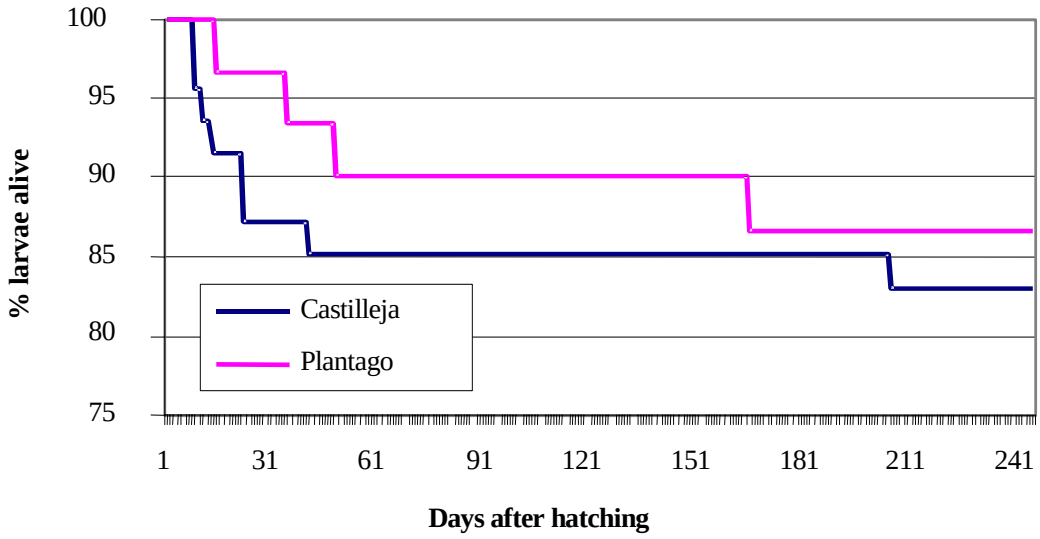


**Figure 1. Larval survival on *Castilleja hispida* vs. *Plantago lanceolata*.**

There was no difference in survival or weight between 5 or 10 larvae reared per container (5 larvae reared mean weight = 2.53 mg; 10 larvae reared mean weight = 2.60 mg; Student's *t* test, $P = 0.602$). Hand-raised larval mortality often occurred when an individual failed to molt along with the rest of the group. Larval mortality occurred more often in early instars and less often as the larvae entered the fourth instar and diapause.

Of the nine Taylor's checkerspot larvae that were placed on the potted *C. hispida* plant, four made it through the winter and began feeding again on warmer days in late February. They moved within the enclosure during the winter, down into a gap between the pot and the potting soil during colder weather, and up onto the wooden supports or leaf surfaces during warmer weather. The pot was exposed to temperatures as low as -11ºC but was slightly protected under the eaves of a building.

One group of five hand-raised, refrigerator-diapause, *C. hispida*-fed larvae was removed on 27 February 2004. They showed interest in *C. hispida* leaves, and one began feeding within one hour of coming out of the refrigerator. Four others began feeding over the course of the day.

Several mardon skipper results are of interest, but the number of larvae was relatively small, so statistical inference is limited. These results, however, suggest that some areas require further testing. Low egg production, asynchrony in larval development, and early eclosion by pupae all will require further investigation.

A total of 61 eggs were laid by the first 10 captive skippers. Three of these skippers did not lay any eggs. Six additional individuals were captured late in the flight period, and none of them laid eggs. The females that laid the most (24) and second most (13) eggs were in different sized cages with different treatments. An average of six eggs per female is clearly below the species' reproductive potential, and females died with fully formed eggs still in their oviduct. The additional strategies that were attempted to encourage egg laying were unsuccessful. When potted *F. roemeri* was placed in three of the cages, the skippers did not lay eggs on the plants, although two of the butterflies did lay eggs in the cage (one laid three eggs, the other five) when the plant was in the cage. No butterflies laid eggs when held outdoors. Cages placed in partial direct sunlight were sometimes exposed to temperatures that spiked above 30°C, although temperatures did not exceed 35°C. The corrugated surfaces added for tactile stimulation were unused for egg laying, but these surfaces were used only with the six butterflies that were captured late in the flight period. Increased nectar concentrations did not lead to an increase in egg laying activity, but the skippers had some difficulty with the stickiness of the dried nectar and had to have their legs and proboscis washed. Butterflies did nectar on the cut flower nectar sources, but they did not lay eggs. When eggs were laid, they were placed somewhat haphazardly in the cage. Some were stuck to the screen lid, some were on the bottom of the cage, and quite a few were in crevices along the edge of the cage.

The last group of mardon skippers captured did not lay any eggs, and all died relatively shortly after entering captivity. This group was captured right at the end of the flight period and appeared lethargic compared to earlier captures. Even after having their proboscis unrolled with a pin, they generally refused to eat. They have been excluded from further analysis because it appears that they were very near the end of their lives and are probably representative only of what nearly dead mardon skippers can contribute to captive rearing.

It is possible to get fair survival of mardon skippers larvae when they are reared in petri dishes on *F. roemeri*. Of the original 33 larvae, 12 survived to pupate. In a previous small pilot study, 9 of 13 larvae from a lowland Puget Sound site survived when reared in this manner. One of the pupae eclosed an adult male seven days after pupating in November. None of the pupae eclosed after being placed in refrigeration.

The larval skippers developed in a highly asynchronous manner. Each of the mardon groups, with the exception of the outdoor-rearing groups, was treated in an identical manner. Despite this, their time to pupation varied wildly. All hatched within a 15-day period from 24 July to 8 August. The first pupa was found on 15 October, and all but one had pupated by 20 December. The last larvae continued to eat and did not pupate until two months later on 14 February.

## Conclusions

Taylor's checkerspots have been relatively easy to captive rear, thus far. The methods used have worked remarkably well in rearing caterpillars from egg through diapause. Some larvae

have begun to feed after diapause indicating that they are likely to successfully complete their life cycle. Initial results are promising, but winter diapause emergence, eclosion timing, and healthy adults have not been produced. The methods used for mardon skippers have been proven to rear eggs through to adults, although significant problems still need to be addressed in timing eclosion, and some improvement could be made in larval survival. Because this captive-rearing attempt was exploratory in nature, it was possible that it would fail entirely. Future attempts can build on the natural history information generated by this exploratory attempt and can allow increasingly rigorous comparisons to be made among different rearing protocols.

The petri dish rearing system has been effective for both butterflies. The low volume and slow but adequate ventilation, combined with a large bottom for dispersing frass, keeps the larvae moist enough while at the same time reducing mold formation. There have not been any escapes, and the dishes are easy to handle. At a larger scale, petri dish racks could be used to stack the containers, which would ease handling and minimize space requirements.

Taylor's checkerspot hand rearing has been successful through late diapause, and it appears from the first group removed that larvae are emerging from diapause and feeding normally. *P. lanceolata* and *C. hispida*, at least in captivity, appear to work equally well as host plants, and larvae will eat either when both are available in the wild. This is somewhat encouraging because even though there are significant efforts underway to plant *C. hispida* in proposed reintroduction areas, *P. lanceolata* is widespread. Whether or not there are any sort of tertiary effects, such as reduced levels of compounds protecting caterpillars against predation, is unknown. Fifty fairly well established *C. hispida* plants were just barely enough for the 50 caterpillars that fed on this host. A ratio of two plants for every larvae would be better and would reduce the stress on plants from excessive cutting. *P. major* is obviously not a suitable host given the fairly rapid mortality of larvae that reared on it.

Group size did not affect rearing weight of the Taylor's checkerspot; therefore, because it is less labor intensive to rear larvae in large groups, future rearing will use the larger group size. It might be possible to increase the group size even more in hand rearing. This would need to occur in a larger sized rearing dish. The space per larvae available for 10 larvae in a 13.5-cm petri dish is 13 $cm^2$. This should probably not be reduced for fourth or fifth instars because of waste generation unless the petri dish is to be changed once a day or more.

Outdoor enclosure rearing of the Taylor's checkerspot is promising. Low temperatures were the likely cause of mortality for five of the nine larvae as there were at least seven larvae alive prior to the cold temperatures. An insulated retreat area would likely reduce this mortality. Taylor's checkerspot larvae are much more visible and accessible than mardon skipper larvae grown in this manner because the host plant has an open growth form, and the larvae do not seem to hide. Not all larvae can always be seen, so there is a loss of information on their exact numbers; however, enough larvae can be seen to indicate if there has been a catastrophic change. Potentially, a raised bed of *C. hispida*, *Collinsia parviflora*, *Plantago lanceolata*, and *Plectritis congesta* could be constructed and netted so that larvae could be raised in an inexpensive and

more natural situation. This could reduce both costs and artificial selection leading to butterflies that are fit for captivity but not for survival in the wild. Excluding predators from such an enclosure is difficult, but it should be possible to eliminate most of them.

Some results from the mardon skipper rearing were baffling. Because the larvae are from a higher elevation population that has a later flight period than Puget Sound populations, they may develop differently than the lowland populations. Mardon skipper captive rearing has thus far been hampered by low rates of egg laying by females. It is important to get additional egg production from each female. Current rates of oviposition in captivity are unlikely to provide even replacement of animals that are taken into captivity given that some mortality will occur. There is little information available on getting skippers to oviposit in captivity. The one escapee was the butterfly that laid the most eggs. We do not know what drives mardon skipper oviposition, but it is possible that they need movement. Other insects have 'odometers' that trigger specific behaviors (Srinivasan et al. 2000). This may also be true for mardon skippers. Other techniques to get mardon skippers to oviposit have been suggested by other workers; future efforts will test these methods. Results from this study suggest that for captive-rearing purposes, females taken early in the flight period are preferable to those captured late in the flight.

The source population of mardon skippers is usually under snow at least some of the time starting in late October. It is quite odd that the captive-reared larvae were still feeding when the larvae in the source population must surely have been pupating. The reason for this is probably due to a dramatic difference between daytime temperatures and insolation at the higher elevation, warmer, and drier site in the south Cascades and in the shaded, cooler captive-rearing room. This makes sense because the captive-reared larvae may not have had the same number of degree days as larvae in the wild. Beyond the lateness of the first pupation, the extreme asynchrony of pupation indicated that something was not right with the captive conditions. Earlier work with the Puget lowland mardon skippers did not produce this result. It is unknown why the first mardon skipper pupated in mid-October, probably at least a month after wild populations, and the last did not pupate until mid-February, but temperature and larval food source are likely causes. Temperature has an overwhelming effect on larval development for many insects. In terms of food sources, larvae in the wild feed on *Festuca* and other grasses, and nutrients provided by those other species may be needed for rapid growth. In previous rearing, larvae were fed 'lawn grass', which gave them a larger variety of grass species to choose from. Future rearing should compare the use of *Festuca* vs. *Festuca* plus other grass species, and different temperature regimes in achieving synchronicity in mardon skipper development within the captive population and with animals in the wild.

Survival rates for mardon skippers from first instar larvae through pupae compare favorably with survival rates for other captive-reared endangered butterflies such as the Oregon silverspot, although survival was much reduced compared to earlier rearing of lowland populations. The slow development compared to wild populations may have increased mortality. Mardon skippers that eclosed in the fall, both in this project and in a previous one, appeared to have normal

morphology, indicating that they can be reared through to adult. If timing issues can be resolved, captive rearing of eggs for reintroduction is certainly possible. The results of winter diapause survival will indicate whether or not the methods described above will actually be sufficient for rearing enough butterflies for effective reintroduction.

Bundling up fescue takes time; therefore, it would be good to find a way to reduce the labor required. In future efforts, it may be useful to try placing loose fescue on moist filter paper. This would require less handling time, but it might create difficulties for larvae when constructing nests since they appear to need parallel blades of grass. It may also make it difficult for the larvae to feed. Unfortunately, the use of outdoor rearing has significant problems. It is not possible to check on the larvae without destroying the host and having a high likelihood of killing larvae or pupae due to the host plant's dense growth habit and the larvae's tunneling behavior.

Scaling up rearing gradually minimizes the loss of animals and provides opportunities to learn additional information about the species' natural history. At this point, with more than 60 larvae of each type of butterfly in captive rearing, a few inferences between different methods can be drawn. In future years, with even more larvae in the captive-rearing program, investigation of rearing using adaptive management techniques will become more powerful and rigorous. Future captive rearing will be done in conjunction with a zoo that is experienced in butterfly rearing.

Success in getting all the butterflies through diapause and finding ways to ensure that they eclose at the correct time will indicate whether or not the lessons learned in this captive-rearing effort can be increased in scale and used to reintroduce populations into suitable habitat. None of the butterfly rearing methods are ready for large scale captive rearing and reintroduction. The use of experimental adaptive management method development at a mid-sized scale (100–500 eggs) should lead to repeatable successful methods and may provide enough butterflies for a single reintroduction attempt. With persistent efforts to investigate rearing methods, rearing of both butterflies should be scalable to levels that reliably support reintroduction for extirpated populations in the near future.

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