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Interspecific Variation in Size, Diapause Intensity, and Moisture Responses of First-Instar *Speyeria* (Lepidoptera: Nymphalidae) Larvae

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ABSTRACT Egg weights of *Speyeria* (Nymphalidae) species from California were measured to estimate relative sizes of first-instar larvae. First-instar larvae were compared for diapause intensity and for their physiological and behavioral responses to atmospheric humidity and free water. Diapause intensity, measured by number of days between first instar and molt to second instar, ranged from 7.6 d (*Speyeria nokomis*) to 40.7 d (*Speyeria callippe*). Among species, diapause intensity was uncorrelated with egg weight, but within three species (*S. nokomis*, *Speyeria mormonia*, and *Speyeria zerene*), diapause intensity was positively correlated to egg weight. There was significant interspecific variability in the desiccation tolerance of diapause larvae to low (11% RH) humidity. The least and most desiccation-tolerant species, respectively, were *S. nokomis* (LT_{50} = 1.9 d) and *S. callippe* (LT_{50} = 13.6 d). Interspecific desiccation tolerance was uncorrelated with egg weight. Larvae of *S. nokomis* moved from lower to higher humidity within a humidity gradient, whereas larvae of *S. callippe* did not move toward higher humidity. Desiccated larvae of *S. callippe* and *S. zerene* rehydrated by imbibing free water. Exposure of *S. callippe* and *S. zerene* larvae to 100% RH in the absence of free water did not result in a body weight increase, but high humidity conditions reduced the rate of water loss.

KEY WORDS *Speyeria*, diapause, desiccation, behavior, overwintering

Speyeria (greater fritillaries) overwinter as first-instar larvae in diapause. Diapause larvae are very small (1–2.5 mm) and, following eclosion and consumption of their egg shell, they do not feed until the following spring. The diapause larvae of *Speyeria* species from xeric habitats are exposed to considerable desiccation stress, especially during the summer and fall months. In California, desiccation stress is especially severe among those species occurring in summer dry habitats where adults of species such as *Speyeria callippe* often emerge in May and conclude oviposition before July (Brittnacher et al. 1978). Little is known about the physiology and behavior of diapause larvae, yet appropriate adaptations are critical to successful overwintering within each habitat. We examined interspecific differences in size, diapause intensity, and desiccation resistance of first-instar larvae and related them to preferred species habitats. Observations were also made on the physiological and behavioral responses of diapause larvae to moisture. The questions we asked were 1) how do species differ in size, diapause intensity, and their ability to survive desiccating conditions? 2) do larvae respond to gradients in atmospheric humidity? and 3) what mechanism(s) do larvae use to replenish lost body fluids?

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Materials and Methods

Insects. Adult *Speyeria* collection locations in California and Nevada (NV) were as follows: Anthony Peak, Mendocino Co., 2100 M (AP); Boggs Mountain State Forest nr. Cobb, Lake Co., 850 M (BM); Devils Gate Pass, Mono Co., 2230 M (DG); Donner Pass, NV Co., 2100 M (DP); Kings Canyon, Carson City Co., NV 1600 M (KC); Lang Crossing, NV Co., 1600 M (LC); Round Valley, Inyo Co., 1400 M (RV); Sagehen Creek, 8.4 miles N Truckee, NV Co., 1960 M (SC); and Yuba Pass, Sierra Co., 2000 M (YP).

The species studied and collection locations were: *S. callippe* (Boisduval) (CA-AP, CA-BM), *Speyeria coronis snyderi* (Skinner) (CO-LC, CO-YP), *Speyeria cybele leto* (Behr) (CY-SC, CY-KC), *Speyeria egleis* (E-DP, E-YP), *Speyeria hesperis irene* (Boisduval) (H-AP, H-DP), *Speyeria hydaspe* (Boisduval) (HY-AP), *Speyeria mormonia* (Boisduval) (M-DP), *Speyeria nokomis apacheana* (Skinner) (N-DG, N-RV), *Speyeria zerene zerene* (Boisduval) (ZZ-BM), and *Speyeria zerene conchyliatus* (Comstock) (ZC-DP, ZC-YP).

Field-collected adult females were transported back to the laboratory in glassine envelopes under refrigeration in insulated coolers. Females were handled, and ova obtained, as previously described by Sims (1979, 1984). Briefly, adults were fed once daily on a 10% honey–water solution and maintained, in 1-liter cardboard containers over leaves of *Viola papilionacea* Pursh, at 24 ± 1°C and a photoperiod of 15:9 (L:D) h within a Percival environmental chamber.

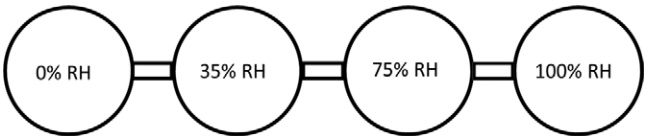

Fig. 1. Experimental design used for studying humidity responses of first-instar *Speyeria* larvae.

Ova and first-instar larvae were exposed to 24 ± 1°C, 30–40% RH, and a photoperiod of 14.5:9.5 (L:D) h. Larval behavior tests were performed at the same photoperiod and temperature. Multiple strips of moist paper toweling in 118-ml glass baby food jars provided a substrate for larvae maintenance before testing.

Egg Weights and Estimation of Larval Sizes. First-instar larval size was estimated by determination of ova weight. Groups of 45–50, 3–5-d-old ova were weighed to determine mean live weight. There were three to six replications per species representing the ova from ≥10 females. Following live weight determination, eggs were dried for 72 h at 75°C in a vacuum oven and then reweighed; average dry weight and water loss were estimated. To evaluate the relationship between fresh ova weight, dry ova weight, and newly eclosed first-instar larval weight, larval weights were determined for two species (H-DP, E-DP).

Diapause Intensity—Interspecific Differences. Diapause intensity was studied in 2–4-d-old larvae of CA-AP, CO-YP, CY-SC, E-YP, H-AP, HY-AP, M-DP, N-RV, and ZC-YP. A camel hair brush was used to transfer larvae that were confined on top of three lightly moistened pieces of filter paper within a 100- by 25-mm styrene plastic petri dish. A small leaf of *Viola papilionacea* with the cut end of the petiole inserted into a small vial of water was also placed in the dish. The filter paper and leaf were replaced at 2–3-d intervals to minimize fungal contamination. Lighting was continuous from a 60-W bulb in a gooseneck lamp positioned ≈10″ (≈25 cm) away. Laboratory temperatures were 24°C (±2°C). The test was scored daily and diapause was considered to be terminated when larvae molted to the second instar.

Diapause Intensity—Intraspecific Effects of Larval Size. Intraspecific analysis of the influence of larval weight on diapause intensity was conducted on three species: M-DP, N-DG, and ZZ-BM. Ova were collected from the first 3–5 d of oviposition and from the final 5–7 d of oviposition (estimated from wing wear and number of days following capture) in the laboratory. Groups of 50–100 ova were weighed to the nearest 10 μg and the mean egg weight was calculated. Mean weights of the earliest laid eggs always exceeded those laid near the end of female life. Four groups of larvae per species from the early, heavier eggs, and late, lighter eggs, were tested for diapause intensity in the same manner as described earlier.

Desiccation Stress Larval Survival. Groups of ≈50 larvae from nine species (CA-AP, CO-YP, CY-SC, E-DP, H-DP, HY-AP, M-DP, N-RV, and Z-YP) were maintained at 25 ± 1°C and 11% RH. There were three to five replications per species. The 11% RH was maintained within a heavy glass 200 mm ID desiccator (Fisher, Pittsburg, PA) using a saturated LiCl salt solution (Greenspan 1976). Mortality was scored daily for the first 7 d and at 2-d intervals thereafter. Data were analyzed using POLO PC probit analysis (LeOra Software 2002) and LT_{50} and LT_{90} values were determined. Relationships between desiccation LT_{50} values, diapause intensity, and egg weights were studied using the Spearman rank order correlation (SAS Institute 1999).

Humidity Responses. Larval responses of *S. callippe* (CA-AP) and *S. nokomis* (N-DG) to a humidity gradient were determined using a multiple-chamber tube made from four plastic vials (38 mm OD by 64 mm in height). The vials were connected in a linear row using 15 mm lengths of 5 mm ID clear Tygon tubing. A smaller-diameter plastic tube formed the base for a tight-fitting fine nylon mesh bottom within each larger vial. A schematic (representation) of the device is shown in Fig. 1. Approximate relative humidities (RH) are indicated by numbers on the chambers. RH inside the vials and the substance or saturated salt solution used to maintain them were as follows: 0–5% RH, Drierite desiccant (W. A. Hammond Drierite Co. Ltd., Xenia, OH); 35% RH, $CaCl_2$; 75% RH, $NaCl_2$; and 100% RH, H_2O (Greenspan 1977). Salts were obtained from Sigma-Aldrich (St. Louis, MO) and were of >95% purity. A 1.25-cm-wide strip of color indicator humidity paper (Humidial Corporation, Colton, CA) with a humidity accuracy of ±5% was placed inside each vial to verify RH. This level of accuracy was judged to be adequate because larval response to an RH gradient was the key variable. Humidity preference (HP) determinations were made by placing 15 or 30 first-instar larvae (20-d-old) into each of the four different RH vials. After 48 h, the number of larvae in each of the RH vials was counted. Three replications of the test were done per species and the results were combined for analysis. Dead larvae were not counted. Deviations from an expected 1:1:1:1 (i.e., 25% in each tube) distribution of larvae could reflect larval activity level and HP. Active larvae without a distinct HP should randomly distribute themselves according to the frequency of vials containing each humidity condition. Here, one would expect 25% of the larvae in each of the 100, 75, 35, and 0% RH tubes. Sluggish or inactive larvae should tend to remain within the vial into which they were introduced and also give a 1:1:1:1 distribution. A positive larvae HP response by active larvae would be supported by an excess of larvae in the higher RH vials and a deficiency in the low RH vials. The significance of differences between observed versus the expected 1:1:1:1 larval distribution was analyzed using a chi-square (χ^2) goodness-of-fit test.

Table 1. Egg weights of *Speyeria* species from California locations

Species–location	*n*	Fresh egg wt μg-mean (SE)	Dry egg wt μg-mean (SE)	Percent water	Fresh larval wt μg-mean (SE)	*n*
S. coronis-YP	150	188.7 (3.1)	58.3 (1.7)	69.1	–	–
S. zerene-YP	246	198.6 (2.1)	61.0 (0.3)	69.2	–	–
S. cybele leto-SC	97	209.5 (2.6)	68.7 (0.5)	67.2	–	–
S. mormonia-DP	246	237.7 (2.0)	79.2 (0.4)	66.7	–	–
S. hesperis-DP	298	267.6 (5.2)	87.2 (1.4)	67.4	170.8 (6.9)	53
S. egleis-YP	286	271.8 (5.1)	89.0 (1.5)	67.3	171.5 (8.8)	70
S. callippe-AP	298	282.9 (6.2)	97.0 (1.7)	65.7	–	–
S. hydaspe-AP	300	295.0 (7.9)	98.5 (2.9)	66.6	–	–
S. nokomis-RV	350	298.6 (1.9)	104.7 (0.7)	64.9	–	–

Larval Replenishment of Body Liquids. The mechanisms by which *Speyeria* larvae replenish depleted body fluids are unclear. Mechanisms might be limited to simple drinking or could include physiological adaptations such as absorption of water through the cuticle (O'Donnell and Machin 1988). These possibilities were studied using partially desiccated 7–8-d-old first-instar *S. callippe* (CA-AP) and *S. zerene* (ZC-YP) larvae previously maintained at 24 ± 1 C and 35 ± 5% RH. Samples of larvae were weighed before and after treatment using a Mettler Analytical Balance (0.01 mg readability) and mean weight loss (gain) calculated. Tests were conducted at 24 ± 1°C and test duration was 72 h. Two test conditions were studied. The first condition was 35% RH with direct larval contact and access to water. A glass petri dish bottom was lined with four thicknesses of Whatman number 1 filter paper (Whatman Inc., Piscataway, NJ) saturated with water and covered with fine mesh netting. Larvae were allowed direct contact with the wet filter paper surface. The second condition was 100% RH without larval access to free water. Vials similar to those previously described but without tubing holes were used to evaluate response to 100% RH.

Water Consumption by Desiccated Larvae. Observations were made, at 50× magnification using a Wild M5 Stereomicroscope, of desiccated larvae of *S. callippe* (CA-BM) and *S. zerene* (ZZ-BM) when placed in direct contact with filter paper saturated with water containing 1% carmine dye (Ward's Natural Science, Rochester, NY).

Results

Egg Weights and Estimation of Larval Sizes. Fresh and dried ova weights from nine *Speyeria* species are presented in Table 1. Mean fresh egg weights ranged from 188.7 μg (*S. coronis*) to 298.6 μg (*S. nokomis*). Water loss was similar among ova of all species (range, 64.9–69.2%) and the order of dry ova weights corresponded to that of fresh weights. Fresh larval weights of *S. hesperis* and *S. egleis* were 63–64% of fresh ova weight ($n = 75$ ova and larvae per species, three replications). These data support the use of ova weight as an index for first-instar larval weight.

Diapause Intensity—Interspecific Differences. Diapause intensities of first-instar *Speyeria* larvae are shown in Table 2. Diapause was most easily terminated in *S. nokomis* (7.6 d) and was most intense in *S. callippe* (40.7 d). Mortality during the experiment exceeded 70% for all species, except *S. nokomis* and *S. mormonia.* Diapause intensity could not be calculated for *S egleis* and *S. hydaspe* because mortality was 100%. Diapause intensity was not significantly correlated with egg size (Spearman's rho $= -0.143$, $n = 7$, $P = 0.720$).

Diapause Intensity—Intraspecific Effects of Larval Size. The influence of larval weight on diapause intensity for *S. mormonia*, *S. nokomis*, and *S. zerene* is shown in Table 3. For each species, the heavier, earliest laid ova produced larvae with stronger diapause than the lighter larvae from ova produced near the end of female reproduction. The correlation between egg weight and diapause intensity was highly significant ($r > 0.90$, $P < 0.01$) in each species.

Table 2. Diapause intensity of first-instar *Speyeria* larvae

Species–location	Starting sample size	No. breaking diapause	Mortality %	Days to break first-instar larval diapause[a,b]
S. nokomis-DG	203	180	11.3	7.6 (0.2)
S. zerene-BM	219	60	72.6	10.6 (0.4)
S. mormonia-DP	114	68	37.7	10.7 (0.4)
S. hesperis-DP	126	18	85.7	17.8 (1.7)
S. coronis-LC	124	14	88.7	19.4 (1.7)
S. cybele leto-KC	78	11	85.9	35.3 (2.4)
S. callippe-BM	218	38	82.6	40.7 (2.6)
S. hydaspe-AP	65	0	100	–
S. egleis-YP	78	0	100	–

[a] First–instar larva, at 24°C (100% RH), begins feeding on *Viola* leaf and molts to second instar.
[b] Mean (SE).

Table 3. Relationship between egg size and first-instar larval diapause intensity in three species of *Speyeria*

Species–location	Sample size	Mean egg wt (μg)	No. breaking diapause	Mortality %	Days to terminate diapause[a,b]
S. nokomis-DG	101	290	101	0	6.3 (0.2)
S. nokomis-DG	102	322	79	22.6	9.4 (0.4)
S. zerene-BM	42	207	11	73.8	8.3 (0.9)
S. zerene-BM	96	253	49	49.0	11.1 (0.5)
S. mormonia-DP	48	195	37	35.4	9.2 (0.3)
S. mormonia-DP	66	235	31	39.4	12.6 (0.7)

[a] First-instar larva, at 24°C (100% RH), begins feeding on *Viola* leaf and molts to second instar.
[b] Mean (SE).

Survival of Larvae Under Desiccation Stress. Times to 50 and 90% mortality of larvae (LT_{50} and LT_{90}, respectively) at 11% RH are shown in Table 4. *S. nokomis* was least resistant to desiccation (LT_{50} = 1.9 d, LT_{90} = 3.7 d), whereas *S. callippe* had the greatest desiccation resistance (LT_{50} = 13.6 d, LT_{90} = 29.6). Desiccation resistance was not correlated to egg size (Spearman's rho = 0.150, $n = 9$, $P = 0.676$) but desiccation resistance and diapause strength were correlated (Spearman's rho = 0.750, $n = 7$, $P = 0.038$).

Humidity Responses. Diapause first-instar *S. callippe* larvae were relatively inactive, with few moving from the release vials to adjacent vials. Total *S. callippe* larvae in the 100, 75, 35, and 5% vials were 51, 48, 59, and 42, respectively. There was no significant deviation from an expected 1:1:1:1 distribution ($\chi^2 = 3.0$, $P > 0.05$). In contrast, *S. nokomis* larvae were very active and total larvae in the 100, 75, 35, and 5% vials were 70, 32, 17, and 20, respectively. The observed larval distribution of *S. nokomis* showed preference for high humidity and was different ($\chi^2 = 51.3$, $P < 0.01$) from the expected 1:1:1:1 distribution.

Larval Replenishment of Body Liquids. *S. callippe* and *S. zerene* larvae showed little evidence for the ability to absorb water through their cuticle or via respiration (Table 5). A small amount of weight gain of larvae at 100% RH may be attributable to consumption of condensation on the lower sides of the vials. A more dramatic weight increase occurred as a result of drinking water. *S. callippe* larvae gained >33% of their original weight in water, whereas *S. zerene* weight increased >58%. Desiccated first-instar larvae of *S. callippe* and *S. zerene* on moistened filter paper containing red carmine stain immediately placed their mouthparts (labrum, maxillae, palps, spinneret, and mandibles) into a droplet of water and imbibed a sufficient amount to color the alimentary canal red. The stain could be observed through the pale intersegmental areas of the integument. Water uptake was rapid, lasting for 1–4 min during the initial drink, and the larvae rapidly expanded in size from the water gain. No mouthpart movement was observed when drinking was from droplets or small pools, but mandibular movement was noted when either a wet surface was tested by the larvae or when the water was not in pools. A characteristic body posture adopted by the drinking larvae was an arched stance with only the first or the first and second pairs of thoracic legs and the anal prolegs touching the substrate.

Table 4. Response (days to 50 and 90% mortality) of first-instar *Speyeria* larvae to desiccation stress at 11% RH

Species–location	*n*	LT_{50} (95% CI)	LT_{90} (95% CI)
S. nokomis-RV	170	1.9 (1.0–2.4)	3.7 (2.9–5.9)
S. zerene-YP	264	2.7 (2.4–3.0)	6.5 (5.9–7.2)
S. cybele leto-SC	143	3.2 (1.4–4.6)	10.9 (7.3–32.5)
S. egleis-YP	232	3.9 (3.2–4.6)	12.2 (10.5–14.6)
S. mormonia-DP	148	4.5 (2.4–6.3)	12.6 (8.8–31.2)
S. coronis-YP	261	5.8 (5.0–6.5)	16.4 (14.5–18.9)
S. hesperis-DP	381	11.3 (9.5–13.0)	24.2 (20.5–31.1)
S. hydaspe-AP	372	11.6 (9.6–13.6)	26.6 (21.5–37.7)
S. callippe-AP	187	13.6 (10.6–13.5)	29.6 (23.7–42.8)

Discussion

Speyeria is a popular group for studies on taxonomy and speciation but most research has focused on the showy adults (Brittnacher et al. 1978, Hammond 1990/1991, Dunford 2009), whereas there have been few studies on larval biology and behavior. This lack of knowledge is particularly acute for the first-instar larvae, which probably suffer the greatest life-stage-specific mortality (Zalucki et al. 2002). Adaptive responses of first-instar larvae are a critical component of *Speyeria* survival. All *Speyeria* species enter a first-instar diapause during the summer or fall, overwinter, and resume larval growth and development on their *Viola* host plants during the spring or summer of the following year. Important physiological adaptations of first-instar larvae to their summer and overwintering habitat include desiccation resistance, diapause strength, and tolerance to freezing temperatures (which was not studied here). We focused on a comparative analysis of the diapause strength and desiccation resistance of nine *Speyeria* species found in California. These represent >50% of the 16 currently recognized *Speyeria* species in North America (Opler and Warren 2005), but three of the species studied (*S. hesperis*, *S. callippe*, *S. zerene*) have numerous subspecies occupying a diverse array of habitats over a wide geographical range (Hovanitz 1943, Grey and Moeck 1962, Arnold 1985, Hammond 1990/1991, Dunford 2009). Therefore the larval characteristics of the subspecies studied may not be typical of larval responses of other subspecies and populations from different habitats.

Table 5. Weight gain of first-instar *Speyeria* larvae in response to humidity and free water

Species–location	Total sample size[a]	Treatment	Weight (μg) before treatment, mean (SE)	Weight (μg) after treatment, mean (SE)	Percent wt gain, mean (SE)
S. callippe-AP	7	35% RH, 24°C, contact moisture	139 (6)	186 (5)	33.8 (2.5)
S. callippe-AP	20[b]	35% RH, 24°C, contact moisture	112 (2)	154 (4)	37.7 (2.3)
S. callippe-AP	5	100% RH, 24°C	140 (6)	139 (5)	−0.7 (1)
S. zerene-YP	5	35% RH, 24°C, contact moisture	106 (3)	168 (4)	58.5 (5.6)
S. zerene-YP	2	100% RH, 24°C	101 (1)	103 (4)	2.0 (4.2)

[a] Replications of 50 larvae.
[b] Individual larvae.

Among the *Speyeria* studied, we found a positive relationship between larval diapause strength and desiccation resistance. However, in the interspecies analysis, neither diapause strength nor desiccation resistance was related to larval size. Karlsson and Wiklund (1985) reported similar results in a group of five Satyridae species that showed no correlation between either egg weight and desiccation resistance or first-instar larval weight and starvation resistance.

S. nokomis, with the heaviest larvae, had the least intense diapause and lowest desiccation resistance. *S. nokomis* inhabits lush wet montane meadows where adults emerge and oviposit during August and September. Although *Viola* hosts in these wet meadow habitats may remain suitable for larval development in late summer, oviposition timing of female *S. nokomis* would preclude completion of a second generation (Ferris and Fisher 1970; Scott and Mattoon 1981, 1982). First-instar larvae of *S. nokomis* are exposed to desiccating conditions for a relatively brief period. In addition, our results indicate that larvae will actively seek locations within the meadow habitat with the highest relative humidity and lowest desiccation stress. The combined effect of late season larval emergence and moisture seeking behavior would reduce water loss and increase survival. *S. callippe* had the most intense diapause and highest desiccation resistance. *S. callippe* typically inhabits the warmest and driest habitats of any California *Speyeria* and many low-elevation California populations initiate adult flights as early as May. At this time, their *Viola* host plants have already flowered and begun to senesce. First-instar larvae hatching in spring/early summer are therefore exposed to 4+ months of severe drought and high temperatures before cooler/moister conditions. *S. cybele leto* and *S. mormonia* are found in meadow habitats at moderate to high elevations (Hammond 1981/1983, Boggs 1987) and their larvae have relatively low resistance to desiccation. *S. cybele leto*, however, had a strong larval diapause more typical of xeric species, suggesting adaptation to a longer summer exposure under more arid conditions. The remaining species are found in xeric habitats at various elevations. *S. hesperis* is mainly found in lodgepole pine-red fir forests, *S. egleis* occurs in open dry habitats above 2100 M, *S. hydaspe* occupies dry habitats below 1,900 M and broadly overlaps the higher elevation habitats of *S. callippe* (Hammond 1981/1983, Hammond and Dornfeld 1983). *S. hesperis* and *S. hydaspe* both have high desiccation resistance, which is consistent with the dry habitats in which they occur. Larvae of *S. egleis* are significantly less resistant, suggesting adaptation to late season oviposition at higher elevations and perhaps cooler-moister microhabitats. *S. coronis* and *S. zerene*, from mid elevation habitats, have relatively weak desiccation resistance. Females from both of these species can delay oviposition until late summer/early fall via a reproductive diapause (Sims 1984, James and Pelham 2011), reducing the duration of larval exposure to desiccation. Reproductive diapause is not unique to western *Speyeria. Speyeria idalia*, from prairie habitats in the Midwest, also exhibits a reproductive diapause, and there is evidence that *Speyeria diana* may delay oviposition until fall (Kopper et al. 2001, Adams and Finkelstein 2006, Wells et al. 2011).

The physiology of first-instar diapause in *Speyeria* larvae has not been studied. Diapause appears to fit the definition of "obligate" in most species but there is significant intra- and interspecific variation in diapause strength. One species (*S. nokomis*) has a diapause more similar to "quiescence" (Tauber et al. 1986, Danks 1987). In most species, normal diapause termination appears to require an interval of exposure to low temperatures (Mattoon et al. 1971, Hodek 2002, James 2008). In this study, species with stronger diapause, and possibly longer low-temperature termination requirements, suffered higher mortality under "forced" diapause termination conditions. Under natural conditions, termination probably occurs in winter followed by a temperature maintained quiescence that would serve to synchronize larval activity with the emergence and availability of violets. For those *Speyeria* species occurring in montane habitats, adaptations to freezing winter temperatures must also exist, but the details of these adaptations are unstudied.

Diapause strength varies significantly among *Speyeria* species and for species such as *S. zerene*, which occur over a considerable altitudinal range, it probably also varies among populations. We did not find a significant interspecific relationship between diapause strength and larval size but diapause strength can vary between progeny of individual females producing eggs/larvae of different sizes. Within three species, larger larvae (those produced earlier in female life and earlier in the season) had relatively stronger diapause than small larvae produced near the end of female oviposition. These findings suggest that although larvae produced late in the flight season may have a relatively weaker diapause, and perhaps less

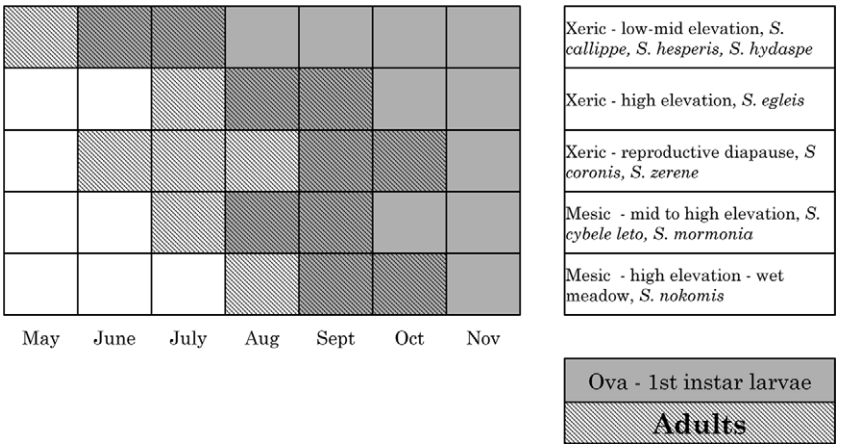

Fig. 2. Major life history strategies, from spring to fall, of *Speyeria* species in California.

desiccation resistance, they also encounter a shorter period of drought stress.

Desiccation resistance is a component of larval adaptation related to the severity (high temperatures, drought duration, etc.) of the summer habitat. For diapause larvae, behavioral, biochemical, and/or physiological adaptations are essential for minimizing water loss and enhancing survival in a desiccated state. Other summer dormant insect species use one or more adaptations to maintain water balance. These adaptations include absorption of water vapor, low metabolic rate, increases in osmolytes, tolerance of low body water levels, cuticular hydrocarbon changes leading to decreased cuticular permeability, and cell membrane restructuring (Beament 1964, Danks 2000, Benoit 2010). In addition, modifications in respiratory transpiration may help to decrease larval water loss (Chown 2002). Details of the physiological water balance adaptations in *Speyeria* await further study. We demonstrated that two xeric species (*S. callippe* and *S. zerene*) lack the ability to rehydrate by absorbing atmospheric moisture. However, desiccated larvae of both species readily drink free water and this may be a major route of rehydration under natural conditions. *S. nokomis* larvae were not tested for ability to absorb atmospheric water, but their positive movement toward high humidity would help to reduce water loss. For xeric-adapted species without predictable access to free water during summer diapause, avoidance of excess heat and reduction of water loss are critical. One simple method that larvae could use would be to seek habitats within the soil, below ground level, or within accumulations of organic material that are shaded with relative humidity higher and temperature cooler than ambient. Scott (1986), for example, observed that first-instar larvae of some *Speyeria* can hibernate inside grass stems. James and Nunnallee (2011) found first-instar larvae of *S. zerene* overwintering inside a *Viola* seed case and noted that larvae of other species have been found inside dry plant material.

Speyeria species have evolved a number of distinct adaptations and strategies for synchronizing larval development with growth of their *Viola* host plants and maximizing the survival of the desiccation-sensitive first-instar larvae over prolonged periods. These strategies include combinations of larval diapause strength and desiccation resistance, larval behavior, reproductive diapause in adult females, and habitat selection (See Fig. 2). Mesic habitat species have achieved this by a combination of late season flight times, moist ground level conditions, and, perhaps, larval moisture-seeking behavior. Xeric habitat species survive using either a "wait it out" strategy in which larvae are extremely desiccation resistant or by reducing the larval drought exposure period through a reproductive diapause or by favorable habitat selection (e.g., high altitude, coastal environment, microhabitat selection).

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