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COMPARATIVE STUDIES ON THE IMMATURE STAGES AND DEVELOPMENTAL BIOLOGY OF FIVE *ARGYNNIS SPP.* (SUBGENUS *SPEYERIA*) (NYMPHALIDAE) FROM WASHINGTON

DAVID G. JAMES

Department of Entomology, Washington State University, Irrigated Agriculture Research and Extension Center, 24105 North Bunn Road, Prosser, Washington 99350; email: david_james@wsu.edu

ABSTRACT. Comparative illustrations and notes on morphology and biology are provided on the immature stages of five *Argynnis spp.* (*A. cybele leto, A. coronis simaetha, A. zerene picta, A. egleis mcdunnoughi, A. hydaspe rhodope*) found in the Pacific Northwest. High quality images allowed separation of the five species in most of their immature stages. Sixth instars of all species possessed a fleshy, eversible osmeterium-like gland located ventrally between the head and first thoracic segment. Dormant first instar larvae of all species exposed to summer-like conditions (25 ± 0.5° C and continuous illumination), 2.0–2.5 months after hatching, did not feed and died within 6–9 days, indicating the larvae were in diapause. Overwintering of first instars for ~ 80 days in darkness at 5 ± 0.5° C, 75 ± 5% r.h. resulted in minimal mortality. Subsequent exposure to summer-like conditions (25 ± 0.5° C and continuous illumination) resulted in breaking of dormancy and commencement of feeding in all species within 2–5 days. Durations of individual instars and complete post-larval feeding development durations were similar for *A. coronis, A. zerene, A. egleis* and *A. cybele* (54.1–55.5 days from post-diapause first instars to adulthood). Development of *A. hydaspe* was significantly faster averaging 47 days. Larvae of all species readily accepted *Viola adunca* and *V. glabella* as host plants. *Viola labradorica* was also accepted by all instars of *A. egleis*, however, its acceptance was limited in the other species to later instars. Domesticated pansies (*Viola tricolor*) were accepted by sixth instars of *A. egleis, A. coronis* and *A. zerene*, but only limited feeding occurred with sixth instar *A. cybele*.

Additional key words: morphology, osmeterium, development, diapause, host plants, overwintering, instar durations

Nine species of Greater Fritillary (*Argynnis spp.*) occur in Washington: *A. cybele* (F.), *A. coronis* (Behr), *A. zerene* (Boisduval), *A. callippe* (Boisduval), *A. egleis* (Behr), *A. hesperis* (Edwards), *A. atlantis* (Edwards), *A. hydaspe* (Boisduval) and *A. mormonia* (Boisduval) (Guppy and Shepard 2001, Pyle 2002, Warren 2005). Until recently, these species, along with all North American Greater Fritillaries were considered to belong to the genus *Speyeria*. However, recent morphological and molecular studies by Simonsen (2006) and Simonsen *et al.* (2006) showed that the North American species are better treated as members of the large, unified genus, *Argynnis* with *Speyeria* relegated to a sub-genus. The immature stages of *Speyeria* spp. are generally infrequently encountered and are thus poorly described or illustrated. Detailed morphological studies and descriptions of *Argynnis* eggs, larvae and pupae may yield important distinguishing characteristics that could be useful along with adult characteristics for resolving taxonomic issues with the many subspecies and putative subspecies that occur in this genus (Warren 2005). Similarly, the biology of immature *Argynnis* spp. is also imperfectly known and offers a field rich in potential for understanding mechanisms of diapause, defense, host plant relationships etc.

This paper provides biological information on, and detailed illustrations of the immature stages of five Washington *Argynnis* spp., *A. cybele* leto (Behr), *A. coronis* simaetha dos Passos and Grey, *A. zerene* picta (McDunnough) , *A. egleis* mcdunnoughi (Gunder) and *A. hydaspe* rhodope (W. H. Edwards). Of the five, only the endangered Oregon coast subspecies of *A. zerene* (*A. z. hippolyta* (W. H. Edwards)) has received detailed attention to its immature stages within the past 25 years (McCorkle 1980, McCorkle and Hammond 1988). The early stages of eastern North American *A. cybele* and *A. egleis* from Nevada were described more than 125 years ago by W. H. Edwards (Edwards 1879, 1880). Descriptions and illustrations of late instars only, are available for *A. cybele, A. coronis, A. zerene* and *A. hydaspe* (Comstock and Dammers 1931, Dornfeld 1980, Scott 1986, Allen 1997, Guppy and Shepard 2001, Miller and Hammond 2003, Allen *et al.* 2005, Wagner 2005). Allen *et al.* (2005) described the late instar larva

of *A. egleis* but did not illustrate it. Aside from *A. z. hippolyta*, very little has been reported on the biology and ecology of the immature stages of these five species (Pyle 2002, Warren 2005).

During August 2005 to April 2006, *A. cybele*, *A. coronis*, *A. zerene*, *A. egleis* and *A. hydaspe* were reared in the laboratory, for photography of all the immature stages (including each instar). Notes on coloration, patterning and dimensions of eggs, larvae and pupae were made. Observations were also made on aspects of biology such as overwintering mortality, diapause, host plant acceptance and developmental duration.

MATERIALS AND METHODS

Gravid females of *A. cybele leto* (2), *A. coronis simaetha* (4), *A. zerene picta* (10), *A. egleis mcdunnoughi* (4) and *A. hydaspe rhodope* (4), were obtained during August 2005 from the Umatilla National Forest in SE Washington (*A. c. leto*, *A. z. picta*, *A. e. mcdunnoughi*) and Wenatchee National Forest on the eastern edge of the Cascade mountains (*A. c. simaetha*, *A. h. rhodope*). Females were placed in plastic buckets (31 cm deep, 28 cm diameter) with muslin-covered lids and held at 21–28° C under natural daylength. Butterflies were provided with potted violets (*Viola labradorica* Schrank) or dessicating violet foliage (*Viola adunca* Sm., *V. labradorica*, *Viola glabella* Nutt.) and paper toweling as oviposition substrates and tissue pads soaked in sugar/water solution for nourishment. Butterflies oviposited freely under these conditions. Eggs were measured, photographed and left in the buckets to hatch. First instars were also measured and photographed and along with all violet foliage and paper toweling, were transferred to plastic boxes (30 × 23 × 10 cm) with muslin lids. The boxes were held at 20–28° C under natural daylength until September 3 when they were transferred to shaded outdoor conditions until October 31 (10–25° C). During this period, larvae were exposed to fine water-misting every 2–3 weeks.

Larval diapause termination experiment. On October 31, five first instars of *A. coronis*, *A. zerene*, *S. cybele* and *S. hydaspe* were transferred to summer-like (25 ± 0.5° C, constant fluorescent illumination) conditions and placed on fresh, detached *V. adunca* leaves laid upperside down on wet cotton wool in a muslin-covered plastic Petri-dish (13 cm diameter). Observations on behavior and mortality were made daily.

On November 1, plastic boxes containing remaining dormant larvae were transferred to a dark constant temperature room set at 5 ± 0.5 C for overwintering. Relative humidity was maintained at 75 ± 5 %. Overwintering larvae were transferred to summer-like conditions (25 ± 1° C, constant fluorescent illumination) during 11–19 January. One group of 12 *A. coronis* larvae was transferred on January 4 to 15–21 ° C/ 9 hrs light. First and second instars were reared on detached *Viola* leaves placed upper surface down on moist cotton wool in muslin-covered plastic Petri dishes (13 cm diameter). Dried leaf debris was provided as shelter for the larvae. Third-sixth instars were reared in plastic boxes (25 × 15 × 6 cm) with muslin lids. Cut *Viola* spp. with stems in water was provided for food and shelter. Pre-pupal sixth instars were placed in larger boxes (30 × 23 × 10 cm) with a greater amount of foliage to provide pupation sites. All instars and pupae were photographed. Observations on larval morphology, coloration, behavior, development, host plant acceptance and mortality were made daily until pupation.

Photographs were taken using a Canon EOS 1DS Mark II, digital SLR camera mounted on a tripod. A Canon MP-E 65 mm 1 X – 5 X macro lens was used together with a Macro Twin Lite MT – 24 EX flash lighting system.

RESULTS

Morphology of immature stages. Eggs, instars 1–6 and pupae of *A. coronis simaetha*, *A. zerene picta*, *S. egleis mcdunnoughi*, *A. cybele leto* and *A. hydaspe rhodope* are shown in Figs. 1–2. Eggs of all species were creamy white when freshly laid, turning orange or pinkish/tan/brown with development after 2–3 days. Egg dimensions were comparable between species, being 0.9–1.0 mm in height and 0.8–1.0 mm in diameter (Table 1). The eggs of *A. zerene* were generally the smallest (0.9 × 0.8 mm) and were noticeably more ovoid than eggs of the other species. Eggs of *S. egleis* and *S. cybele* were flattened basally, while the eggs of *S. coronis* and *S. hydaspe* were more cylindrical (Figure 1).

First instars of all species measured approximately1.5 mm after hatching but increased to 1.75–2.0 mm after imbibing water droplets. Lengths of larvae at the beginning and end of each instar for the five species are shown in Table 1. Generally, there was good correlation of instar sizes between species with an approximate doubling of size with each instar from one to three, a lessening of growth rate in instars 4 and 5, then an increase in the sixth instar. Mature larvae of *A. cybele* were largest at 45 mm with the other four species similarly sized, ca. 35–38 mm (Table 1). Coloration of unfed first instars varied with *S. coronis* and *S. hydaspe* generally lighter colored than the other three species (Figure 1). All species had black head capsules. In general the black tubercles running longitudinally down the body were darker and more prominent in first

instars of *S. egleis* and *S. cybele* than the other three species. Fine dark hairs arose from these tubercles and appeared in all species to secrete a small droplet of fluid at the distal end. Second instars were characterized by the development of spines, replacing the hairs rising from the tubercles. Head capsules were black and the five species were generally similarly-colored in this instar. A light colored dorsal band was evident in *A. cybele* and all species had a lower lateral row of orange/tan-colored tubercles. Third instars showed greater spine development, particularly in *A. hydaspe* (Fig.1) and the lower lateral row of orange tubercles was more prominent in all species. In addition, the upper lateral row of tubercles was also orange in this instar. This was particularly pronounced in *A. coronis* and *A. zerene*. Head capsules remained solid black except for *A. hydaspe* which showed limited orange/brown marking. All species except *A. hydaspe* had a prominent pale dorsal band in the third instar with a central darker colored intermittent stripe running through the center. The lower lateral row of orange tubercles was further developed in the fourth instar of all species. The upper lateral row was also strongly developed in *A. cybele*, *A. coronis* and *A. zerene* but virtually lacking in *A. hydaspe* and *A. egleis*. The pale dorsal band with intermittent central dark stripe was also further developed in fourth instar *A. coronis*, *A. zerene* and *A. egleis*, but absent in fourth instar *A. cybele* and *A. hydaspe*. Ground color of fourth instar *A. coronis*, *A. zerene* and *A. egleis* was gray/white rather than black as in *A. cybele* and *A. hydaspe* (Fig. 1). Head capsules of fourth instars were largely black with varying amounts of orange-brown on dorsal surfaces, most pronounced in *A. cybele* and *A. hydaspe* and least in *A. coronis*. Fifth instars were very similar to fourth instars in coloration, although *A. coronis* tended to be a little darker in this instar. The orange-brown dorsal markings on the head capsule were more pronounced in *A. cybele*, *A. hydaspe* and *A. egleis* in the fifth instar. Lateral and dorsal views of sixth instar larvae are shown in Fig. 2. The upper lateral row of orange tubercles was most developed in *A. cybele* and *A. egleis*, although they tended paler, almost white, in the latter species. In *A. zerene* the color of these tubercles was brown or black and blended in with background coloration (Fig. 2). The same was true for *A. coronis* except on the first two abdominal segments where the upper row of tubercles remained orange. The upper lateral tubercles on the first two abdominal segments of *A. egleis* sixth instars were also more vividly colored than the rest of this row. Dorsal coloration of *A. zerene* and *A. coronis* was palest contrasting with the black ground color of *A. cybele* and *A. hydaspe*. The dorsal ground color of *A. egleis* was intermediate lacking the distinctive gray/tan/white blotches of *A. zerene* and *A. coronis* (Fig. 2). The pale dorsal band containing an intermittent central dark stripe was still present in *A. coronis* and *A. zerene* but absent in the other species. Dorsal orange-brown coloration of the head capsule extending laterally was most developed in *A. cybele* and *A. egleis*, with only very minor orange coloration on the

TABLE 1. Sizes (mm) of immature stages of five *Argynnis* spp. Egg dimensions are height × width. Larval dimensions are lengths measured at commencement and end of each instar. Egg and larval data were obtained from examination of 2–4 individuals. Variation was generally less than 0.1 mm. Pupae were measured from cremaster to tip of head (Mean ± SE) (number of pupae examined in parentheses).

	S. coronis simaetha	***S. zerene picta***	***S. egleis mcdunnoughi***	***S. cybele leto***	***S. hydaspe rhodope***
Egg	1.0 × 0.9	0.9 × 0.8	1.0 × 1.0	0.9 × 0.9	1.0 × 0.8
First instar	1.5 – 3.0	1.5 – 2.5	1.5 – 3.0	1.5 – 3.0	1.5 – 3.0
Second instar	3.0 – 6.0	2.5 – 4.0	3.0 – 5.0	3.0 – 5.0	3.0 – 6.0
Third instar	6.0 – 10	4.0 – 8.0	5.0 – 8.0	5.0 – 9.0	6.0 – 10
Fourth instar	10 – 15	8.0 – 13	8.0 – 13	9.0 – 15	10 – 15
Fifth instar	15 – 20	13 – 20	13 – 20	15 – 25	15 – 19
Sixth instar	20 – 35	20 – 38	20 – 35	25 – 45	19 – 37
Pupa	23.5 ± 0.2 (4)	23.6 ± 0.6 (10)	22.7 ± 0.2 (7)	28.5 ± 0.2 (4)	22.0 ± 0.9 (4)

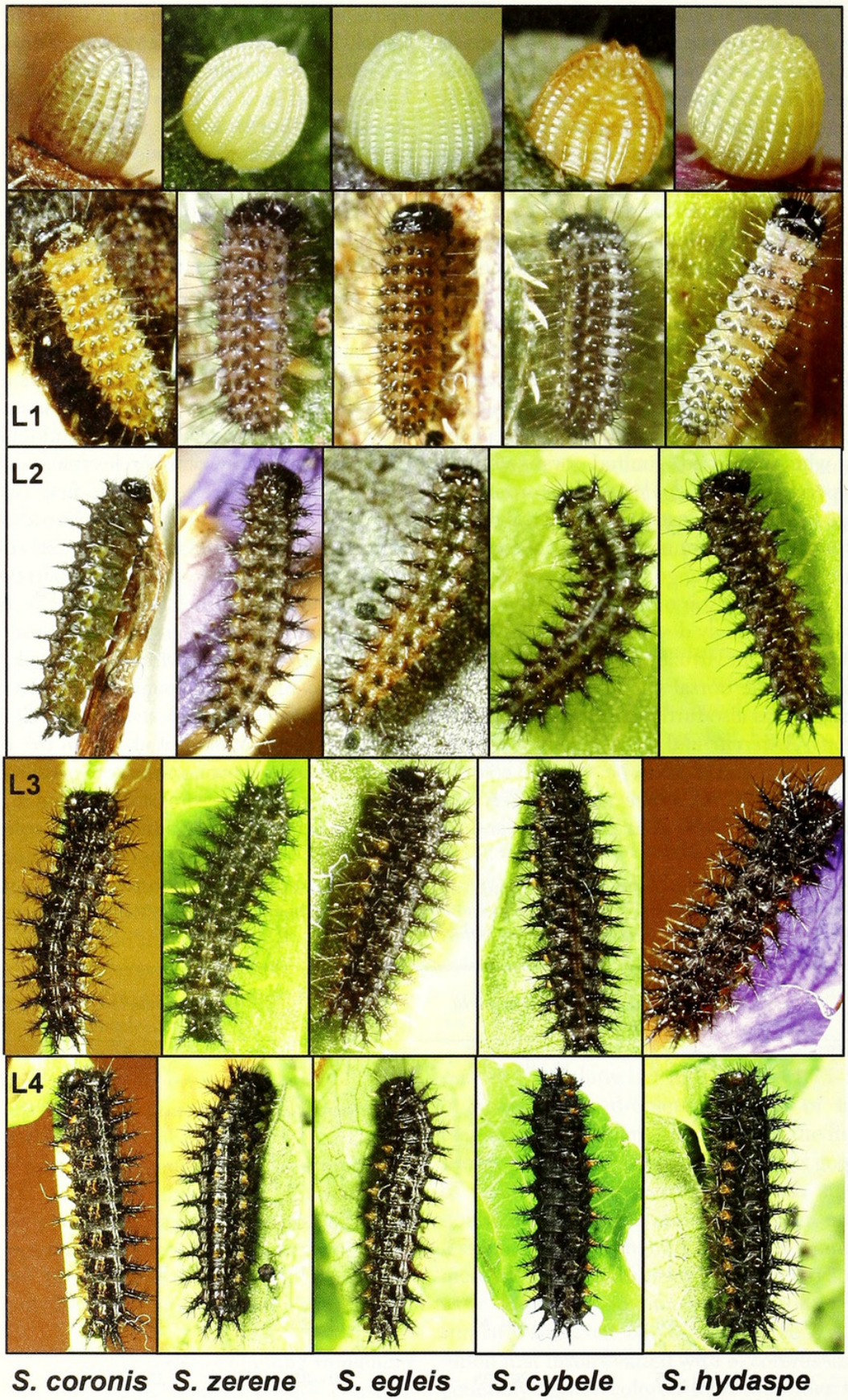

FIG. 1. Eggs and first four instars of *A. coronis simaetha*, *A. zerene picta*, *A. egleis mcdunnoughi*, *A. cybele leto* and *A. hydaspe rhodope*.

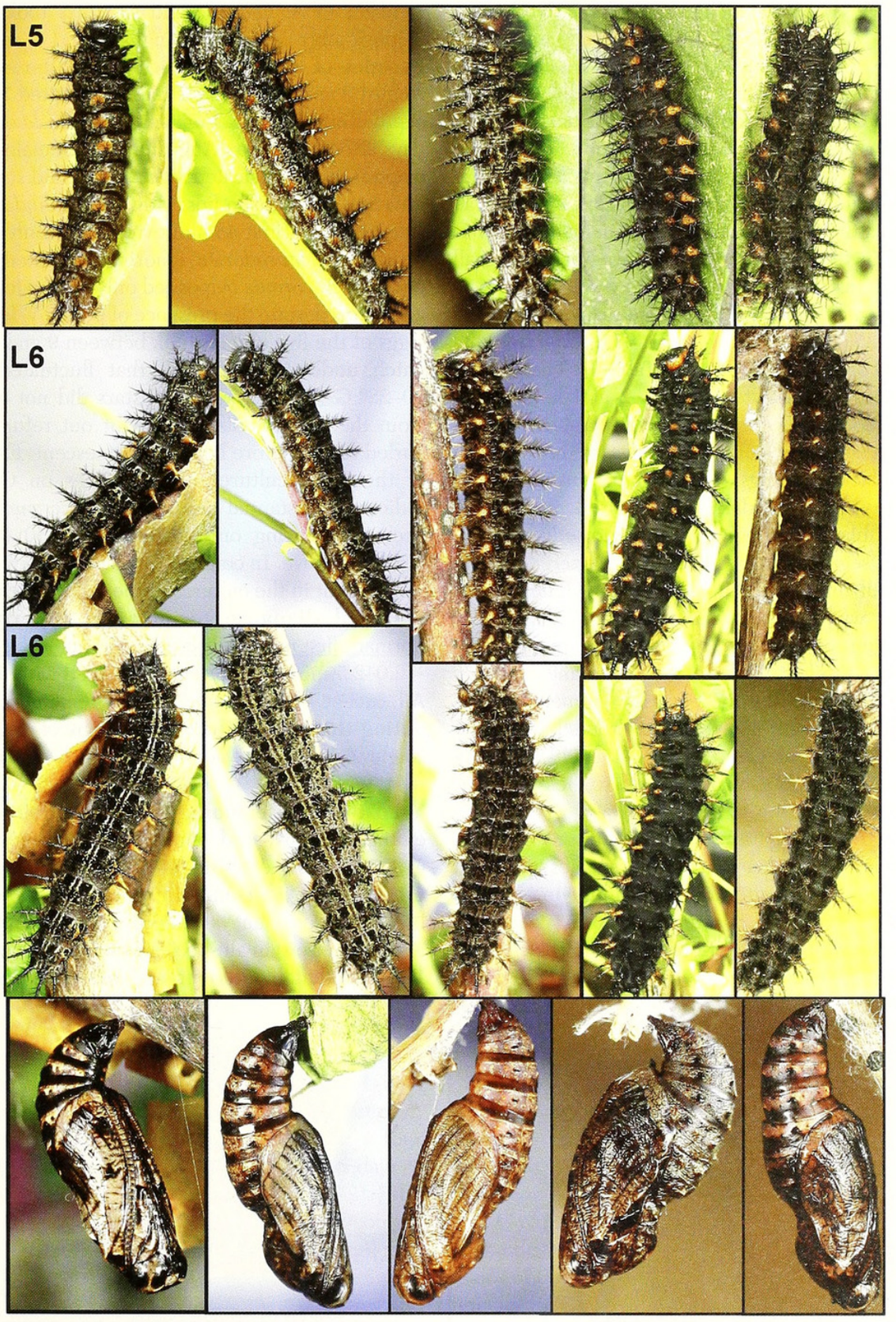

FIG. 2. Fifth and sixth (ventral and dorsal views) instars and pupae of *A. coronis simaetha*, *A. zerene picta*, *A. egleis mcdunnoughi*, *A. cybele leto* and *A. hydaspe rhodope*.

head capsules of *A. zerene* and *A. hydaspe*. No orange coloration was seen on the all black head capsules of sixth instar *A. coronis*.

Sixth instars of all species possessed a fleshy, eversible osmeterium located ventrally between the head and first thoracic segment (Fig. 3). A musky odor emanating from the organ was apparent when the larvae were handled roughly. Further examination of *A. egleis* larvae showed that the organ was not present in first instars but appeared in the second instar and was present in all subsequent instars.

Sixth instars were mature at maximum recorded lengths of 35 (*A. egleis*, *A. coronis*), 37 (*A. hydaspe*), 38 (*A. zerene*) or 45 mm (*A. cybele*) (Table 1). The pupa of each species is illustrated in Fig. 2 and lengths are shown in Table 1. The pupae of *A. coronis* and *A. zerene* were most similarly colored (shades of brown with variable black markings) although *A. coronis* pupae tended to be more darkly pigmented with more prominent black banding on the anterior edge of each abdominal segment. Wing venation in *A. zerene* pupae was generally highlighted in black (Fig. 2). The pupa of *A. egleis* was light brown with the least dark pigmentation of the five species. The pupa of *A. hydaspe* was similar to those of *A. coronis* and *A. zerene* although was generally more darkly pigmented. The pupa of *A. cybele* differed from the others by having a rougher texture and greater girth, as well as having greater length. It was similarly colored to the pupa of *A. hydaspe*.

Oviposition, egg and pre-diapause biology. Oviposition by females of the five species occurred between 2 and 8 days after caging, resulting in ~50 eggs each for *A. egleis*, *A. coronis* and *A. cybele*. Approximately 30 eggs were obtained from *A. hydaspe* females and an estimated 250 from *A. zerene* females. Females of *A. hydaspe* and *A. egleis* took 2 days to oviposit after capture on August 1 and 3, respectively. Females of *A. zerene* took 6 days after capture on August 3 and *A. coronis* females oviposited 7 days after capture on July 30th. Females of *A. cybele* oviposited 8 days after capture on August 20. All females generally oviposited on dessicated *Viola* leaves and stems, paper toweling, and muslin lids. However, *A. hydaspe* and *A. egleis* initially (first 48 h of oviposition) only oviposited on potted *V. labradorica*, ignoring dessicated foliage. In contrast, *A. coronis* oviposited only on dessicated leaves and stems despite the presence of potted *V. labradorica*. Eggs of the five species took between 9 and 14 days to hatch under temperatures that fluctuated between 20–28° C (Table 2). First instars did not wander far from their egg shells but sought out refugia such as curled leaves before becoming quiescent. Examination of the larval cultures of all species on October 31 indicated substantial mortality of *A. zerene* larvae had occurred, leaving only an estimated 50 (from 250) larvae still alive. In contrast, very little (< 5 %) mortality was evident in the cultures of the other species.

Larval diapause termination experiment. All dormant first instars exposed to summer-like conditions ~ 2.0–2.5 months after hatching, died within 6–9 days. All except one *A. cybele* larva remained quiescent during this period showing no sign of feeding. The single *A. cybele* larvae wandered a little but did not feed.

Post-diapause biology. Examination of the larval cultures in early-mid January indicated good survival (>90%) of all species under 5° C conditions. Exposure of the larvae to summer-like conditions resulted in breaking of dormancy (using commencement of feeding as the criterion) in all species within 2–5 days (Table 2). Developmental durations and overall mortalities for

FIG. 3. Ventral gland of sixth instar *A. coronis simaetha*.

TABLE 2. Developmental durations (days) for eggs, larvae and pupae of five *Argynnis* spp. reared at 20-28° C (eggs) or 25 ± 0.5° C under continuous illumination (larvae and pupae). Pre-feeding durations represent the time between introduction of diapausing larvae into summer-like conditions and commencement of feeding. Instar duration data were obtained from first appearance of each instar among species cohorts. First-adult durations were calculated from introduction of first instars to summer-like conditions to adult eclosion (Mean ± SE) (number of individuals completing development in parentheses). *Indicates significant difference from

	A. coronis simaetha	***A. zerene picta***	***A. egleis mcdunnoughi***	***A. cybele leto***	***A. hydaspe rhodope***
Egg	14	13	14	12	9
Pre-feeding period 25°C	3	5	2	4	3
First instar	9	9	8	9	9
Second instar	7	5	5	6	8
Third instar	8	6	5	6	3
Fourth instar	4	6	5	8	3
Fifth instar	4	6	6	5	3
Sixth instar	8	11	13	10	9
Pupa	12	13	12	13	12
First - Adult	54.2 ± 0.7 (4)	55.0 ± 0.3 (10)	54.1 ± 0.2 (7)	55.5 ± 1.0 (4)	47.0 ± 1.0* (4)
% Survival First-Adult	50	50	50	50	75

larvae during post-diapause development at 25 ± 0.5° C/ 24 h light are shown in Table 2. Due to limitations in host plant availability, starting cohort sizes for each species were necessarily small (4–30 larvae). Additional larval cohorts of *A. egleis* (3) and *A. zerene* (10) exposed to these conditions from January 11 instead of January 19, were slower to start feeding and took three weeks to reach the second instar instead of just over a week for the later group. An extra group of 12 *A. coronis* larvae exposed to 15–21 °C and short days (9 h) from January 4–19 failed to break dormancy and did not feed. Durations of individual instars and complete post-larval feeding development were similar for *A. coronis*, *A. zerene*, *A. egleis* and *A. cybele* (Table 2). From their introduction to 25 ± 0.5° C and continuous illumination on January 19, these four species averaged 54.1–55.5 days to reach adulthood. In contrast, development of *A. hydaspe* was significantly faster than the other four species under the same conditions, averaging 47 days ($P < 0.05$, Mann-Whitney Rank Sum Test) (Table 2). Fifty per cent survival was obtained for all species cohorts except *A. hydaspe* in which 3 of the 4 larvae reached adulthood (Table 2).

Larvae of all species readily accepted *V. adunca* and *V. glabella* as host plants. *Viola labradorica* was also accepted by all instars of *A. egleis*. However, its acceptance was limited in the other species to later instars (5 and 6). First instars of *A. coronis* and *A. zerene* would not feed on *V. labradorica*. Later instars of these two species fed preferentially on *V. adunca* when supplied in combination with *V. labradorica*. The sagebrush violet, *V. trinervata* Howell, was provided to *A. egleis* third instars only and was not accepted as a host. Domesticated pansies (*Viola tricolor* L.) were accepted by sixth instars of *A. egleis*, *A. coronis* and *A. zerene*. Only limited feeding on pansy occurred with sixth instar *A. cybele*, and *A. hydaspe* was not evaluated.

Mature larvae of all five species constructed 'leaf tents' from strategically silked leaves for pupation, in which they spun a silk pad for cremaster attachment. This behavior was strongly entrenched, taking place even when insufficient space within the 'tent' was available for 'hanging'. The silken pads or cremaster attachments were insufficient in many cases with pre-pupae or pupae falling to the ground. Pupal development durations are provided in Table 2. The pupae of *A. cybele* were noticeably more active (wriggling at slightest provocation) than the pupae of the other species. Relative humidity of ~ 40% at 25 ± 0.5° C caused desiccation of many pupae. Noticeable protandry occurred only with *A. zerene* and *A. cybele*; males of these species emerged ~ 4–5 days earlier than females.

DISCUSSION

Comparative illustrations and notes on the morphology and biology of immature stages of five *Argynnis* spp. commonly found in the Pacific Northwest

are provided for the first time. High quality images allowed separation of the five species in most of their immature stages. Prior to this study, only the endangered, coastal *A. zerene hippolyta* had received detailed attention to its immature stages including aspects of biology (McCorkle 1980, McCorkle and Hammond 1988). Aside from some very old descriptions of the early stages of *A. cybele* and *A. egleis* (Edwards 1879, 1880), only descriptions/illustrations of late instars were available previously for the five species covered here (Comstock and Dammers 1931, Dornfeld 1980, Scott 1986, Allen 1997, Guppy and Shepard 2001, Miller and Hammond 2003, Allen *et al.* 2005, Wagner 2005).

Oviposition was obtained readily within a few days by females of *A. coronis*, *A. zerene*, *A. egleis* and *A. hydaspe* collected in late July or early August. Most *Argynnis* spp, including those in this study, are thought to undergo an adult reproductive diapause for 3–5 weeks after eclosion (Sims 1984). This study indicates reproductive diapause in *A. coronis*, *A. zerene*, *A. egleis* and *A. hydaspe* terminates by early August in Washington populations. The eggs of *Argynnis* spp. do not appear to have been depicted in publications previously, except as line drawings (e.g. Scott and Mattoon 1982). Despite their obvious similarity, some subtle differences in shape appeared to be consistent between species, especially the basal flattening of *A. cybele* and *A. egleis* eggs. Edwards (1880) also noted the broad base of *A. cybele* eggs. Coloration also appeared to differ between species both in newly laid and developing eggs, but more study is needed to characterize this. Similarly, very little attention has been paid previously to first instar *Argynnis* with this study indicating at least some differences in coloration occur between species. Ground color of first instar *A. zerene*, *A. egleis* and *A. cybele* ranged between brown-purple/black while *A. coronis* and *A. hydaspe* larvae were generally cream-yellow. In all species, the unbranched setae or hairs of first instars usually carried a droplet of fluid at the distal end as is characteristic of pierid larvae (Allen *et al.* 2005). In *Pieris rapae* L. these droplets contain defensive chemicals (Smedley *et al.* 2002) and it is possible that the droplets on *Argynnis* first instars also have a defensive function. Second instars were the least species-differentiated stage, but clear differences in markings and coloration began to appear in third instars and continued until larval maturity. These differences allow good separation of species based on head capsule and body ground color, presence or absence of dorsal bands and the extent of lateral tubercle coloration. Generally, previously published descriptions/illustrations of the five late instar *Argynnis*, matched the current observations. Earlier descriptions tended to describe tubercle coloration as 'yellowish', whereas in this study, the color was clearly more orange than yellow in most cases.

The existence of a ventral osmeterium-type organ in *Argynnis* larvae, analogous to the well-known eversible dorsal defense organ of papilionid larvae (Honda and Hayashi 1995), was first observed by McCorkle and Hammond (1988) in *A. zerene hippolyta* and was also reported by Scott (1986). This study is the first to illustrate the organ. This gland, likely to be defensive in function, probably occurs in larvae of all *Argynnis* spp. Similar ventral glands were also observed in late instars of *Nymphalis vaualbum* (Denis & Schiffermuller), *Nymphalis antiopa* (L.) and *Boloria selene* (Denis & Schiffermuller) (James, unpublished observation). Scott (1986) reports occurrence of ventral glands in other nymphalid genera including *Historis*, *Smyrna* and *Anartia* and suggests they also occur in "some Pieridae, Danainae, Libytheidae, skippers and probably others" (page 71, Scott 1986). The chemical ecology of ventral glands in Washington *Argynnis* spp. is currently being researched (James, in prep).

All *Argynnis* spp. overwinter as unfed first instars which are presumed to be in diapause (Mattoon *et al.* 1971). Diapause is a physiologically defined and controlled mechanism ensuring resumption of development does not occur prematurely (Danks 1987). First instar *Argynnis* presented with normally favorable conditions of temperature during late summer and autumn do not commence feeding and development. Daylengths are declining during this time and host plants are of poor quality or unavailable and it may be these factors that directly prevent development. Exposure of first instar *A. zerene*, *A. coronis*, *A. cybele* and *A. hydaspe* in this study to summer-like conditions of temperature, photoperiod and host plant availability in late Fall, did not succeed in 'breaking' diapause in any species. This is the first experimental evidence confirming the existence of physiological diapause in first instar *Argynnis*, rather than a more flexible dormancy state (or 'quiescence' as suggested by Mattoon *et al.* (1971)) cued by declining daylength and/or host plant inadequacy. *Argynnis* spp. are among the few lepidopteran genera that have been documented to possess diapause in two life stages, larvae and adults (Sims 1984, James 1999). Approximately 80 days of exposure to a constant (5 ± 0.5°C) cool temperature and darkness, resulted in the termination of diapause within a few days in all five species when subsequently exposed to summer-like conditions. Interestingly, exposure to these conditions a week earlier resulted in much slower breaking of

diapause in *A. coronis*, suggesting that diapause development at this time was less complete. Survival of diapausing first instars confined in plastic boxes amongst leaf debris at 5 ± 0.5°C and 75 ± 5 % r.h. for ~ 80 days was high. However significant mortality (> 50%) occurred with *S. coronis*, *S. zerene* and *S. cybele* larvae held for 135 days under these conditions. In contrast, *S. egleis* larvae still showed little mortality after 135 days. This overwintering technique was a lot simpler than the small hollow wooden block technique reported by Mattoon *et al.* (1971). In the latter procedure blocks are soaked weekly to maintain high relative humidity. The key to good overwintering survival of first instar *Argynnis* appears to be adequate moisture/humidity levels but requirements may differ between species. Dry ambient conditions during September–October may have caused the substantial mortality observed among *A. zerene* larvae, although the other species were not adversely affected.

Post-diapause development rates of immature *A. coronis*, *A. zerene*, *A. egleis* and *A. cybele*, were very similar with each species taking about 54–55 days or 7.7–7.8 weeks to reach adulthood at 25° C. Although only three *A. hydaspe* larvae completed development, their mean duration of 47 days or 6.7 weeks from post-diapause first instar to adult was significantly less than for the other four species. These developmental durations are similar to what has been reported previously for *Argynnis* spp. For example, McCorkle and Hammond (1988) reported 'most subspecies of *A. coronis* and *A. zerene* required six to seven weeks for males and seven to eight weeks for females', when reared at 21–23° C. They also noted that *A. cybele* required a similar period of time. These authors did not mention *A. hydaspe*, but the duration recorded here for this species compares to the fastest rates reported in their paper for certain forms of *A. atlantis*, *A. egleis* and *A. callippe*.

Although no systematic host acceptance or preference studies were conducted, it is likely as reported previously (Mattoon *et al.* 1971), that *Argynnis* spp. differ in their acceptance of different *Viola* spp. All five species readily accepted the two Pacific Northwest endemic *Viola* spp., *adunca* and *glabella* provided to them. *Viola adunca* is recorded as a natural host for four of the five species, with *A. coronis* as the exception. *Viola glabella* is recorded as a natural host for *A. hydaspe*, *A. cybele* and *A. zerene* but not for *A. egleis* and *A. coronis* (Hammond 1983, Scott 1986, Pyle 2002, Warren 2005).

In Washington, *A. coronis* is reported to feed mostly on *V. trinervata* (Pyle 2002, Warren 2005) but *V. adunca* and *V. glabella* also occur in many *A. coronis*-occupied habitats particularly on the eastern slopes of the Cascade mountains. This study suggests the larvae of this species would have no problem utilizing this host if they encounter it. *Viola trinervata* was not accepted by *A. egleis* as a host in this study and is unlikely to grow in habitats occupied by *A. egleis*. *Viola glabella* occurs in the Blue Mountain habitat from which females were collected for this study and could be utilized as a host, given its acceptability as a larval host recorded here. *Viola labradorica* is native to the north eastern USA, and varied considerably in its acceptability to Washington *Argynnis*. First instar *A. coronis* and *A. zerene* would not feed on it and later instars of these species and *A. cybele* and *A. hydaspe* only accepted it when given no choice. In contrast, *A. egleis* accepted this host readily in all instars. Late in rearing it became necessary to supplement the diets of four species with pansies (*V. tricolor*). *Argynnis egleis*, *A. coronis* and *A. zerene* accepted this host readily, but *A. cybele* did not.

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