

# Life Cycle and Growth of Senita Moths (Lepidoptera: Pyralidae): A Lepidopteran with Less Than Four Instars?

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**ABSTRACT** Despite great variation in instar number among Insecta, no Lepidopteran has been observed to have less than four larval instars. I report in this work on the life cycle and growth of the senita moth, *Upiga virescens* Hulst, which forms an obligate pollinating predispersal seed-eating mutualism with senita cacti (*Lophocereus schottii* Engelmann) in the Sonoran Desert of North America. From 1996 to 1999, I studied larval growth and life cycle associations of *U. virescens* with *L. schottii* in the field by labeling cohorts of eggs laid in flowers and following them through pupation. All life stages of *U. virescens* were associated with flowers, fruit, or cactus stems of *L. schottii*. Among the five cohorts studied, larval growth consistently conformed to Dyar's rule. Only three larval instars were identified among the >500 larvae for which head capsule widths were measured. I discuss and dismiss the feasibility of a fourth undetected instar. I then discuss selection pressures that may have contributed to the evolutionary loss of an instar, including a time and/or size constraint on larval growth, as well as the nutritional quantity and quality of larval food.

**KEY WORDS** Dyar's rule, life history, plant-insect interaction, *Upiga virescens*

LIFE CYCLES OF INSECTS vary greatly among ametabolous, hemimetabolous, and holometabolous species. Accompanied with this diversity in life cycles is great variation in the number of molts or instars within and among species. Ametabolous insects can molt 10, 20, and even up to 60 times before reaching sexual maturity, while hemimetabolous species most commonly have 4–6 nymphal molts before the adult stage. Holometabolous insects can have as few as 2 instars or as many as 20. Intraspecific variation in the number of molts and instars results from differences in food quality and quantity, wounding of larvae, temperature, and, among others, photoperiod (Philogene and Benjamin 1971, Wigglesworth 1972, Nijhout 1975, Barbosa and Capinera 1977, Taylor 1984, Bellinger and Pienkowski 1987, Fantinou et al. 1996). Despite such variation in life cycles and number of molts, no moth or butterfly has been observed to have less than four instars. In this study, I report data that indicate only three larval instars in a Lepidopteran. Specifically, I studied the life cycle and growth of the senita moth, *Upiga virescens* Hulst, which forms a pollinating predispersal seed-eating mutualism with senita cacti (*Lophocereus schottii* Engelmann). Both senita moths and senita cacti are endemic to the Sonoran Desert of northwestern Mexico and southern Arizona. Interactions between senita cacti and senita moths are most ecologically and evolutionarily similar to those between yucca and yucca moths (Pellmyr et al. 1996, Fleming and Holland 1998). Senita cactus reproduction is dependent on senita moth pollination, and the

growth of larvae emerging from eggs laid in flowers is obligately dependent on fruit resources resulting from moth pollination (Holland and Fleming 1999).

## Materials and Methods

Data were collected near Bahia de Kino, Sonora, Mexico (29° N, 110° W), from 1996 to 1999. The senita moth, *U. virescens*, is a pyralid moth with forewings 7–10 mm in length. *U. virescens* is in the subfamily Glaphyriinae and is the only species in its genus. Until recently (Holland and Fleming 1999), the only report of *U. virescens* was its species account (Munroe 1972). I studied the life cycle and growth of *U. virescens* by following cohorts from eggs to pupae. Because moths typically lay one egg per flower (Holland and Fleming 1999), I was able to study individual eggs by tagging individual flowers. I labeled one cohort of eggs in 1996 ( $n = 586$  eggs) and four cohorts in 1998 ( $n = 140, 634, 612, \text{ and } 252$  eggs). Every 1–2 d during development of eggs to adults, I destructively sampled a random subset (12–72) of individuals in each cohort by collecting flowers, immature fruit, or areoles (spine-bearing pads that produce flowers). For each sample, I dissected the corolla, immature fruit, and/or areole to identify life stage associations of *U. virescens* with the senita cactus and to collect preadult life stages. Samples of *U. virescens* were preserved in 70% ethanol. To determine whether the first instar observed in the field was the same as that emerging from eggs, fresh eggs were collected in 1998 ( $n = 102$ ) and 1999 ( $n = 133$ ).

Each egg was placed in a pill capsule to collect emerging larvae. I measured head capsule widths (HCWs) of intact larvae of preserved specimens for the 1996 cohort ( $n = 82$  larvae) and the four cohorts of 1998 ( $n = 48, 197, 53,$  and  $18$  larvae), using a dissecting microscope with an ocular micrometer calibrated with a stage micrometer.

## Results

**Number of Larval Instars.** The frequencies of HCWs measured from larvae collected in 1996 were clustered into three distinct size classes, suggesting three larval instars (Figs. 1A and 2). In 1998, I collected larvae from an additional four cohorts. Frequencies of HCWs of those cohorts also clustered into three distinct size classes (Figs. 1B–D and 2). Hereafter, I refer to these size classes of HCWs as instar 1 (mean  $\pm$  1 SE;  $0.428 \pm 0.011$  mm), instar 2 ( $0.637 \pm 0.005$  mm), and instar 3 ( $1.04 \pm 0.011$  mm). Notably, little variation occurred in HCWs for the three instars within or among cohorts (Fig. 2). The coefficients of variation among the five cohorts for the three instars were 5.5, 1.7, and 2.4%, respectively.

Dyar's rule (Dyar 1890) states that larval growth progresses geometrically and by a relatively constant factor. Dyar's rule predicts that a linear measure of size increases by a constant factor from one instar to the next. This growth factor is calculated by dividing the linear measure of size for one instar by that measure of size for the previous instar. This produces a growth ratio for each larval molt. When insect growth follows Dyar's rule (which it often does not [e.g., Goettel and Philogene 1979, Jobin et al. 1992]), growth ratios are commonly  $\approx 1.4$ . According to Dyar's rule, a strong linear relationship is predicted between the log of a linear measurement of size and instar number. Figure 3 shows this relationship for each of the five cohorts of *U. virescens*. In all five cases, there was a strong linear relationship between log HCW and instar number (Fig. 3). For each of the five cohorts, the linear regression line captured 99–100% of variation in HCW. Moreover, slopes of these regression lines varied little among cohorts (Fig. 3), indicating similar increments in larval growth among cohorts. These analyses suggest that larval growth of *U. virescens* does follow a geometric pattern consistent with Dyar's rule (Figs. 2 and 3). However, the mean ( $\pm$  1 SE) growth ratio of the five cohorts from instar 1–2 was 1.49 ( $\pm 0.019$ ) and from instar 2–3 was 1.63 ( $\pm 0.008$ ). These average growth ratios do not deviate greatly from the 1.4 generally observed for insects, but they are statistically different from one another ( $t = 4.36$ ,  $df = 8$ ,  $P < 0.05$ ). Hence, while larvae of *U. virescens* do grow geometrically (Fig. 2) and by a constant factor (Fig. 3), there is slight, but significantly greater growth from the second to third instar than from the first to second.

**Association of Life Stages with Senita Cactus.** Female senita moths invariably lay their eggs in open flowers of senita cacti, which open at sunset and close within 8–12 h. The chorion presumably has a substance on it for adhesion to flowers; eggs are not easily

blown or knocked off flowers. Larvae can hatch within hours after the egg is laid, but may take up to 3 d. Of freshly laid eggs collected from flowers in 1998 ( $n = 102$ ) and 1999 ( $n = 133$ ), 100 and 92% were viable, respectively, producing first instars. Only 3% of collected eggs showed any sign of a larva having eaten the chorion, and even then, nearly all of the chorion remained uneaten. These larvae had a mean ( $\pm$  1 SE) HCW of  $0.43$  mm ( $\pm 0.003$ ), which was not different from first instars collected from cohorts studied in 1996 and 1998 ( $0.43 \pm 0.002$ ;  $n = 166$  larvae).

Larvae of *U. virescens* are eruciform. The first stadium can last up to 7 d, but typically is 4 d long (Fig. 4). First instar larvae move down wilting corollas of flowers toward developing ovules and immature fruit. Larvae typically do not feed until they reach the bottom of the corolla and the top of the developing fruit. Ecdysis, and the appearance of the second instar, is associated with consumption of tissue as larvae bore into developing fruit. However, if a larva does not reach the bottom of the corolla and the top of the developing fruit within 5–6 d, tissue at the corolla-fruit interface becomes too hard for a larva to bore into it. Dead first instars are commonly found at the base of the corolla after days 5–6 (Holland and Fleming 1999).

Second instars feed within the immature fruit on developing ovules and fruit tissue. The second molt, and emergence of the third instar, occurs within the fruit, usually at 8 d of age (Fig. 4). There is never more than one larva per fruit, and a larva rarely consumes  $>50\%$  of developing ovules (Holland and Fleming 1999). As second and third instars feed, they work their way from the top of the immature fruit toward the bottom, where the fruit is attached to the areole of the cactus stem. Usually at  $\sim 12$  d of age, but occasionally as late as 17 d, third instars bore a hole out of the base of fruit where the fruit is attached to the areole, through the areole, and into the cactus stem. This invariably induces those fruit to abscise and fall off cacti. A larva then creates a small gallery within the cactus stem by consuming stem tissue. Larval feeding has not been observed after the creation of galleries in cactus stems. Third instars either enter into the pupal stage, in which case they emerge as adults from the cactus stem within the same flowering season, or they diapause as prepupal third instars and emerge as adults during a subsequent flowering season. Pupae are obovate and can occur as early as 17 d of age. Moths exit galleries through the holes made by larvae boring into the cactus stem. Bodies harden and wings fill with hemolymph as moths sit on spines of cacti. Mating also takes place on spines.

## Discussion

Head capsule widths of the 504 larvae measured in this study clustered into three distinct size classes (Figs. 1–4), indicating that *U. virescens* breaks, rather than conforms, to the general rule of no less than four larval instars in Lepidoptera. This is the first study, to my knowledge, to show a lepidopteran with only three larval instars. There are at least three places in the life

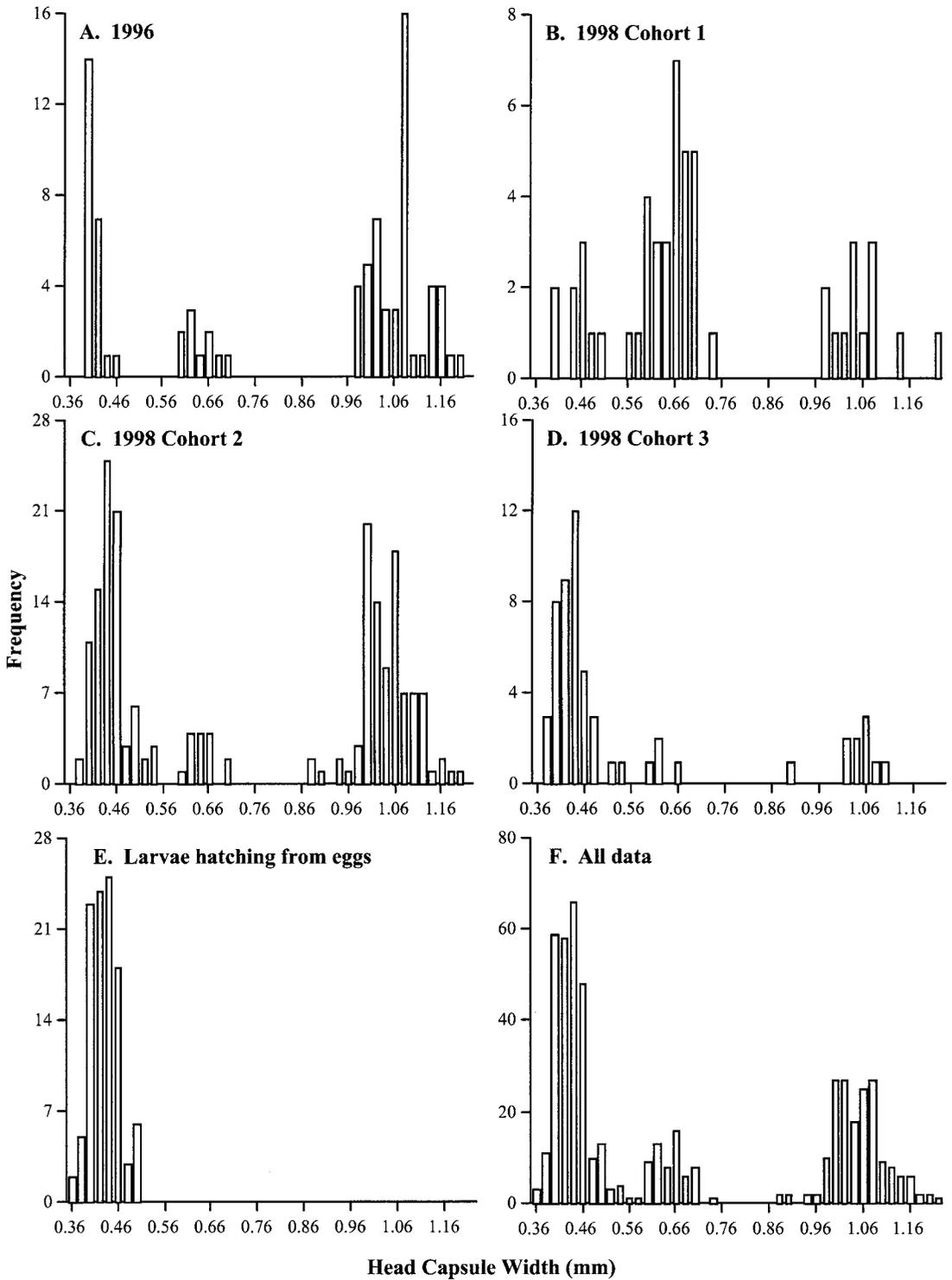


Fig. 1. Frequency distribution of head capsule widths (mm) of *U. virescens* (Pyralidae) for a cohort in 1996 (A); cohorts 1 (B), 2 (C), and 3 (D) in 1998; first instar larvae emerging from eggs (E); and all head capsule widths from the cohort in 1996, the four cohorts in 1998, and larvae emerging from eggs. A separate figure for head capsule widths for cohort 4 of 1998 is not included because of small sample size. The relative frequency of different size classes is an artifact of sample size, not the relative abundance of individuals within size classes.

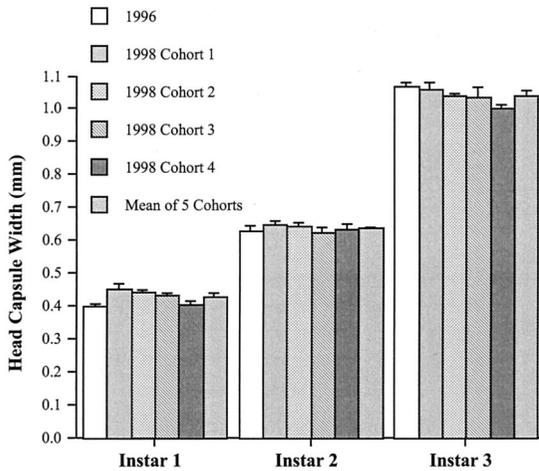


Fig. 2. Average ( $\pm 1$  SE) head capsule widths (mm) of *U. virescens* for each of three discrete size classes, identified as instar 1, 2, and 3. Data are presented for each of the five cohorts studied and the mean of these five cohorts.

cycle of *U. virescens* in which an additional instar may have occurred, but was undetected: 1) between instars 1 and 3; 2) before instar 1; and 3) after instar 3. If larval growth of *U. virescens* progresses geometrically and by a fairly constant factor, as suggested by Dyar's rule, then I can deduce whether a size class of HCWs is missing between instars 1 and 2 or 2 and 3 based on predicted changes in HCWs. The average ( $\pm 1$  SE) growth ratio empirically observed for *U. virescens* was 1.56 ( $\pm 0.03$ ;  $n = 10$ ). Based on an HCW of 0.428 mm observed for instar 1, the predicted HCW for instars 2 and 3 are 0.668 and 1.042 mm, respectively. These predictions deviate little from observed values for instars 2 (0.637 mm) and 3 (1.042 mm). Hence, a size class of HCW representing another instar cannot occur between what I have identified as instars 1, 2, and 3.

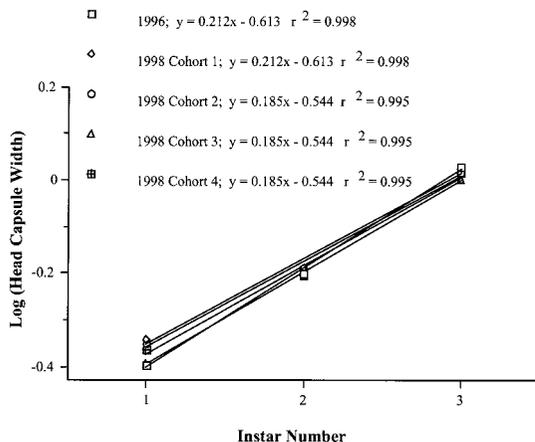


Fig. 3. Log of head capsule width of *U. virescens* as a function of instar number for the five cohorts studied. Regression coefficients and linear regression equations are in the legend.

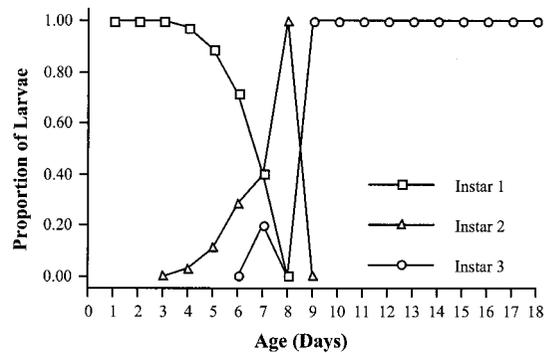


Fig. 4. Proportion of *U. virescens* larvae in each of three instars as a function of larval age (days).

Based on Dyar's rule, if an instar occurs before 0.43 mm HCW, then it is predicted to have an HCW ranging from 0.263 to 0.306 mm, with an average of 0.274 mm. No larva that I measured had an HCW  $< 0.36$  mm. Yet, it remains possible that the sampling method precluded detection of such an instar all together. For this reason, I collected eggs to compare HCWs of emerging first instar larvae (Fig. 1E) with HCWs of the smallest size class collected in the field. The mean ( $\pm 1$  SE) HCW of larvae ( $n = 102$ ) emerging from eggs was 0.43 mm  $\pm 0.003$ . This HCW was not different from the smallest size class of larva (0.43 mm  $\pm 0.002$ ;  $n = 166$  larvae) collected from the five cohorts in the field. Hence, the instar emerging from eggs is what I have identified as instar 1.

Based on Dyar's rule, if an instar occurs after what I identified as instar 3 ( $\approx 1.04$  mm HCW), then it is predicted to have an HCW ranging from 1.46 to 1.70 mm, with an average 1.62 mm. No larva collected had an HCW  $> 1.22$  mm, which is substantially below that predicted for an additional instar. It is unlikely that another undetected instar occurs after what I have identified as instar 3 (HCW  $\approx 1.04$  mm). Recall from the life cycle of *U. virescens* that a larva bores a hole out of the immature fruit, through the areole, and into the cactus stem. Within the cactus stem, the larva consumes tissue creating a small gallery ( $\approx 1$  cm<sup>3</sup>). Stem tissue hardens and forms hard calluses when damaged, such as occurs with other columnar cacti (e.g., "boots" of saguaro, *Carnegiea gigantea* Engelman). These hard calluses prohibit further feeding by larvae. I dissected  $> 1000$  areoles and galleries over 8 yr, but never observed an HCW  $> 1.22$  mm. Yet, within these galleries I have observed pupae, pupal cases, and emerging moths. Hence, the instar identified as instar 3 is the terminal prepupal instar.

For holometabolous insects, it is not the number of instars that determines when metamorphosis occurs. Rather, a threshold size determines that a particular instar is the last (Nijhout 1975), and within this last instar, a critical weight determines when metamorphosis occurs (D'Amico et al. 2001). The number of instars required to reach this critical weight can vary with the nutritional quality and quantity of food resources (Davidowitz et al. 2003). For example, indi-

viduals feeding on a nutritionally poor food, such as low-dietary nitrogen, may require more instars to reach their critical weight than individuals feeding on a more nitrogen-rich diet (Chapman 1982, Taylor 1984, Nijhout 1994). Amino acids and proteins of developing seeds in senita cactus fruit provide a nutritionally-rich diet for moth larvae. Tissue of senita cactus stems has high concentrations of alkaloids. Because *L. schottii* is the only known host plant of *U. virescens*, it is not unreasonable to hypothesize that larvae can break down these alkaloids and possibly use the nitrogen-rich by-products. Nutritional quality of fruit and stem tissue of *L. schottii* most likely has not inhibited the evolutionary loss of an instar. Moreover, the constancy in the quality and quantity of larval food resources may explain why there is strikingly little variation in growth and size (HCW) of larvae within instars and among cohorts (Fig. 2).

Assuming that *U. virescens* can obtain sufficient nutritional quality and quantity from senita cacti within three instars, there are at least three hypotheses of selection pressures for the evolutionary loss of an instar. First, there may be a size constraint on larvae. If larvae continue to grow and molt after entering the cactus stem, adult moths could be too large to emerge from the hole in the areole of the cactus stem made by an earlier instar with a small HCW. Second, there may be a constraint on time in which larval development can occur. Fruit can mature quickly, within 21 d, and seed coats can begin to harden as quickly as within  $\approx 16$  d, making them unavailable as food for larvae. This short time for larvae to feed on fruit may explain why they only consume  $\approx 50\%$  of developing ovules before boring into cactus stems (Holland and Fleming 1999). Finally, the loss of an instar would shorten the life cycle of *U. virescens* and allow more generations to fit within flowering seasons of senita cacti. Thus, the data presented in this study indicate that *U. virescens* is the first Lepidopteran documented to have only three larval instars. Both the nutritional quality of senita cacti and selection pressures on the evolution of its life cycle are consistent with a shortened developmental period.

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