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Cover illustration: A caterpillar of *Junonia coenia* moves to a favorable feeding spot on the leaf of *Mimulus guttatus* at a spring in northern Arizona.

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POPULATION BIOLOGY OF THE SAND FOREST SPECIALIST BUTTERFLY *HELICONIUS HERMATHENA HERMATHENA* (HEWITSON) (NYMPHALIDAE: HELICONIINAE) IN CENTRAL AMAZONIA)

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ABSTRACT. The present paper describes the population parameters and natural history of *Heliconius hermathena hermathena* (Hewitson, [1854]), a sand forest specialist nymphalid butterfly. Population biology was described based on a 14-month mark-recapture program in a site of open forest in Pará state, northern Brazil. The population was constant through the year, with no marked peaks of abundance for both sexes, with females always less abundant. The range of the population size was 70–150 individuals (with a maximum near 200 individuals). Sex ratio was male biased, with males dominating most of the time. Average residence time was of 35 days for males and 31 days for females, with a maximum of 139 days recorded for males and 129 days for females, with both sexes presenting survival curves approaching the type II survival curve. Males presented wing sizes greater than females in all months. Adults were observed visiting five species of flowers as nectar and pollen sources and establishing communal nocturnal roosting aggregations on small shrubs. Considering the fragility of the Amazonian white sand forests, understanding the population patterns of *H. h. hermathena* can help future conservation planning for these potentially threatened habitats.

Additional key words: Campina, Campinarana, Heliconiini, white sand forest

Studies of population ecology of tropical butterflies are extremely scarce in the literature (see Vlasanek et al. 2013, Vlasanek & Novotny 2015 and references therein), and besides some recent efforts, most published studies of tropical butterflies are restricted to species of Nymphalidae and Papilionidae (e.g. Ramos & Freitas 1999, Freitas et al. 2001, Uehara-Prado et al. 2005, Tufto et al. 2012, Beirão et al. 2012, Vlasanek & Novotny 2015). This general lack of data on dispersal and demography of tropical butterflies hinders our capacity to understand ecology and functioning of plant-insect systems in tropical forests and to propose adequate measures for the conservation of endangered tropical butterflies (Freitas 1996, Freitas & Marini-Filho 2011, Vlasanek et al. 2013).

For *Heliconius* Kluk (Nymphalidae) butterflies, however, the situation is different. These are by far the most studied tropical butterflies, and concerning population ecology, a relatively large literature is available, including several different species and populations from Florida to Southern Brazil (Turner 1971, Ehrlich & Gilbert 1973, Cook et al. 1976, Araujo 1980, Brown 1981, Mallet & Jackson 1980, Romanowsky et al. 1985, Quintero 1988, Gilbert 1991, Ramos & Freitas 1999, Andrade & Freitas 2005, Sobral-Souza et al. 2015 and references therein). All these studies helped us to construct a general picture about *Heliconius* population patterns through space and time and to review the early ideas of low-density constant populations, which are typical of those populations from tropical sites (Ramos & Freitas 1999, Andrade & Freitas 2005, Sobral-Souza et al. 2015).

Nevertheless, although *Heliconius* butterflies are well known in terms of population ecology, published studies are restricted to a dozen of the approximately 40 described species in the genus (see references above), most of them in lowland tropical forest habitats (but see Fleming et al. 2005 for a study in an urban garden in Florida). In fact, most known *Heliconius* are typical of forested habitats, although some species such as *Heliconius erato* (Linnaeus) are able to persist in several different vegetation types (Araujo 1980, Ramos & Freitas 1999).

Contrary to its congeners, *Heliconius hermathena hermathena* (Hewitson) is associated with open vegetal formations, including the white-sand vegetation known locally as "Campina" or "Campinarana" (see detailed description of these habitats in Ducke & Black 1953, Anderson 1981 and Adeney et al. 2016), where the high-light, low- humidity, and often harsh conditions are restrictive for almost all other species of *Heliconius* (Brown & Benson 1977). In a detailed and extensive study, Brown & Benson (1977) presented comprehensive information on the systematics, biogeography, natural history (including host plant and immature stages) mimicry and ecology of this peculiar species of *Heliconius*. However, in the above study, demographic data for *H. hermathena* was restricted to a limited mark-recapture session during a few days, where little population data has been recorded (see Brown & Benson 1977).

The present paper describes the population biology of *H. hermathena hermathena* (Hewitson, [1854]) in central Amazonia based on a 14-month mark-recapture program. Given the fragile situation of the Amazonian white sand forests (Adeney et al., 2016), the results provide information that could aid in future management of this butterfly species and its fragile and unprotected habitats.

STUDY SITES AND METHODS

A mark-release-recapture (MRR) study of *Heliconius hermathena hermathena* was carried out in the "Parque Zoobotânico das Faculdades Integradas do Tapajós" (02°27'38''S, 54°43'59''W; ca. 25–30 m a.s.l.) (Figs. 1A, B), in the city of Santarém, Pará, Northern Brazil. The study area is covered with a mosaic of "terra firme" (never floodable forest) and open forests in different degrees of succession. Annual rainfall reaches 2100 mm and the average annual temperature is 26°C (INMET 2016) (a climagram for the study area is presented in Fig. 2). Butterflies were marked and recaptured in a trail (1.8 km long, divided into 49 sectors varying from 40 to 100 m, Figs. 1A, B) inside the forest during 14 months, from January 7, 2012 to February 26, 2013, for a total of 107 days (approximately 4 hours/day). Butterflies were net-captured, individually numbered on the underside of both forewings with a black permanent felt-tipped pen, and released. Characteristics of each individual (wing size, point of capture, sex and food sources) were recorded for later analysis (as in Ramos & Freitas; 1999 and Beirão et al. 2012).

TABLE 1. Permanence of males (n = 524) and females (n = 395) of marked *H. hermathena hermathena*. Days elapsed between marking and last recapture represent the minimum permanence (MP) for each individual.

MP (days)	Males	(%)	Females	(%)
1–20	214	40.8	172	43.5
21–40	133	25.4	112	28.4
41–60	80	15.3	68	17.2
61–80	41	7.8	26	6.6
81–100	29	5.5	8	2.0
101–120	21	4.0	5	1.3
> 120	6	1.2	4	1.0

The MRR data were analyzed using the Joly-Seber method for estimating population parameters (Francini 2010a, b). Males and females were analyzed separately. To estimate the number of individuals present per day, recaptured individuals were considered to be present on all previous days since the first capture (i.e. marked animals at risk, following Freitas & Ramos 2001). Time of permanence in population (i.e. minimum permanence, an indirect measure of longevity) was calculated as days elapsed between marking and last recapture (following Brussard et al. 1974). The sex ratio was calculated through the monthly means of daily proportions in number of individuals captured per day.

RESULTS

Population Dynamics. A total of 2014 individuals of *Heliconius hermathena hermathena* (1095 males and 919 females) (Figs. 1C, D) were captured between January 2012 and April 2013. The number of individuals captured per day varied from one to 53 for males (mean = 20.9; SD = 10.29; n = 107 d), and from two to 47 for females (mean = 17.2; SD = 7.84; n = 107 d). The number of individuals present per day varied from one to 96 for males (mean = 56.4; SD = 18.38; n = 107 d), and from six to 66 for females (mean = 37.7; SD = 10.78; n = 107 d). The population was constant through the year, with no marked peaks of abundance for both sexes, with females always less abundant (Fig. 3). In general, estimated population numbers were not greater than the number of individuals present per day, especially for females (Fig. 3).

TABLE 2. Number of recaptures for all marked individuals of males and females *H. hermathena hermathena*.

Number of recaptures	Males	Females
0	571	524
1	261	190
2	113	85
3	65	41
4	38	32
5	64	64
6	10	8
7	10	5
8	2	5
9	3	1
10	3	2
11	1	1
12	0	3
13	1	1

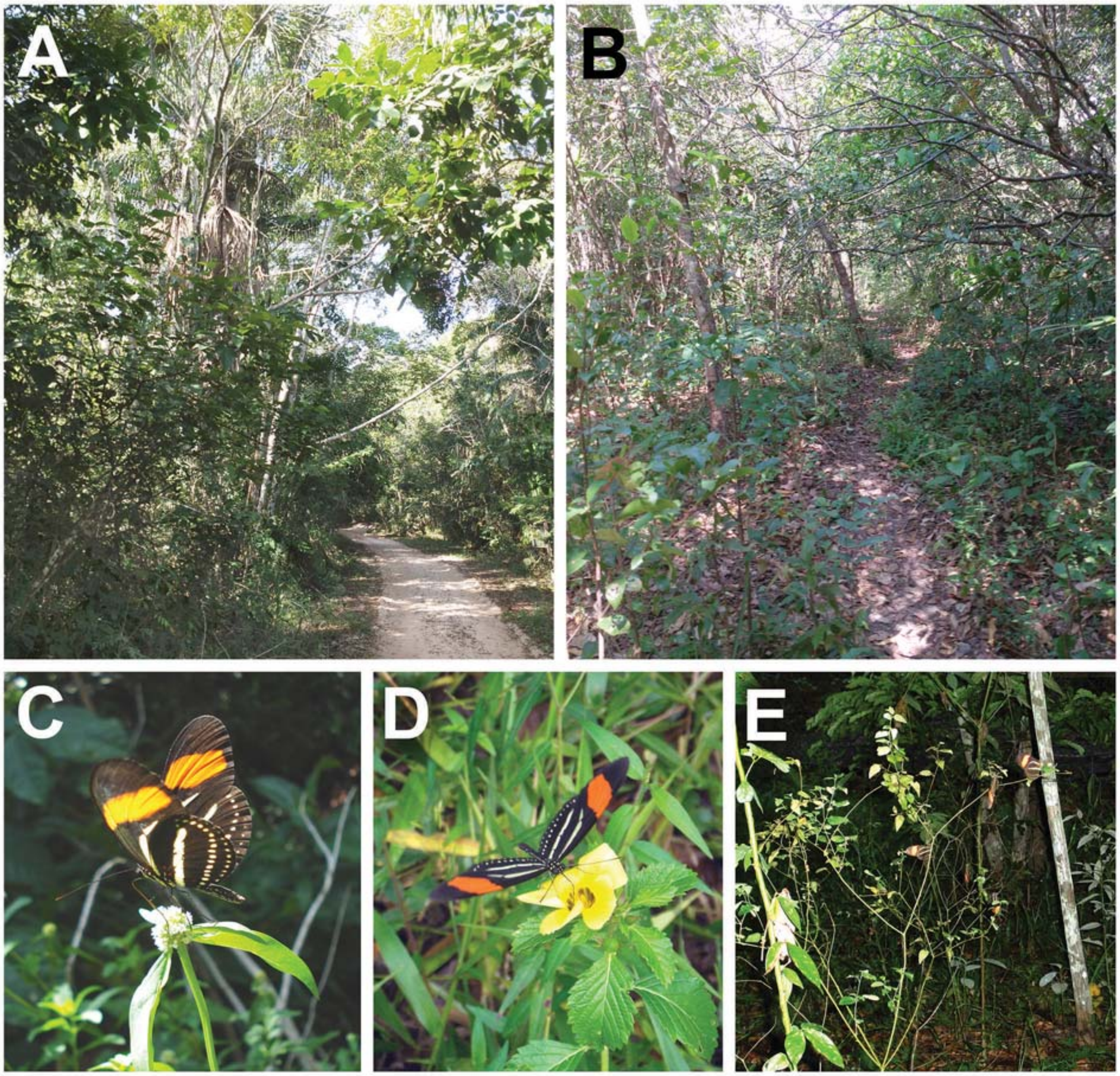

FIG. 1. **A, B**. Two views of the trails where the mark-release-recapture study of *H. hermathena hermathena* had been carried out; **C, D.** Males of *H. hermathena hermathena* visiting flowers of *Spermacoce capitata* (Rubiaceae) and *Turnera ulmifolia* (Turneraceae), respectively; **E.** A nocturnal roosting aggregation of *H. hermathena hermathena*.

Residence Time. The residence time (based on recaptured individuals) varied from two to 139 days for males (mean = 34.9 d; n = 524) and from two to 129 days for females (mean = 30.5 d; n = 395) (Table 1). Life expectancy (following Cook et al. 1967) was 50.8 days for males and 22.5 days for females. Survival curves (following Ehrlich and Gilbert 1973) are similar for both sexes (Kolmogorov - Smirnov test, $P > 0.05$, df = 2), approaching a type II survival curve (Fig. 4).

Sex Ratio. The sex ratio of individuals captured and marked was male biased (sex ratio of 1.2:1), with 1095 males and 919 females marked ($X^2 = 15.38$; df = 1; $P < 0.0001$), with males dominant in most months (Fig. 5). Both, males and females were recaptured from one to 13 times (Table 2); 524 males (47.9%) and 395 females (42.9%) were recaptured at least once, with males recaptured more than females ($X^2 = 4.78$; DF = 1; $P = 0.032$).

Wing Size. The forewing length ranged from 31.0 to 46.0 mm in males and from 30.0 to 45.0 mm in females. The average forewing length of males (mean = 42.1 mm, SD = 1.74, n = 1094) was greater than that of females

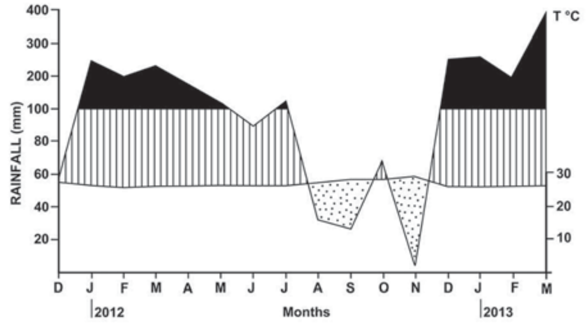

FIG. 2. Climatic diagram of the study site (see methods) during the study period (format following Walter 1985). Dotted = dry periods, hatched = humid periods, black = superhumid periods.

(mean = 41.6 mm, SD = 1.69, n = 919) (t = 6.32, df = 2011, P < 0.0001). The mean forewing length of both sexes were constant along the year (Fig. 6).

Natural history and behavior. In the study area, *H. h. hermathena* was common and easily observed throughout all the year. The adults were commonly observed near forest edges, flying from close to the ground (0.5–1 m high) to 2–3 m searching for flowers. Flower resources were not quantified but adults were observed visiting five species of flowers as nectar and pollen sources, including species in the family Costaceae (*Costus* sp.), Rubiaceae (*Spermacoce capitata* Ruiz & Pav., Fig. 1C), Turneraceae (*Turnera ulmifolia* L., Fig. 1D), Verbenaceae (*Stachytarpheta cayennensis* (Rich.)) and Vitaceae (*Cissus erosa* L. Rich.). Activity started before 0700 h in the morning and ceased around 1730 h in the afternoon; most flower visits were observed from 0800 to 1030 h in the morning, with the activity decreasing after 1200 h in the morning, when temperature became very hot in the study site and all adults moved to the shadow of the vegetation. Adults were observed establishing communal nocturnal roosting aggregations on small shrubs (Fig. 1E). The only reported host plant in the study site was *Passiflora hexagonocarpa* Barb. Rodr. (Passifloraceae).

DISCUSSION

Besides the distinct ecological requirements of *H. hermathena hermathena* compared with other studied *Heliconius*, population parameters here described are similar to those described for other species in the genus, which includes the constant populations through the year and long-lived adults with clear generation overlap (see Turner 1971, Ehrlich & Gilbert 1973, Quintero 1988, Ramos & Freitas 1999).

The male biased sex ratio reported here is a pattern usually reported for butterflies in general and for *Heliconius* in particular (Mallet & Jackson 1980, Ramos & Freitas 1999, Andrade & Freitas 2005, Herkenhoff et al. 2013, Sobral-Souza et al. 2015 and references therein). Because sex ratios are near to 1:1 in laboratory breeds, behavioral differences between sexes have been suggested as the reason for male biased sex ratios in population studies of tropical butterflies (Ehrlich & Gilbert 1973, Mallet & Jackson 1980, Ehrlich 1984, Freitas 1993, Brown et al. 1995). This is true for most nectar feeding species, where males are more easily captured along trails and forest edges where they come to visit flower resources, while females are supposedly looking for host plants inside the forest (e.g. Freitas 1993, 1996, Ramos & Freitas 1999, Francini et al. 2005). For most heliconians, which are nectar and pollen feeding, behavioral differences among sexes should be responsible for this pattern of male biased sex ratios (see above).

As described for other studied *Heliconius*, adults of *H. hermathena hermathena* live about one month on average, with some individuals living up to four months or more. These values are equivalent to those reported for previous studies with *Heliconius* in both, stable tropical (Turner 1971, Benson 1972, Ehrlich & Gilbert 1973, Quintero 1988, Ramos & Freitas 1999) or seasonal sites (Araujo 1980, Romanowsky et al. 1985, Flemming et al. 2005, Andrade & Freitas 2005). Even considering the small differences reported in previously mentioned studies, present results confirm the general pattern of long adult lifespans of species of *Heliconius* when compared to other tropical butterflies (Freitas & Ramos 1999, Uehara-Prado et al. 2005). Also in accordance with previous studies, males are more likely to be recaptured and present higher residence times than females, both possibly related to the above-mentioned behavioral differences among sexes (see Ramos & Freitas 1999 and references therein).

For tropical butterflies in general, females present greater wing sizes compared to males, a pattern reported in Pieridae (Jones 1992, Vanini et al. 1999, Ruszczyk et al. 2004), Papilionidae (Freitas & Ramos 2001, Beirão et al. 2012, Herkenhof et al. 2013, Scalco et al. 2016), and several groups of Nymphalidae (Kemp & Jones 2001, Uehara-Prado et al. 2005, Francini et al. 2005, Tourinho & Freitas 2009, Cavanzón-Medrano et al. 2016). Conversely, in *Heliconius*, female biased sexual size dimorphism is rare; males present greater wing lengths than females (Ramos & Freitas 1999 and present study) or differences are not significant (Andrade & Freitas 2005). A notable exception is *Heliconius sara* (Fabricius) whose males are smaller

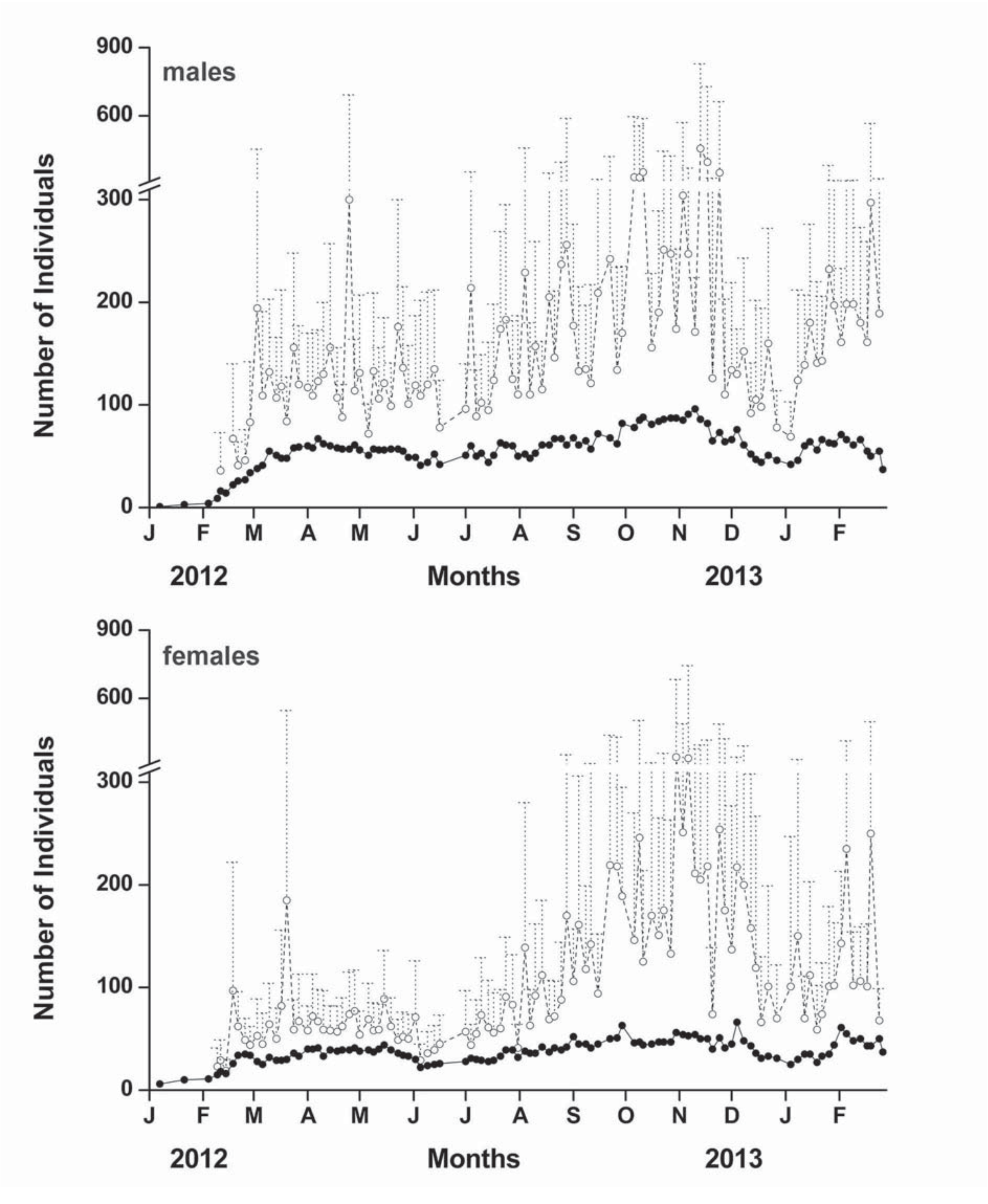

FIG. 3. Number of males (above) and females (below) of *H. hermathena hermathena* from January 2012 to February 2013 in the study site in Santarém, Pará. Solid circles = number of individuals present per day, open circles = estimated number based on Joly-Seber (bars = 1 standard error).

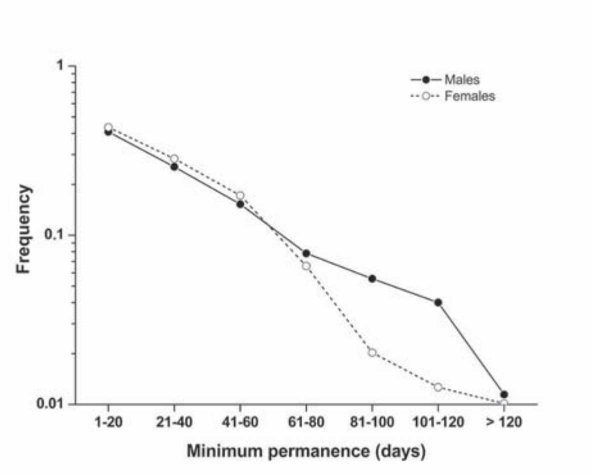

FIG. 4. Survivorship curves for *H. hermathena hermathena* males and females (following Ehrlich and Gilbert 1973). The frequencies of males and females are plotted on log scale against permanence categories (based on data presented on Table 1).

than females (and territorial advantage is associated with small sizes, see Hernandez & Benson 1998). Female-biased sexual size dimorphism is the more common pattern for insects in general (Stillwell et al. 2010) and for Lepidoptera in particular (Allen et al. 2011), and is related with larger fecundity in bigger females (Allen et al. 2011 and references therein). Although territorial behavior could partially explain this pattern (see Benson et al. 1989), the reasons for male-biased or no sexual size dimorphism in *Heliconius* are unknown and a topic to be further investigated.

Interestingly, besides the marked seasonality of the study area (with a prominent dry season), the studied population of *H. hermathena hermathena* was quite constant along the 13 months of study. This pattern is very similar to that reported in non-seasonal sites, such as for *Heliconius erato phyllis* (Fabricius) in coastal Brazilian Atlantic Forest (Ramos & Freitas 1999) and for *Heliconius ethilla* (Godart) in Costa Rica (Ehrlich & Gilbert 1973). In seasonal sites, conversely, populations of some species of *Heliconius* showed marked population fluctuations, with peaks of high densities of adults alternating with periods of extremely low population numbers. This pattern was reported in subtropical sites with a marked cold season, such as Southern Brazil (Araujo 1980, Romanowsky et al. 1985) and Florida (Fleming et al. 2005) and in seasonal forests with a marked dry season (Quintero 1988, Andrade e Freitas 2005). Conversely, *Heliconius sara apseudes* (Hübner) was reported as strongly seasonal in a stable tropical site in southeastern Brazil (Sobral-Souza et al. 2015).

Based on the present available information, four different population syndromes have been documented for *Heliconius*: 1) ecologically plastic species occurring in several different habitats, whose populations could be either, constant or seasonal, depending on the local climate—examples are *H. erato, H. ethilla* (Ehrlich & Gilbert 1973, Ramos & Freitas 1999, Andrade & Freitas 2005) and *Heliconius charitonia* (L.) (see Cook et al. 1976, Quintero 1988, Gilbert 1991 and Fleming et al. 2005); 2) species presenting marked seasonality independent of the climatic conditions, such as *H. sara apseudes* (Sobral-Souza et al. 2015, AVLF unpublished); 3) species from cooler montane forests, presenting marked seasonality, such as *Heliconius besckei* (Ménétriés) (AVLF unpublished) and *Heliconius nattereri* C. Felder & R. Felder (Brown Jr., K. S., pers. comm.); and 4) specialized tropical species restricted to tropical warm forests, with constant populations even in seasonal sites (such as *H. hermathena hermathena* in the present study).

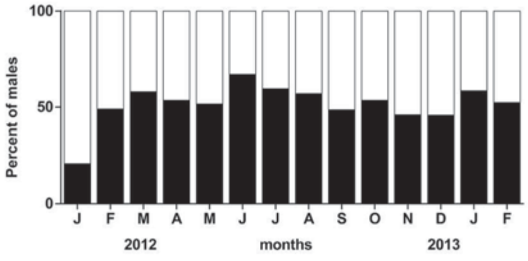

FIG. 5. Sex ratio of *H. hermathena hermathena* from January 2012 to February 2013 in the study site in Santarém, Pará. Data presented as percent of males (in black) by month (based on means of each days' captures).

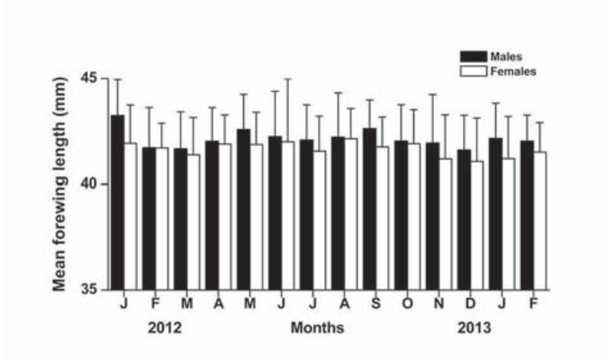

FIG. 6. Mean forewing length of males (solid bars) and females (open bars) of *H. hermathena hermathena* in the study site in in Santarém, Pará, from January 2012 to February 2013 (based on monthly recruitment). Bars = monthly means, line extensions = standard deviations.

However, information about *Heliconius* population ecology is limited to very few studies focusing on a restricted subset of less than dozen of the over 40 described species in the genus. Consequently, very few species could be assigned to the above-proposed fourth category, which was for a long time considered as a model of population dynamics in *Heliconius*. The typical example is *H. ethilla*; the first studied species of *Heliconius* from a population point of view and an example of a tropical butterfly with low-density constant populations throughout time (Ehrlich & Gilbert 1973, Ehrlich 1984). Three decades later, Andrade & Freitas (2005) showed that population parameters of the same species (*H. ethilla*) in a seasonal tropical site with a marked dry period are quite distinct: the population was not constant, showing a marked peak of abundance in the rainy season and a period of extremely low population numbers during most of the dry season.

Gilbert (1991) provided demographic data for eight species of *Heliconius*, all presenting constant populations in a tropical site in Costa Rica. From these, two of them (*H. erato* and *H. charitonia*) were shown to fit in the first population syndrome when additional population data become available (see above). The remaining six species, however, are all restricted to tropical Amazonian and Central American habitats (with one also occurring in the northern tropical portion of Atlantic Forest—*Heliconius melpomene* (Linnaeus)) and could be good examples of species fitting in the fourth population syndrome (and maybe *Heliconius xanthocles* H. Bates, see Mallet & Jackson 1980).

In this sense, the present demographic data for *H. hermathena hermathena* is relevant by adding information that can help in understanding the above proposed population syndromes for the genus *Heliconius*. In addition, because *H. hermathena* is a sand forest specialist, it would be important to obtain data from other populations of this same species in different localities in the Amazonia. Finally, understanding the population patterns of this species will add information that can help in future conservation planning for these fragile and potentially threatened habitats.

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ORNATIVALVA ERUBESCENS (WALSINGHAM) (LEPIDOPTERA: GELECHIIDAE) INTRODUCED IN NORTH AMERICA

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ABSTRACT. *Ornativalva* (Lepidoptera: Gelechiidae) is an Old World genus known to be associated with the plant genus *Tamarix* (Angiosperm: Tamaricaceae) which was introduced to North America and became widely distributed in the Southwestern United States. *Ornativalva erubescens*, introduced from the Old World, is reported from the Southwestern United States. *Ornativalva erubescens* is redescribed. Adults and male and female genitalia are illustrated.

Additional key words: *Tamarix*, *saltcedar*, Arizona, Nevada, Texas, potential biological control, riparian habitats

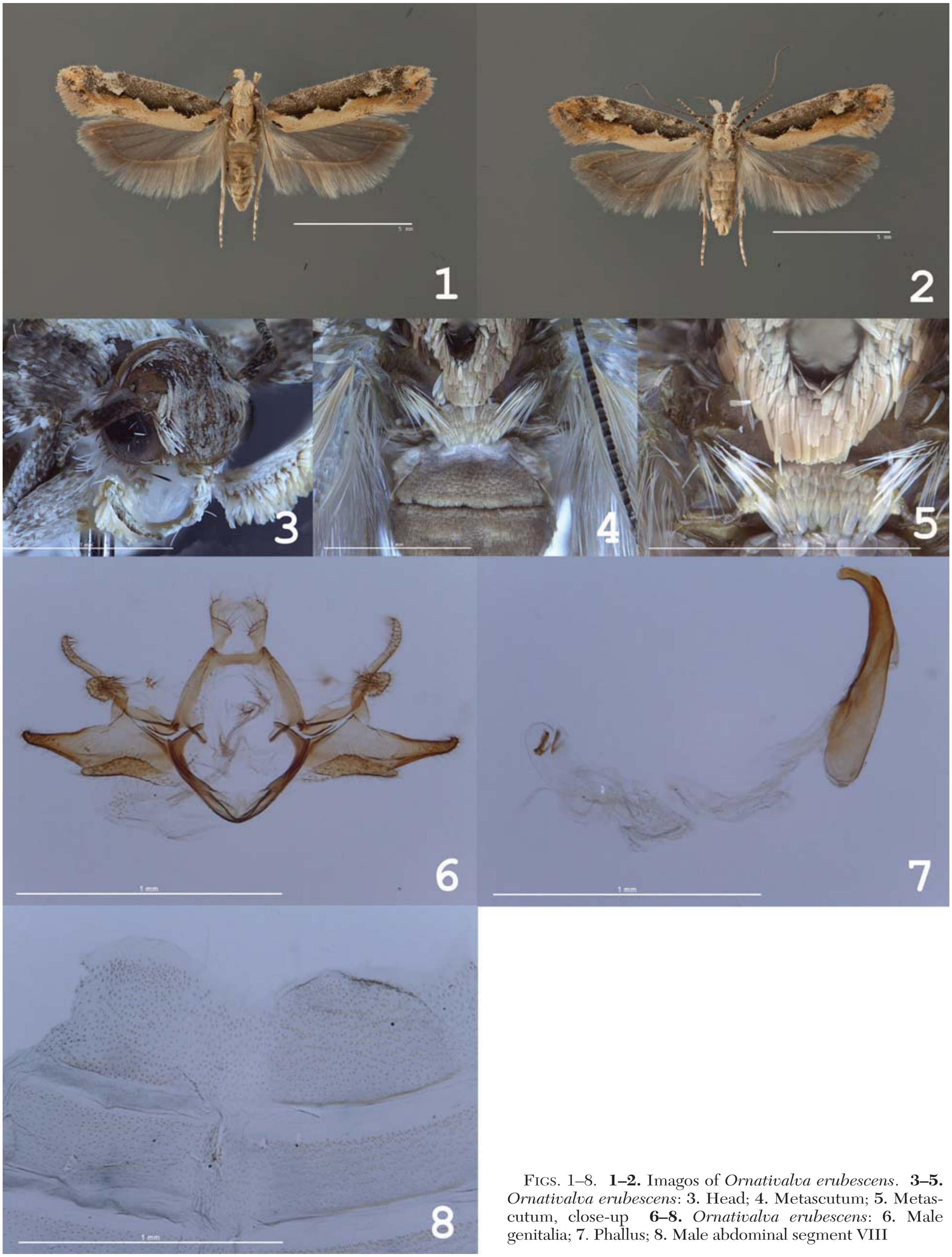
Ornativalva Gozmány is an Old World genus in the family Gelechiidae (Lepidoptera). The genus was revised by Sattler (1967, 1976) and contains 60 described species from the Palaearctic Region and one species from South Africa (Bidzilya 2009; ftp.funet.fi Feb. 27 2017, Li 1991, Li & Zheng 1996, Sattler 1967, 1976).

According to the literature, the genus *Ornativalva* is closely correlated with the plant genus *Tamarix*, commonly called saltcedar, based on two lines of evidence. First, the distribution of *Ornativalva* and *Tamarix* are highly similar. Second, a number of *Ornativalva* species were reared from *Tamarix* host plants. The other known host plant is *Frankenia* (Frankeniaceae) (Sattler 1976). Saltcedar is native to xeric areas of Eurasia and Africa and was introduced into the United States in the 1800s, where it was planted for erosion control along river systems in the Southwestern United States. *Tamarix ramosissima*, *T. chinensis* and hybrids between these are now considered undesirable invasive plant species in the Southwestern United States, particularly in desert regions of California and many riparian habitats in New Mexico. Land managers and owners of riparian areas all across the Southwestern United States, as well as many state, public, and private organizations in Mexico, are struggling to deal with saltcedar. Thousands of dollars are spent every year trying to control or attempt to eradicate saltcedar in the White Sands Missile Range, Holloman Air Force Base and White Sands National Monument in Otero County New Mexico (E. Metzler, personal communication). Currently the distribution of saltcedar in the United States is reported along major rivers and reservoirs from southern California east through Texas and western Oklahoma, and north to Nevada, Colorado and Utah. Some years ago, the USDA brought one of the natural consumers of saltcedar, *Ornativalva grisea* Sattler, from China and tested it in quarantine, but any further updates are not reported (Bossard et al. 2000, Chew 2009, Nagler et al 2011). The herein reported introduction of *O. erubescens* (Walsingham) could be considered as an accidental potential biological control.

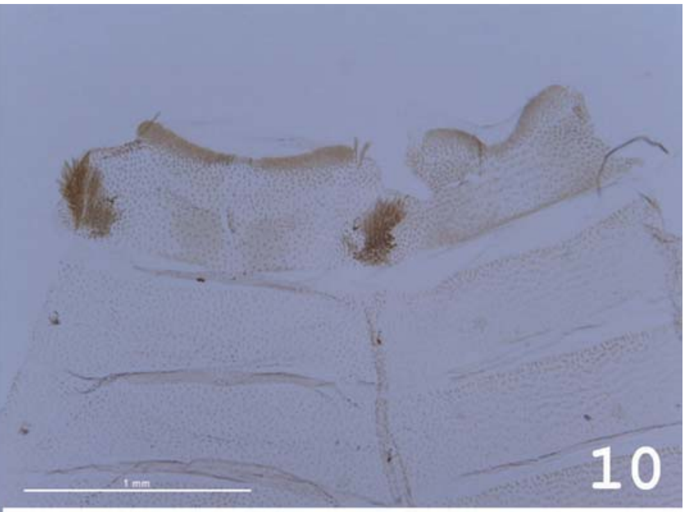
Ornativalva erubescens, naturally distributed in Northern Africa to Western Asia, was recently collected in the Southwestern United States, with an especially high abundance of individuals from the state of Arizona. The introduced species is redescribed and illustrated below, and known locality records from the United States are given.

MATERIALS AND METHODS

Imago specimens were captured at a 12 Volt, 15 Watt Ultraviolet (UV) light. The lectotype female (Wlsm no. 96596) of *O. erubescens* deposited in the Natural History Museum, London (NHM) was examined. Dissection and slide mounting methods for genitalia followed Clarke (1941), except preparations were stained with eosin and mounted in Euparal. A Leica M205 A Stereo-Microscope (with magnifications 7.76-159X) was used



FIGS. 1–8. **1–2.** Imagos of *Ornativalva erubescens*. **3–5.** *Ornativalva erubescens*: **3.** Head; **4.** Metascutum; **5.** Metascutum, close-up **6–8.** *Ornativalva erubescens*: **6.** Male genitalia; **7.** Phallus; **8.** Male abdominal segment VIII



Figs. 9–10. *Ornativalva erubescens*: **9.** Female genitalia; **10.** Female abdominal segment VIII

for examining specimens and slide mounts. Images were made with the Passport II Imaging System with a Canon MPE 65 mm 1–5X micro-photography lens, and with a Leica stereoscope with Leica Application Suite 4.6©. Specimens are deposited in the Arizona State University Hasbrouck Insect Collection (ASUHIC), at least one male and one female deposited in the U.S. National Museum of Natural History (USNM), and several specimens in the British Museum Natural History, London (BMNH) (Ian Watkinson, personal communication, June 26, 2017).

Genus *Ornativalva* Gozmány, 1955

Ornativalva Gozmány, 1955, Ann. Hist.–nat. Mus. natn. Hung. 47: 308-309 [keys], 310.

Type species: *Gelechia plutelliformis* Staudinger, 1859

Pelostola Janse, 1960, Moths S. Afr. 6(2): 188.

Type species: *Pelostola kalahariensis* Janse, 1960

Most *Ornativalva* species are characterized by having frontal modifications or processes on the denuded head, however some species are free of modifications. The metascutum bears paired patches of long hair-like scales near its posterior margin, like most gelechiid species, however some species of *Ornativalva* are found with modified short broad scales. The forewing bears a characteristic W-shaped line in the fold. The genitalia in both sexes are diagnostic; the male valvae are divided into two to five branches, and in the female a signum is always present in the corpus bursae.

***Ornativalva erubescens* (Walsingham, 1904)** Figs. 1–10

Gelechia erubescens Walsingham, 1904, Entomologist's mon. Mag. 40: 265.

Ornativalva erubescens (Walsingham) Sattler, 1964: 578; Sattler, 1976: 108; Li & Li, 2005: 247.

Description (Figs. 1-5). Wing length 5.6-6.2 mm. Head pale or whitish ochreous, brown along eye; without developed arc above the transfrontal sulcus. Vertex without enlarged scale bases or frontal process. Labial palpus pale or whitish ochreous; outer surface of second segment with scattered light brown rings at base and apex; third segment with scattered light brown scales. Antennae dark brown with pale ochreous rings. Thorax ochreous or pale ochreous; metascutum with a paired group of hair-like scales near its posterior margin. Tegula dark brown with white mottled. Forewing with a short dark brown basal streak; anterior half of wing dark brown like a band running from the base slightly widening toward the apex and the termen, W-shaped markings in the fold, posterior half of wing ochreous or pale ochreous, dark dot at end of cell with short dark streak extending towards apex, pale ochreous broad spot extending towards costa along anterior streak, pale ochreous streak at four fifths

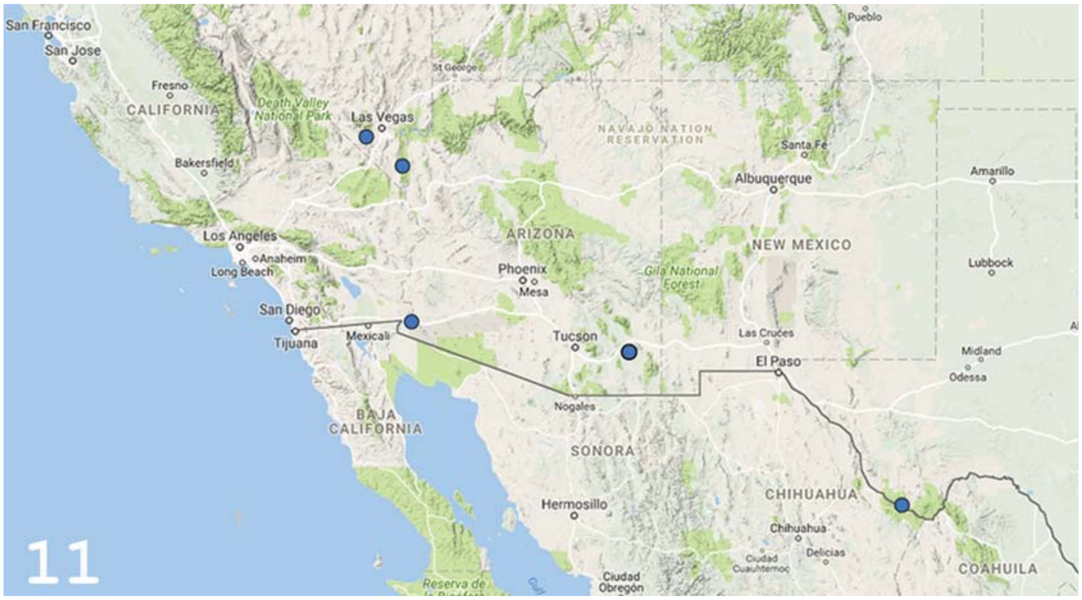


FIG. 11. Distribution map of *Ornativalva erubescens*. Blue dots indicate occurrences in the United States

of costa, sometimes extending towards the dorsum along the posterior half of the wing, base of fringes with distinct dark markings. Hindwing dark brown. Frenulum of female double or triple.

Male genitalia (Figs. 6-8). Uncus short, tubular, truncated. Gnathos absent. Valva broad at base, divided costal and saccular parts widely separated. Costa almost as long as valva, costal half tapering, curved dorsally, digitate-shaped, pointed at apex; harpe almost two-thirds length of costa, bent near middle, broader at apex. Saccular region tapering, apex rounded, turned dorsad. Sacculus digitate-shaped, almost half length of saccular region, enlarged near middle, rounded at apex. Phallus with slightly bulbous base, apical one-third curved, tapering, apex truncated.

Female genitalia (Figs. 9-10). Abdominal segment VIII posteriorly with paired patch of short scales. Apophyses anteriores a little shorter, or almost as long as length of apophyses posteriores. Antrum well developed, sclerotized, funnel-shaped, anterior portion narrowed. Ductus bursae almost twice length of corpus bursae when fully extended, coiled inside the abdomen. Signum with pair of sclerotized teeth, no sclerotized bridge of the signum.

Material examined (37 specimens) (Fig. 11). 3(♂), 3(♀), USA: AZ: Yuma Co., Yuma, Elev. 200', 16.vi.2016, at blacklight, leg. I. Watkinson (male genitalia slide No. SLEE0875); 12(♂), 12(♀), USA: AZ: Cochise Co., Willcox Playa Wildlife Area, Kansas Settlement Rd., 32.142360, -109.758675, Elev. 4177ft., 5.viii.2016, UV light, leg. S. Lee and F. Skillman (male genitalia slide No. SLEE0873; female genitalia slide No. SLEE0874); 1(♀), USA: TX: Brewster Co., N29.265, W103.790, 12.iii.2014, leg. J. Vargo; 5(♀), USA: NV: Clark Co., N35.495, W114.69, 5.x.2016, leg. J. Vargo; 1(♀), USA: NV: Clark Co., N36.01, W115.488, Elev. 5400ft., 6.x.2016, leg. J. Vargo. Slides deposited in ASUHIC.

Diagnosis. The forewing pattern with the dark brown band-like anterior half, short dark basal streak or W-shaped markings in the fold, is similar to *O. arabica* Sattler, *O. aspera* Sattler, or *O. pulchella* Sattler. The male genitalia are rather similar to *O. frontella* Sattler especially with the harpe bent near middle. *O. erubescens* is specifically separated from those similar ones by the combination of characters: head without enlarged scale bases or frontal process; forewing with a short dark brown basal streak, anterior half of wing dark brown, posterior half of wing ochreous or pale ochreous, pale ochreous broad spot extending towards costa along anterior streak, pale ochreous streak at four fifths of costa; male with harpe bent near middle, sacculus digitate-shaped, enlarged near middle, rounded at apex; female with funnel-shaped antrum, signum with pair of sclerotized teeth, sclerotized bridge absent.

Biology. Host plant unknown, however suspected to be *Tamarix* (Angiosperm: Tamaricacea). Imago moths were collected in March, June, August and October in Southwestern United States.

Distribution. North Africa (Morocco, Algeria, Tunisia, Libya, Sudan) and the Middle East (Egypt, Israel, Saudi Arabia, Iran) to Pakistan (ftp.funet.fi Feb. 27 2017; Sattler 1976). United States (Cochise and Yuma Counties in Arizona, Clark County in Nevada, and Brewster County in Texas).

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NOVEL OBSERVATIONS OF LARVAL FIRE SURVIVAL, FEEDING BEHAVIOR, AND HOST PLANT USE IN THE REGAL FRITILLARY, *SPEYERIA IDALIA* (DRURY) (NYMPHALIDAE)

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ABSTRACT. *Speyeria idalia* is a prairie specialist that has experienced dramatic population declines throughout its range. *Speyeria idalia* is nearly extirpated from the eastern portion of its former range; however, populations within Kansas are relatively stable. We made several previously undescribed field observations of late-instar larvae and post-diapause female *S. idalia* in north-eastern Kansas during 2014–2016. We report finding late-instar larvae at locations that were burned within weeks of detection. The observations of larvae shortly following a burn suggests that *S. idalia* larvae are capable of surviving fire and contradicts our current knowledge of this species. Additionally, we describe a feeding behavior characteristic of late-instar larvae. Larvae observed in the field and lab stripped leaves of host plants leaving only stems. This strip-style feeding behavior provided unique feeding evidence that was valuable to detecting the presence of larvae in the field. Finally, we documented larvae and post-diapause, egg depositing females using *Viola sororia*. The use of this relatively widespread and common plant by *S. idalia* populations in the Central Great Plains has only been implicitly documented but may have important conservation implications. These novel observations further our knowledge of the ecology of this imperiled species and provide timely information that may improve research and conservation management efforts directed toward *S. idalia* populations.

Additional key words: conservation, feeding sign, grasslands, Great Plains, violet

The regal fritillary, *Speyeria idalia* (Drury, 1773) (Nymphalidae), is a large univoltine and non-migratory butterfly. Adult flight begins with the emergence of males in late May and continues through September when females begin to oviposit (Klots 1951, Tilden & Smith 1986, Wagner et al. 1997). *Speyeria idalia* mate shortly after they emerge in late May–early June; however, females postpone oviposition until late August to early September (Scott 1986, Wagner et al. 1997, Zercher et al. 2002). Oviposition site selection appears to be somewhat casual with eggs deposited near, but rarely on host plants (Scott 1986, Kopper et al. 2000, Swengel & Swengel 2001). It is generally suspected that females cue into factors other than the presence of host plants when determining oviposition locations because host plants are senescing when females begin to oviposit eggs (Wagner et al. 1997, Kopper et al. 2000).

Speyeria idalia eggs hatch in ~ 25 days and 1st instar larvae emerge, consume the chorion, and enter a winter diapause. Larval development resumes in early spring with the emergence of host plants and lasts ~ 6–7 weeks. There are six larval instars followed by pupation in late spring and a pupal stage that lasts 2.5 to 4 weeks (Edwards 1879, Hammond 1974, Wagner et al. 1997). *Speyeria idalia* larvae are oligophagous and feed on a variety of violet (*Viola* spp.) host plant species (Klots 1951, Hammond 1974, Ferris & Brown 1981). Local populations are often associated with specific violet species and larvae in the Central Great Plains are reported to preferentially feed on birdfoot, *Viola pedata* (Linnaeus) (Violaceae) or prairie violet, *Viola pedatifida* (G. Don) (Violaceae) (Swengel 1997, Kelly & Debinski 1998, Dole et al. 2004).

The historic range of this once abundant butterfly of N. American prairie communities extended from Oklahoma to the border of Canada and east to the Atlantic coast. Populations have declined by approximately 99%. *Speyeria idalia* is now nearly extirpated from the eastern portion of its former range and western populations have experienced dramatic declines (NatureServe 2016). *Speyeria idalia* was listed as a Category II species under the United States Endangered Species Act (ESA) until this category was removed in 1996 (U.S. Fish and Wildlife Service 1996).

FIG. 1. A late-instar *Speyeria idalia* larva feeding on *Viola pedatifida* leaves in the Flint Hills of northeastern Kansas, USA.

FIG. 2. The characteristic feeding sign exhibited by late-instar *Speyeria idalia* larvae on *Viola pedatifida* in the Flint Hills of northeastern Kansas, USA. On the left is a *V. pedatifida* leaf before being consumed by a larva. On the right are the remnants of a *V. pedatifida* leaf after a late-instar larva strips away the leaflets until only a small portion of leaf and stem remain.

Rapid, range-wide declines continued, prompting the U.S. Fish and Wildlife Service to initiate a status review under the ESA in September 2015. However, some locally abundant populations persist in the west and the species is considered stable in Kansas (Ely et al. 1986, Marrone 2002, Selby 2007).

While it is suspected that *S. idalia* population declines are caused by habitat loss, fragmentation and land management practices such as heavy grazing, frequent and intensive burning, and haying, the direct causes are unclear (Schultz & Crone 1998, Davies et al. 2005, Ferster & Vulinec 2010). It is estimated that the Great Plains region has lost 70% of its grasslands and only 37% of the historic extent of tallgrass prairie in the Flint Hills of Kansas remains (Samson et al. 2004). Native tallgrass prairie communities have succumbed to anthropogenic land conversion, development, invasive weeds, and plant succession (Samson & Knopf 1996). Historically, unrestricted grazing by herbivores and wildfire played important roles in the maintenance of prairie ecosystems in the Great Plains (Fuhlendorf et al. 2009). However, in the absence of these ecological drivers, remnant tracts of prairie are often maintained by prescribed land management (Shuey 1997, Samson 1999, Toombs 2012). This is crucial to maintaining grassland ecosystems and a lack of management threatens the persistence of remaining prairie remnants (Fuhlendorf and Engle 2004). In particular, prescribed fire can suppress invasive plant species and control woody encroachment, and is considered a primary tool for maintaining the open vegetative structure of grasslands (Fuhlendorf & Engle 2001, Ditomaso et al. 2006).

The effect of fire on prairie insects is contentious. Some studies suggest that prescribed fire benefits prairie specialist butterflies (Selby 2007, Moranz et al. 2014), while others argue that it is harmful (Swengel 1996, 2001, 2004; Swengel & Swengel 2001). In particular, *S. idalia* research often reports that fire reduces or eliminates this species from sites by directly or indirectly killing larvae (Kelly & Debinski 1998, Swengel & Swengel 2001, Powell et al. 2007, among others). Therefore, management recommendations for *S. idalia* populations typically propose prescriptions of mowing, haying, light grazing, rotational burn regimes and the implementation of permanent non-fire refugia (Schlicht & Orwig 1992, Swengel 1996, Schlicht 2001, Panzer 2002, among others).

The uncertainty of the effects of fire on *S. idalia* populations is likely the result of a dearth of autecological information on immature stages. *Speyeria idalia* larvae are inconspicuous, cryptic, and widely dispersed, making them difficult to locate in the field (Scudder 1889, Kopper et al. 2001, TNC 2001). The challenges associated with detecting *S. idalia* larvae have limited field studies on this fundamental life history stage. Consequently, assessments of habitat quality are often conducted by examining relationships between available resources and habitat features and the presence and abundance of adults (Britten & Riley 1994, Smallidge et al. 1996, Grundel et al. 2000, Collinge et al. 2003). However, adult mobility may confound assessments of how the presence and abundance of mature *S. idalia* in an area is affected by management

FIG. 3. A late-instar *Speyeria idalia* larva beginning to feed on the leaves of *Viola sororia* in the Flint Hills of northeastern Kansas, USA.

(Swengel 1996, Ferster & Vulinec 2010, Shuey et al. 2016). Highly vagile adult butterflies can readily move across a landscape to locate resources when conditions change while larvae are generally restricted to the resources and conditions in the area at which they hatched. Thus, habitat features and resources used by adults may not adequately reflect the requirements of the immature stages (Bergman 1999, Lane & Andow 2003, Albanese et al. 2008).

The goal of our study was to investigate the effects of management practices and habitat features on the presence and abundance of *S. idalia* larvae and adults. We made several novel observations of late-instar larvae and post-reproductive diapause female butterflies during this research. Here, we present and discuss the following observations: several late-instar larvae in areas that were recently burned, a distinct and reliable feeding sign exhibited by late-instar larvae, cathemeral larval activity and the use of a less recognized host plant species in our study region by post-reproductive diapause female butterflies and late-instar larvae.

METHODS AND RESULTS

We conducted field and laboratory studies from 2014 through 2016 at the Fort Riley Military Reserve (FRMR) and the Konza Prairie Biological Station (KPBS), in Geary and Riley counties within northeastern Kansas, U.S. These sites are within the Flint Hills, which is characterized by rolling hills, rocky soil, and large tracts of tallgrass prairie (Anderson & Fly 1955, Reichman 1987).

Field surveys for late-instar larvae were conducted in April and May during 2014, 2015, and 2016. Here, we use the term late-instar to describe larvae that were assessed to be in fourth through sixth larval instars when observed. This estimation was based on the period of larval surveys and the relative size of observed larvae. We surveyed ten 2500-m^2 plots for late-instar larvae and the location of surveyed plots were stratified by management regime (i.e., fire, grazing, and haying) and fire-return interval (i.e., low ≥ 10 years, moderate 3–5 years, and high 1–2 years). Six of the plots were located at the FRMR and four were located at the KPBS. Each plot was partitioned into grids of 100-m^2 sub-plots. The 100-m^2 sub-plots included a gradient of violet density. We randomly selected 15 sub-plots within each 2500-m^2 plot for larval surveys each year. Each host plant located within surveyed sub-plots was systematically examined for late-instar larvae and evidence of larval feeding. We also searched the surrounding vegetation, litter, and ground within each sub-plot for larvae.

Surveys for post-reproductive diapause, egg depositing females were conducted from late August to early October during 2014 and 2015. We surveyed 44 line transects for adult females actively depositing eggs. All line transects were ≥500 m in length and stratified by management regime (i.e., fire, grazing, and haying), violet density and fire-return interval (i.e., low ≥ 10 years, moderate 3–5 years, and high 1–2 years). We conducted surveys when weather conditions were appropriate for adult flight using repeated, modified Pollard walks (Pollard 1977). When a female was detected an observer followed its movements and recorded oviposition locations.

Larvae and Fire. We surveyed a total of 353 100-m^2 sub-plots for late-instar *S. idalia* larvae and feeding evidence during this study. Seventy-six of these sub-plots

FIG. 4. An adult *Speyeria idalia* female dragging her abdomen and probing various substrates in search of oviposition locations in the Flint Hills of northeastern Kansas, USA. The broad-leaf plant in the image is *Viola sororia*.

were located in areas that had been burned during the late winter and spring months preceding a larval survey. We detected late-instar larvae and evidence of larval feeding in 16 (21%) of these sub-plots. A total of 22 late-instar *S. idalia* larvae were detected during this study. Twelve (54%) of the larvae observed were in areas that had been burned ≤61 days prior to detection. Seven of these larvae were observed at the FRMR and five at the KPBS study sites.

Larval Feeding Sign and Behavior. We collected a total of five *S. idalia* larvae from the field. In 2014, we collected two larvae on 19 April and a third on 19 May. In 2015, we collected two larvae on 19 April. Larvae were kept outside in small, clear individual enclosures and we observed each larva's behavior for 18 to 35 days. The larvae were reared on both *V. pedatifida* and *Viola sororia* (Willd) (Violaceae) leaves. All five larvae produced a unique strip-style feeding sign on both host plant species. Larvae defoliated stems by consuming the leaves of host plant species in a "type-writer" fashion, feeding back and forth on a single leaf until all that remained was a small portion of the leaf above the stem (Fig. 1). Larvae were active during both day and night but rested at the base of plants or in the folds of leaf litter provided within enclosures when not foraging.

Late-instar *S. idalia* larvae observed in the field also produced this distinctive feeding sign on host plants and exhibited cathemerality. We observed 19 larvae during daylight, two during twilight and one at night during field surveys. These larvae were actively feeding, foraging and resting when observed. Evidence of late-instar larvae feeding between field and lab larvae was consistent. Late-instar larvae observed in the field also defoliated host plants by stripping away and consuming the leaves until only a small portion of the leaf above the stem remained (Fig. 2). This feeding sign was detected in all of the 100-m^2 sub-plots in which larvae were detected.

***Speyeria idalia* and *Viola sororia*.** Among the ten 2500-m^2 larval survey plots we surveyed for larvae, four contained both *V. pedatifida* and *V. sororia*, and one contained only *V. sororia.* Late-instar *S. idalia* larvae and larval feeding sign was detected on *V. sororia* in three of these plots. The late-instar larvae feeding sign exhibited on *V. sororia* closely resembled the feeding sign we observed on *V. pedatifida* (Fig. 3).

We observed three post-reproductive diapause females oviposit in close proximity (i.e., < 1 m) to *V. sororia* plants during surveys (Fig. 4). All females flew low to the ground and frequently dropped down into vegetation. Upon landing, females maneuvered through vegetation, tasting with their feet and dragging their abdomens while probing various substrates in search of oviposition sites. Females then deposited eggs on the underside of dead vegetation or detritus near *V. sororia* plants. In two of these observations, the only host plant species observed within a 1/4 km of the oviposition location was *V. sororia*.

DISCUSSION

Previous research conducted on *S. idalia* larvae has resulted in relatively small sample sizes of detected larvae (e.g., Barton 1995, n=9; Kopper et al. 2001, n=12; TNC 2000, 2001, n=0), which has limited the application of these data to robust quantitative analysis. However, the identification of a feeding sign distinctive to the larvae of a rare butterfly species can improve larval detection on host plants (Albanese et al. 2007, 2008). The unique feeding sign of late-instar *S. idalia* larvae that we observed was a reliable indication of the presence of larvae and greatly improved our ability to detect this species within host plant patches. Using direct observations of *S. idalia* larvae in conjunction with the feeding sign we describe may improve efforts to conduct field research on this life history stage and ultimately, advance our knowledge of this species' conservation management.

The establishment and spread of invasive plant species is not always detrimental to Lepidoptera populations. For example, both the larvae of wild indigo duskywing, *Erynnis baptisiae* (Forbes, 1936) (Hesperiidae) and Baltimore checkerspot, *Euphydryas phaeton* (Drury, 1773) (Nymphalidae) have adapted to feed on nonnative, invasive plant species (i.e., crown vetch, *Securigera varia* (Linnaeus) (Fabaceae) and English plantain, *Plantago lanceolata* (Linnaeus) (Plantaginaceae), respectively) as alternatives to their native host plants, which have relatively restricted distributions. Subsequently, both butterfly species have increased in abundance (Bowers 1992, Opler and Malikul 1992, Ferge 2008). *Viola sororia* is arguably the most common and widespread *Viola* species in N. America and often considered an invasive weed (Solbrig et al. 1980). Although it is known that *S. idalia* larvae are able to feed on different *Viola* species, direct field observations of larvae feeding on *V. sororia* have not been well documented especially within our study region. Moreover, the selection of oviposition sites by adult females near *V. sororia* further indicates the potential importance of this alternative host plant to *S. idalia* ecology and conservation management. Research suggests that predicted global climate and land use change will continue to facilitate increases in the distribution and abundance of adaptive, generalist species and negatively impact populations of specialist plant and insect species (Sparks 2000, Menendez et al. 2008, Betzholtz et al. 2012). More widespread use of *V.*

sororia by *S. idalia* populations as an alternative to *Viola* restricted to native grasslands (i.e., *V. pedatifida*) may represent an important adaptation toward the reestablishment and range expansion of this imperiled butterfly species particularly in light of predictions of climate and land use change.

Based on our observations of *S. idalia* larvae at recently burned sites, we hypothesize that *S. idalia* larvae have adaptive physiological and/or behavioral mechanisms that facilitate survival of low to moderate intensity surface fires. Previous research has suggested adult *S. idalia* populations are negatively affected by fire (Swengel 1996, Powell et al. 2007) or has presumed that fire is fatal to larvae (Moffat & McPhillips 1993, Kelly & Debinski 1998, Swengel 1998, Huebschman & Bragg 2000, among others) leading to recommendations against prescribed fire as a conservation management tool for this species. However, the response of many species to fire, particularly invertebrates, is complex, inconsistent and driven by a number of different factors. For example, fire increases ant-tending of Fender's blue butterfly, *Icaricia icarioides fendri* (Macy, 1931) (Lycaenidae) larvae and this mutualistic relationship actually increased larval survival the following year (Warchola et al. 2015). Further, some butterfly species evade fire by being semi-fossorial. Immature atala hairstreak, *Eumaeus atala* (Poey, 1832) (Lycaenidae) and frosted elfin, *Callophrys irus* (Godart, 1824) (Lycaenidae) survived fire when pupae were > 1.75 cm below the ground surface (Thom et al. 2015). We observed several larvae resting in dense litter and even underneath rocks at recently burned sites and hypothesize that *S. idalia* larvae use heat as a cue to seek refuge under structures or possibly even underground during fires.

To understand and conserve populations of *S. idalia* and other rare Lepidoptera, we must investigate the ecology of all life history stages. Our observations represent the first time, to our knowledge, that *S. idalia* larvae have been reported in recently burned areas. *Speyeria idalia* populations in the Central Great Plains have evolved with fire for millennia. This species' and its primary host plants' specialization to fire-dependent systems further suggests that *S. idalia* larvae may have adaptations to survive fire and its relationship with fire may be more complex than previously suggested. Our observations highlight the need for additional research investigating the relationship between fire and this species' immature life history stages.

Moreover, adapting to increased use of the more common, generalist *V. sororia* as a host over the prairie restricted *V. pedatifida* may be advantageous to *S. idalia* populations, especially in light of predictions of climate change and the continued loss of prairie communities. Considering the potentially positive implications of host range expansion to conservation efforts, we recommend the documentation and study of the host plant species' used by *S. idalia* larvae across its geographic distribution. The distinctive feeding sign exhibited by late-instar *S. idalia* larvae that we observed may provide a unique tool toward facilitating further field research on this rare species' cryptic immature stages. Given current conservation concerns for *S. idalia* populations, the timely information we provide offers knowledge of this species ecology that can enhance further research and conservation management efforts directed towards this imperiled butterfly.

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PERFORMANCE OF EARLY INSTAR MONARCH BUTTERFLIES (*DANAUS PLEXIPPUS* L.) ON NINE MILKWEED SPECIES NATIVE TO IOWA

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ABSTRACT. Over the past two decades, the population of monarch butterflies east of the Rocky Mountains has experienced a significant decline. Habitat restoration that includes milkweed plants is crucial to boost population numbers in the breeding range. Monarch butterfly larvae use milkweeds as their only host plant, but larval performance on different milkweed species is not well documented. We examined early instar survival and growth on nine milkweed species native to Iowa. These included *Asclepias exaltata* (poke milkweed), *A. hirtella* (tall green milkweed), *A. incarnata* (swamp milkweed), *A. speciosa* (showy milkweed), *A. sullivantii* (prairie milkweed), *A. syriaca* (common milkweed), *A. tuberosa* (butterfly milkweed), *A. verticillata* (whorled milkweed), and *Cynanchum laeve* (honey vine milkweed). In laboratory and greenhouse experiments, larval survival on all nine milkweed species did not differ. Larvae that fed on *C. laeve* plants were an instar behind larvae that fed on any other species, while larvae that fed on *A. verticillata* weighed more than larvae that fed on any other species. Our results show that early instar larvae can survive on all nine milkweed species.

Additional key words: monarch butterfly; milkweed; conservation; larval feeding

Over the last two decades, the populations of monarch butterflies (*Danaus plexippus* L., Nymphalidae) east and west of the Rocky Mountains have experienced a significant decline in overwintering numbers (Brower et al. 2012, Espeset et al. 2016, Inamine et al. 2016). Although recent work has suggested that these declines may not be representative of monarch population size during other stages of monarch phenology or ontogeny (Davis 2012, Davis & Dyer 2015), this decline has been attributed to the loss of milkweed in agricultural fields resulting from the rise of genetically modified crops, increased agricultural herbicide spraying (Oberhauser et al. 2001, Pleasants & Oberhauser 2013), and potentially limited nectar resources (Inamine et al. 2016) as well as the loss of overwintering habitat (The Center for Biological Diversity 2014). Recent models have implicated the loss of milkweeds within the breeding range as the largest threat to the monarch population (Zalucki & Lammers 2010, Flockhart et al. 2015, Zalucki et al. 2016). Monarchs require milkweed species as larval host plants, but apparently feed indiscriminately on nectar from a variety of plants as adults (Brower et al. 2006). Restoration of monarch habitat within the breeding range is of utmost concern to boost population numbers (Oberhauser et al. 2016); roughly 29 milkweed plants will be needed to produce one adult monarch that will be part of the migratory generation (Nail et al. 2015). For that reason, there have been extensive efforts across federal, state, and non-profit groups to establish monarch habitat to boost monarch numbers. These restoration projects have focused on adding milkweeds to the landscape. Most monarchs found at the overwintering sites have originated in the Midwest (Wassenaar & Hobson 1998, Flockhart et al. 2017) and fed on common milkweed, *Asclepias syriaca* (Asclepiadaceae), as larvae (Seiber et al. 1986, Malcolm et al. 1989). However, there are a number of milkweed species in the Midwest that were probably used by monarchs before agriculture dominated the landscape and increased the abundance of common milkweed. These other milkweed species could potentially provide important resources, but more information is needed about monarch larval performance on these milkweed species to ensure the most efficient and effective use of resources.

Since the advent of agriculture, milkweeds that grew in-between crop rows in the Midwest (*A. syriaca*) were among the most heavily used monarch host plants in the North American breeding range (Oberhauser 2001, Pleasants & Oberhauser 2013). Virtually all restoration recommendations to date are based on *A. syriaca*, whereas the historic Midwestern grassland and wetland habitats contained several (2–4) milkweed species (Hayden 1919, Pleasants 2015). There are surprisingly few studies that address larval survival on milkweed species with overlapping ranges. Of the studies comparing larval feeding on milkweed species in North America that do exist, Erickson (1973) measured larval performance and nutrition on four milkweed species, while Schroeder (1976) evaluated an energy budget for larvae that fed on *A. syriaca*. Ladner and Altizer (2005) examined growth differences between monarchs collected from eastern and western North America on widely distributed milkweed species; Yeargan and Allard (2005) examined growth differences of larvae that fed on *A. syriaca* and *Cynanchum laeve*; Zalucki et al. (2012) studied the survival and growth of first instars on milkweeds in southern California; Robertson et al. (2015) focused on larval preference among four desert milkweeds native to California; and Agrawal et al. (2015) compared larval performance on a broad range of milkweed species, some of which were native to North America, to determine the impacts of evolutionary history and latex on milkweed defenses and monarch growth. Because most milkweeds native to the Midwest, especially those with narrow ranges, have not been tested, we examined larval survival on nine milkweed species native to Iowa, which is a high priority area for Midwestern conservation efforts (The Center for Biological Diversity 2014). The species we examined are: *A. syriaca, A. incarnata, A. tuberosa, A. verticillata, A. speciosa, A. exaltata, A. sullivantii, A. hirtella*, and *C. laeve*. These species have overlapping ranges (Woodson 1954), varying concentrations of both cardenolides (Woodson 1954, Roeske et al. 1976, Malcolm 1991, Agrawal et al. 2009, Rasmann & Agrawal 2011, Table 1) and quercetin glycosides (Haribal & Renwick 1996, Agrawal et al. 2009), and different habitat requirements (Woodson 1954, Kaul et al. 1991, Eilers & Roosa 1994, Table 2). We examined larval performance on excised leaves and whole plants of the nine species listed above. An investigation of larval performance on excised leaves separates differences in intrinsic leaf qualities, such as cardenolide content, from the latex found in intact plants, while the data from intact plants addresses latex and overall plant architecture as additional factors in larval performance. Understanding larval performance on each of these milkweed species will be useful in choosing milkweed species for monarch habitat restoration efforts across the Midwestern U.S.

METHODS

Monarch larva used in experiments. A monarch butterfly colony was started by collecting 253 monarch eggs and young larvae on *A. syriaca* and *A. incarnata* plants from May 21 to June 9, 2014 from Boone and Story Counties in Iowa. Larvae were reared on *A. syriaca* through the summer growing season in 2014 and *A. curassavica*, a tropical milkweed, from greenhouse-grown plants through the fall and winter of 2014. Adults were allowed to mate and eggs were collected for propagation of the colony on a weekly

TABLE 1. Cardenolide and quercetin glycoside concentration of nine native milkweeds. Chemical concentrations from Woodson (1954), Roeske et al. (1976), Agrawal et al. (2009), and Rasmann & Agrawal (2011)

Milkweed Species	Cardenolides (mg/gram dried leaves) Woodson (1954)	Cardenolides (mg/gram dried leaves) Roeske et al. (1976)	Cardenolides (% Dry Mass) Agrawal et al. (2009)	Shoot Cardenolides (µg/mg) Rasmann and Agrawal (2011)
A. exaltata	0–0.70	0–0.70	.125	0.735
A. hirtella	n/a	n/a	.208	3.289
A. incarnata	0–0.28	0–0.28	.117	0.511
A. speciosa	0.149	0.15	.227	1.112
A. sullivantii	n/a	n/a	.123	2.149
A. syriaca	0.06–2.64	0.06-2.64	.113	1.573
A. tuberosa	0–0.06	n/a	.064	0.070
A. verticillata	0	n/a	.114	0.031
C. laeve	n/a	n/a	n/a	n/a

basis. Twelve generations of colony breeding preceded the beginning of this experiment (Summer 2014–Spring 2015). All of the resulting larvae from colony matings were reared on *A. curassavica* prior to the beginning of this experiment in late spring 2015. Although the colony was exposed to *A. syriaca* in generations prior to this experiment, we do not think that the colony adapted to a particular host plant because monarchs collected from opposite coasts of the U.S. showed no host preference for milkweeds based on geographic location after colony breeding (Ladner & Altizer 2005).

Excised leaf feeding assay. Milkweeds of all nine species were grown from seed without the use of chemical pesticides in a greenhouse at Iowa State University (21.1–35 °C, 16h photophase, and 56% relative humidity (rh)). During each trial, blocks of petri dishes were set up where each block contained 9 petri dishes, with one replicate of each milkweed species and one larva per petri dish. There were six sets of six blocks throughout this assay. For each group of six blocks, plants of each species were randomly selected, stems were cut, leaves were taken above the cotyledon leaf, and the leaves were immediately placed in water. Leaf material was kept cool and transported to the laboratory in wet paper towels, surface sterilized in 10% bleach (sodium hypochlorite) solution for 10 min., and then rinsed 3 times for 10 minutes (30 minutes total) each with cool running water in order to remove potential pathogens, such as OE. Petri dishes (60 mm × 15 mm) were prepared with water-based agar (2.0% w/v agar to water) to keep plant material moist.

Plant species were randomly assigned within a block (each trial= 6 blocks; 6 trials were included for n=36 total blocks). Plant material was placed into each block of petri dishes and one first instar was added to each petri dish. Larvae were kept on trays in an incubator set at 28°C and 40% RH with a 16:8 hr. photophase. Larvae were monitored daily for survival and surface-sterilized leaf material was provided ad libitum; all leaf material was replaced daily. After five days, larvae were removed from the petri dishes. By conducting this assay over a short five-day period, we were able to avoid large reductions in sample size associated with early instar mortality on some host plants (Hódar et al. 2002). We harvested all larvae after five days throughout the study in order to compare the mass gain and developmental stage for each larva over a fixed amount of time (Agrawal et al. 2015). Larval mass was recorded to the nearest hundredth of a milligram (AND Model GR-202). Head capsule width was measured using a Nikon SMZ 1000 microscope (0.75 × objective, 10× eyepiece with eyepiece grid set with a stage picrometer) and was recorded to the nearest tenth of a millimeter. Instar was determined from head capsule measurements (Oberhauser and Kuda 1997). All larvae were frozen (-28°C) immediately after weighing.

Whole plant feeding assay. Milkweeds of all nine species were grown from seed without the use of chemical pesticides in a greenhouse (21.1–35 °C, 16h photophase, and 56%rh) at Iowa State University. Seeds were sown into 128-cell plug trays (Landmark Plastics, Akron OH) and then at approx. 6 weeks from germination were transplanted into 3.5 inch square deep perennial pots (Kord, Ontario Canada). Plants ranged from 10–30cm in height depending on milkweed species; milkweeds were 8 weeks old when used in each trial. Each plant was watered and placed into a water-filled waxed-paper cup. One neonate was added to each plant. A mesh pop-up hamper cage (57× 37× 55 cm) was placed over the plant and neonate; a no-see-um netting bag was pulled up over the mesh cage and tied on the top with a wire tie. A block in this case included one whole plant of each of the 9 species growing in the pop-up cage. The total number of blocks was 6 per trial, 36 blocks total.

All blocks were kept on the same bench in the greenhouse (21.1–35 °C, 16h photophase, and 56%rh) positioned in a randomized complete block design (6 groups of 6 blocks as in the excised feeding assay). Greenhouse temperature was recorded hourly via Thermocron sensors (iButton, New South Wales Australia). Larval weight (mg), survivorship, and head capsule width (mm) were recorded after 5 days.

Lipid assay. Lipid content was quantified for larvae used in the excised leaf feeding and larvae used in the whole plant feeding assay. Lipid content was quantified using whole bodies of individual larvae that were 5 days old, a mixture of 2nd and 3rd instars, via colorimetric assays with a sulphophosphovanillin reagent, a method that has been demonstrated to provide consistent results for honey bees (Toth et al. 2005, Toth & Robinson 2005). We homogenized whole caterpillars (n=6 per milkweed species for both the excised leaf feeding assay and the whole plant feeding assay, for a total of 108 larvae analyzed) in 2:1 chloroform: methanol solvent in 12 mL glass vials using glass stirring rods to crush each individual. Samples were then left undisturbed for 17 hours to allow the lipids to be extracted into chloroform methanol. After 17 hours, samples were strained through glass wool to remove particulates and leave only lipids dissolved in chloroform methanol. Extracted lipids were then stored in 1mL of 2:1 chloroform: methanol at -20C. One hundred µL of lipid extract was used in each assay. Each sample was dried completely under a stream of air, then 200 µL of 100% sulfuric acid were added, and all samples were heated for 10 minutes in a bath of boiling water. Two ml of a sulfophosphovanillin reagent were added to each sample (Toth et al. 2005). Samples were then briefly vortexed and placed in the dark for 15 minutes to allow the reaction to proceed. Three technical replicates of 200 ul of the resulting solution from each sample were measured for absorbance in a Gen5 2.06 multiwell spectrophotometer at 525 nm. The average of the three replicates was used to estimate lipid quantity by treatment. Estimated quantities of lipids were calculated from standard curves, run alongside the samples, based on known concentrations of cholesterol in petroleum ether (Toth & Robinson 2005, Toth et al. 2009).

Statistical analysis. Data were analyzed using R version 3.1.2 (R Core Team 2014). Data were combined across trials (36 blocks total) within each experiment, as blocks were not significantly different from one another. Differences in survival were determined using a log rank test on the Kaplan-Meier survival estimates for larvae fed each milkweed species. A one-way ANOVA was used to assess differences in larval mass and head capsule width between groups relative to the milkweed species they were fed in both excised feeding and whole plant experiments. A Tukey HSD test was used to assess pairwise differences in larval responses among milkweed species. A linear regression was used to assess the relationship between larval mass and cardenolide content, reported in Agrawal et al. 2009, in the excised feeding assay. Mass and head capsule width were not transformed prior to analysis. A one-way ANOVA was used to assess differences in total percent of lipids between groups relative to the milkweed species they were fed in both excised feeding and whole plant experiments. A Tukey HSD test was used to assess pairwise differences in larval lipid percentages.

RESULTS

Excised leaf feeding assay. Larval survivorship varied from 94–100% across milkweed species, averaging 96% across treatments. Survivorship did not differ among milkweed species ($\chi2=9.8$, d.f. $=8$, $p<0.05$). Larval mass was significantly different among milkweed species ($F=11.65$, d.f. $=8$, $p<0.001$). Larvae that fed on *C. laeve* weighed significantly less that those that fed on *A. incarnata* ($p<0.01$), *A. tuberosa* ($p<0.01$), and *A. verticillata* ($p<0.01$; Figure 1). Larvae that fed on *A. hirtella* weighed significantly less than those that fed on *C. laeve* ($p<0.05$), *A. incarnata* ($p<0.001$), *A. speciosa* ($p<0.01$), *A. sullivantii* ($p<0.001$), *A. syriaca* ($p<0.001$), *A. tuberosa* ($p<0.001$), and *A. verticillata* ($p<0.001$; Figure 1). Larvae that fed on *A. exaltata* weighed significantly less than those that fed on *A. incarnata* ($p<0.001$), *A. tuberosa* ($p<0.001$), and *A. verticillata* ($p<0.001$; Figure 1).

Larval head capsule width was significantly different among milkweed species ($F= 2.56$, d.f. $=8$, $p<0.01$) when all instars were pooled; head capsule width was positively correlated with larval weight. This relationship was significant ($r=0.71$; $p<0.001$). Larvae that fed on *A. incarnata* developed to 4th instars and had the largest head capsule width. Larvae that fed on *A. hirtella* developed to 3rd instars and had a head capsule width that was significantly smaller than those fed on *A. incarnata* ($p<0.05$) or *A. verticillata* ($p<0.05$; Figure 2). All other comparisons were not significantly different.

Whole plant feeding assay. Larval survivorship varied from 81–100% across milkweed species, averaging 90% across treatments. Survivorship did not differ among milkweed species ($\chi2=11.4$, d.f. $=8$, $p>0.05$). Larval mass was significantly different among species ($F=6.956$, d.f. $=8$, $p<0.001$; Figure 3). Larvae fed *A. verticillata* weighed more than larvae fed any other species (Figure 4) and were significantly different from *C. laeve* ($p<0.001$), *A. incarnata* ($p<0.01$), A. speciosa ($p<0.05$), A. sullivantii ($p<0.01$), or A. tuberosa ($p<0.001$). Larvae that fed on *C. laeve* weighed the least. This difference was significant in comparison to *A. hirtella* ($p<0.001$), *A. exaltata* ($p<0.05$), *A. speciosa* ($p<0.05$), *A. sullivantii* ($p<0.05$), *A. syriaca* ($p<0.05$), and *A. verticillata* ($p<0.001$). No other species showed differences in pairwise comparisons.

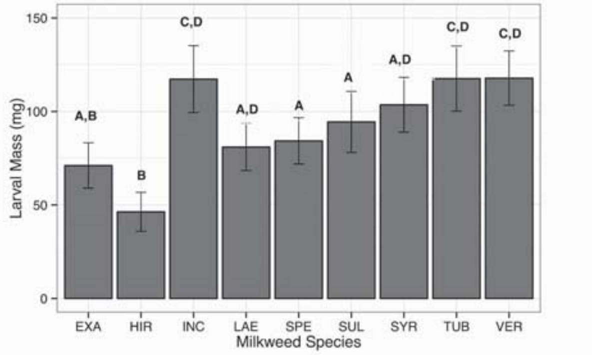

FIG. 1. Differences in mass (mg) among larvae fed excised leaves of nine native milkweed species. This graph represents 6 trials (36 blocks, 315 larvae total). Each bar represents one milkweed species; error bars depict 95% confidence intervals. EXA= *A. exaltata* (n=34 larvae), HIR= *A. hirtella* (n=34 larvae), INC= *A. incarnata* (n=35 larvae), LAE= *C. laeve* (n=36 larvae), SPE= *A. speciosa* (n=34 larvae), SUL= *A. sullivantii* (n=35 larvae), SYR= *A. syriaca* (n=35 larvae), TUB=*A. tuberosa* (n=36 larvae), and VER= *A. verticillata* (n=36). Bars that share a letter are not significantly different from each other at p<0.05.

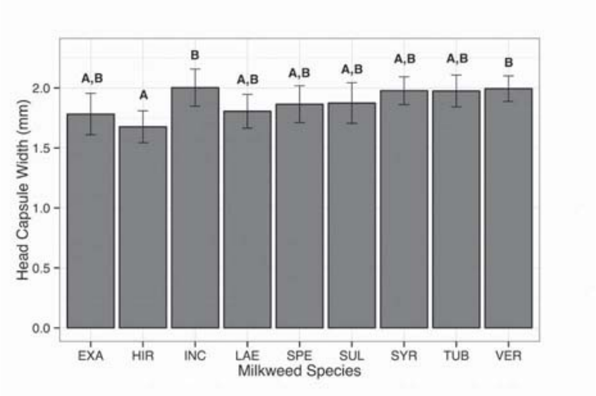

FIG. 2. Differences in head capsule width (mm) among larvae fed excised leaves of nine native milkweed species. This graph represents 6 trials (36 blocks, 315 larvae total). Each bar represents one milkweed species; error bars depict 95% confidence intervals. EXA= *A. exaltata* (n=34 larvae), HIR= *A. hirtella* (n=34 larvae), INC= *A. incarnata* (n=35 larvae), LAE= *C. laeve* (n=36 larvae), SPE= *A. speciosa* (n=34 larvae), SUL= *A. sullivantii* (n=35 larvae), SYR= *A. syriaca* (n=35 larvae), TUB=*A. tuberosa* (n=36 larvae), and VER= *A. verticillata* (n=36 larvae). Bars that share a letter are not significantly different from each other at p<0.05.

Larval head capsule width was significantly different among milkweed species (F=17.25, d.f. =8, p<0.001); head capsule width was positively correlated with larval weight. This relationship was significant (r=0.54; p<0.001). All larvae reached the third instar during the study, with the exception of those fed *C. laeve*. Larvae that fed on *C. laeve* did not reach the third instar. Larvae fed *C. laeve* had a significantly smaller head capsule width in comparison with each of the other 8 milkweed species (p<0.001 for all species). No other species showed differences in pairwise comparisons.

Lipid assay. During excised leaf feeding trials, lipid concentration (lipids as a percentage of total larval mass) was not significantly different among caterpillars that fed on nine different milkweed species (F=0.475, d.f. =8, p>0.05). However, the percent lipid was different among larvae that fed on different species of milkweed plants in the whole-plant assay (F=3.707, d.f. =8, p<0.01). Larvae that fed on *A. incarnata* had a higher percentage of lipids than larvae that fed on *A. exaltata* (p<0.01), *A. hirtella* (p<0.05), *A. sullivantii* (p <0.05), *A. syriaca* (p<0.05), *A. tuberosa* (p<0.05), or *A. verticillata* (p<0.001). All other comparisons were not significantly different.

DISCUSSION

Our findings suggest that young monarch larvae can survive on all nine milkweed species. Eight of the nine species could be used for monarch habitat restoration in the Midwest, provided that each species is planted within its native range and in its appropriate habitat (Table 2). *C. laeve* is not the best choice for such plantings because larvae did not grow as quickly when they fed on this species.

Larvae that fed on excised leaves reached the fourth instar in five days, while larvae that fed on whole plants only reached the third instar in five days in the greenhouse. On average, larval mass after 5 days for larvae that fed on whole plants was 33.4% that of larvae fed on excised leaves. Differences in instar and larval mass are likely due in part to differing temperatures between excised leaf and whole plant experiments. Larvae fed leaf material in petri dishes in the laboratory experienced a stable temperature of 28°C in the controlled environmental chamber while those that fed on whole plants experienced fluctuating temperatures from 23°C to 28°C in the greenhouse. Given that larval growth rates are dependent on temperature (Zalucki & Kitching 1982), the lower temperature in the greenhouse probably resulted in less rapid growth during the whole-plant feeding assay. Larvae that fed on excised leaves also were not exposed to plant latex flow and pressure, which can slow larval growth by up to 50%; larvae in petri dishes also moved less due to a confined space and did not need to negotiate the architecture of the plants (Zalucki & Malcolm 1999, Zalucki et al. 2001a). Larval mortality was minimal throughout the study (96.6% survival excised leaf feeding; 90.4% survival plant feeding), well below ~50% reported elsewhere regardless of whether larvae

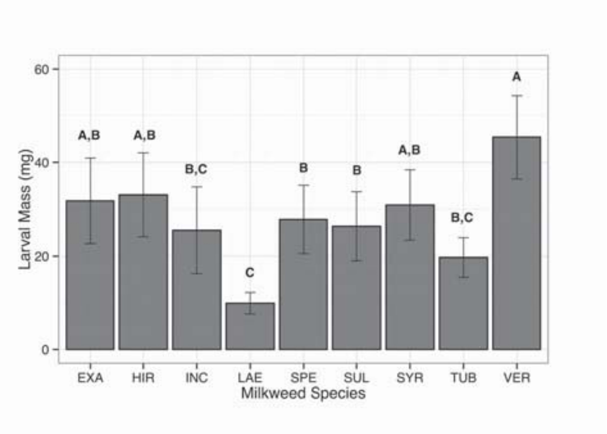

FIG. 3. Differences in mass among larvae fed whole plants of nine native milkweed species. This graph represents 6 trials (36 blocks, 294 larvae total). Each bar represents one milkweed species; error bars depict 95% confidence intervals. EXA= *A. exaltata* (n=31 larvae), HIR= *A. hirtella* (n=32 larvae), INC= *A. incarnata* (n=31 larvae), LAE= *C. laeve* (n=31 larvae), SPE= *A. speciosa* (n=31 larvae), SUL= *A. sullivantii* (n=31 larvae), SYR= *A. syriaca* (n=36 larvae), TUB=*A. tuberosa* (n=34 larvae), and VER= *A. verticillata* (n=36 larvae). Bars that share a letter are not significantly different from each other at p<0.05.

Table 2. Summary of milkweed habitat preferences. Information compiled from Woodson (1954), Kaul et al. (1991), Eilers & Roosa (1994), and USDA-NRCS (2017).

Milkweed Species	Common Name	Habitat Preference	Blooming Period	Soil Moisture	Soil Type	Iowa Distribution
Asclepias exaltata	Poke Milkweed	Woodland edges, upland woods	May–August	Moist	N/A	Northeastern Iowa
Asclepias hirtella	Tall Green Milkweed	Prairie remnants, fields	May–September	Mesic to Dry	Sandy and clayey soils	South Central Iowa
Asclepias incarnata	Swamp Milkweed; Rose Milkweed	Wet meadows, floodplains, riverbanks, pond shores stream banks, wet woods, swamps, marshes, along canal banks, and riparian sites	May–August	Moist to Mesic	Clayey soils, neutral to slightly acidic pH	Entire State
Asclepias speciosa	Showy Milkweed	Pastures, meadows, forest clearings, untilled fields, roadsides, and ditch banks	May–September	Moist to Dry	Sandy, well-drained soils, neutral to slightly acidic pH	Western Iowa
Asclepias sullivantii	Prairie Milkweed	Mesic prairie, alluvial meadows, floodplains, and level roadsides	June–August	Moist to Mesic	Low, moist soils	Central and Western Iowa
Asclepias syriaca	Common Milkweed	Banks or floodplains of lakes, ponds, waterways, prairies, forest margins, roadsides and waste places	June–August	Moist to Dry	Sandy, clayey, or rocky calcareous soils	Entire State
Asclepias tuberosa	Butterfly Milkweed	Prairies, open woodlands, roadsides, and disturbed areas	April–September	Mesic to Dry	Sandy, loamy, or rocky limestone soil	Entire State
Asclepias verticillata	Whorled Milkweed	Prairies, open woodlands, roadsides, and disturbed areas	April–August	Mesic to Dry	Sandy soils	Entire State
Cynanchum laeve	Honeyvine Milkweed	Alluvial woods, cities, waste areas, disturbed areas	July-October	Moist to Mesic	Sandy soils	Southwestern Iowa

fed on excised leaves or whole plants (Oberhauser & Solensky 2004).

Unlike Ladner and Altizer (2005), we found no difference in larval mass or instar size between larvae fed *A. incarnata* and *A. syriaca* (Figures 1 and 3). However, it is possible that differences in larval growth among milkweed plants may be more pronounced during the final instars. We did see evidence, as they did, that *A. speciosa* may produce lighter larvae, but only when larvae fed on excised leaves (Figure 1). This could suggest that young larvae have difficulty processing milkweed leaves with higher cardenolide content, as *A. speciosa* tends to have higher foliar cardenolides compared to some of the other milkweed species (Table 1; Woodson 1954, Roeske et al. 1976, Agrawal et al. 2009, Rasmann & Agrawal 2011) or that these leaves are structurally difficult to eat. We also saw evidence that *A. hirtella* leaves produced lighter larvae than other species (Figure 1), but this could be the result of wilting of the excised leaves during larval feeding or larval difficulty processing leaf material with a high cardenolide content (Table 1; Agrawal et al. 2009, Rasmann & Agrawal 2011). Larvae that fed on *A. hirtella* plants were not significantly lighter than larvae that fed on other species (Figure 3).

Unlike Yeargan and Allard (2005), larvae reared on *C. laeve* plants were significantly smaller and did not grow as quickly as larvae fed other species; larvae fed *C. laeve* did not reach the third instar during the whole plant assay in our study. Our results suggest that larvae can survive on *C. laeve*, but those larvae may not mature as quickly as larvae feeding on other milkweeds. Larvae that fed on *A. verticillata*, a milkweed species that tends to have low cardenolide levels (Figures 1 and 3, Table 1), produced the heaviest larvae. Although we did not measure cardenolide content in our milkweed plants, *A. speciosa* and *A. hirtella* have higher average foliar cardenolides when compared to other milkweed species (Table 1,Woodson 1954, Roeske et al. 1976, Agrawal et al. 2009, Rasmann & Agrawal 2011). Cardenolide content is only one factor that could contribute to the variation in larval mass that we observed. Other factors such as differing latex content and flow, differing amounts of larval movement on various milkweed species, and differing plant architecture among milkweed species also likely contributed to the observed differences in larval mass (Zalucki et al. 2001a,b).

Like Cookman et al. (1984), we observed differences in lipid concentration among larvae reared on different host plants. However, in our study larvae that fed on excised leaves did not show a difference in lipid concentration. Our results suggest that *A. incarnata* may be a more lipid-rich food source for monarch larvae, and that other milkweed species may not be as good a food source for lipid content. Alternatively, monarchs may be able to process toxins from *A. incarnata* more effectively, leading to higher lipid storage (Roeske et al. 1976).

In summary, all nine milkweed species can be used as host plants by the monarch butterfly. Larvae that fed on excised leaves at a controlled temperature weighed more and matured faster than those raised on whole plants in a greenhouse with more variable temperature. Larvae that fed on *A. incarnata* and *A. verticillata* weighed the most, while those that fed on *C. laeve* weighed the least. This is an important finding because milkweeds are needed to boost monarch numbers during the breeding season in the Midwestern U.S (Pleasants & Oberhauser 2013, Flockhart et al. 2015).

Although larvae that fed on *A. incarnata* and *A. verticillata* weighed the most, monarch habitat should include milkweed species with habitat needs that best match the potential restoration site (Table 2). *A. syriaca*, *A. incarnata*, and *A. verticillata* are found across the entirety of Iowa, but *A. syriaca* and *A. verticillata* are found in drier locations than *A. incarnata* (Woodson 1954, Eilers & Roosa 1994). *A. incarnata* is found in wet areas, especially near wetlands and along waterways (Woodson 1954, Kaul et al. 1991, Eilers & Roosa 1994, USDA-NRCS 2017). *A. exaltata* is found in northeastern Iowa in upland woods and along forest edges (Eilers & Roosa 1994). *A. tuberosa* is commonly found in prairie remnants across Iowa, while *A. hirtella* is restricted to mesic remnants in southern Iowa (Eilers & Roosa 1994, USDA-NRCS 2017). *A. speciosa* is found in the western half of Iowa in woodland openings, prairies, and roadside ditches (Woodson 1954, Kaul et al. 1991, Eilers & Roosa 1994, USDA-NRCS 2017). *A. sullivantii* is rare across Iowa, but can be found in mesic prairies and roadsides in mesic soil (Woodson 1954, Eilers & Roosa 1994, USDA-NRCS 2017). *C. laeve* occurs frequently in southwestern Iowa in moist, sandy soils (Woodson 1954, Eilers & Roosa 1994, USDA-NRCS 2017).

In order to provide a complete assessment of the value of different milkweed species, we need to examine adult female egg load and potential fecundity for individuals that have fed on different milkweed species from first instar through adult eclosion. These feeding trails should use mature milkweed plants. We also need to understand the oviposition response and preference of female monarchs for different milkweed species to gauge their potential value in habitat restoration.

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THE LEPIDOPTERAN HERBIVORES OF THE MODEL PLANT *MIMULUS GUTTATUS*

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ABSTRACT. *Mimulus guttatus* is a widespread riparian plant native to western North America. Due to its wide distribution and phenotypic variation it has been the subject of many studies incorporating genetic information into ecological and life history evolution studies. *Mimulus guttatus* defends itself from herbivory with physical defenses (e.g. trichomes) as well as a suite of phytochemical defenses; phenylpropanoid glycosides (PPGs). Despite its importance as a model organism little has been done on the herbivores that feed on this plant. We used literature records as well as field observations to construct a list of the Lepidoptera that utilize *M. guttatus* as a food plant. Sixteen species of Lepidoptera within five families were recorded as feeding on *M. guttatus*. Six of these species are classified as generalist herbivores, while the rest have varying degrees of host plant specialization.

Additional keywords: *Mimulus guttatus*, phenylpropanoid glycosides, specialization, host plant, herbivore

Mimulus guttatus D.C. (syn. *Erythranthe guttata* (Fisch. ex DC.) G.L.Nesom, Family Phrymacea) is a plant species complex native to riparian areas of western North America. This species is an important model organism for studies of evolutionary genomics and ecology having been cited in over 1,000 studies. This use is in part due to its tremendous phenotypic variation, including variation in life history (annual vs. perennial; Hall & Willis 2006), leaf morphology (Wu et al. 2010), and anti-herbivore defenses (Holeski 2007a, Holeski et al. 2013). In addition to its large native range, non-native populations of *M. guttatus* occur in eastern North America, Europe, and New Zealand (Stace 2010, Webb et al. 1988).

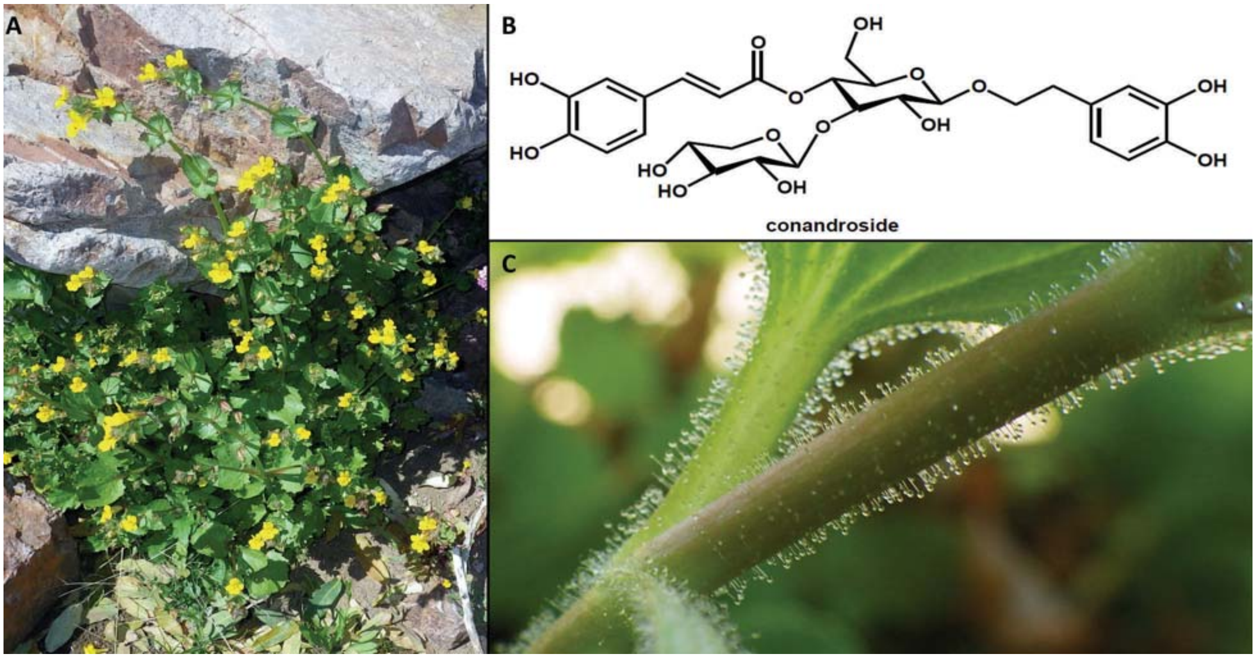

FIG. 1. **A.** *Mimulus guttatus* in a typical habit near flowing water, **B.** Chemical structure of Conandroside, a phenylpropanoid glycoside (PPG) produced by *M. guttatus* that acts as a chemical defense. **C.** glandular *M.guttatus* trichomes, these act as defense from herbivores. Photos by M. Rotter.

Mimulus guttatus possesses both physical and chemical anti-herbivore defenses (Fig. 1). Physical defenses include trichomes. Although trichomes can serve as both a defense against herbivores and in physiological and abiotic interactions, evidence suggests that they function largely as an anti-herbivore defense within perennial and/or coastal *M. guttatus* populations (Holeski et al. 2010). Many trichomes produced by the species are glandular with sticky secretions (Holeski 2007a). *Mimulus guttatus* also produces a suite of foliar secondary compounds, phenylpropanoid glycosides (PPGs). Phenylpropanoid glycosides have been shown to deter feeding by generalist herbivores (Cooper et al. 1980, Mølgaard 1986) while specialist lepidopteran

FIG. 2. Damage to *Mimulus guttatus* from *Junonia coenia* (Maricopa Co. AZ). Photo by M. Rotter.

herbivores may use at least some PPGs as feeding stimulants (Holeski et al. 2013). Many plant species within the Schrophulariacea sensu lato (where Phrymacea was once included) contain PPGs (Mølgaard & Ravn 1988). This shared phytochemistry leads to many of the same specialist herbivores feeding from plants across the Schrophulariacea sensu lato (Bowers 1988). Despite its defenses, herbivory on *M. guttatus* can still be very damaging to plants (Fig. 2).

Lepidoptera have long been an important order in studies of the co-evolution of plants and their insect herbivores (Ehlrich & Raven 1964). The "arms race" between insect herbivores and plants has driven evolution of novel secondary compounds, as well as the means to sequester or detoxify these compounds within many groups of plants and insects, often leading to evolutionary radiations (Cornell & Hawkins 2003, Marquis et al. 2016). For instance, rapid radiation within the Pieridae butterfly family following an adaptive radiation event in its host plant order Brassicales was most likely due to Pieridae specialization on glucosinolates in Brassicales (Braby & Trueman 2006). A clear picture of the evolution within the Lepidoptera thus necessitates a thorough knowledge of host plant use as well as the host plant range of individual species of Lepidoptera. This review uses literature references as well as our own field observations and rearing records to record the diversity of Lepidoptera that feed on *M. guttatus*. We also discuss the ecological context and chemical ecology of these interactions.

METHODS

To locate *M. guttatus* host records for Lepidoptera we consulted field guides with host records, species catalogues, taxonomic treatments of specific groups, and natural history reports. We checked state and regional field guides from countries where *M. guttatus* is present in order to find species that may use *M. guttatus* only locally. This literature search was combined with over three years (2014-2016) of observational records of Lepidoptera feeding on *Mimulus guttatus*. Over 60 populations of *M. guttatus* throughout its range in western North America were searched for caterpillars. These searches included both visual and sweep net surveys. To be included in our list of species that utilize *M. guttatus* as a host, a caterpillar had to be either observed actively feeding on the plant in the field or was collected from the plant and successfully reared to adulthood on a diet of *M. guttatus* in the lab. To classify the degree of host plant specialization for each Lepidopteran, we recorded a representative list of other species of plants that each Lepidoptera species is known to feed upon

RESULTS AND DISCUSSION

We found 16 species of Lepidoptera within five families that utilize *M. guttatus* as a food plant (Tables 1 & 2). Eight of these species were not previously recorded to feed on *M. guttatus* (Table 2). Of the 16 total species, six are broad generalists while four would be considered specialists on plants in the same clade of families as *M. guttatus* (Scrophulariacea sensu lato). One species has been recorded feeding on other plants within the Lamiaceae (*Stachys* spp.), and for two of the species the only known food plant is *M. guttatus*.

Generalist Lepidoptera found feeding on *Mimulus guttatus.*

The six species of generalist herbivores known to feed on *M. guttatus* are all species with large geographic ranges. For example, *Amphipyra tragopoginis* (Clerck, 1759) (Noctuidae) is a common moth throughout the Holarctic region and can be found throughout the northern portions of the native *M. guttatus* range, although the moth itself may have been introduced to North America (Forbes 1954). *Trichoplusia ni* (Hübner, [1803]) (Noctuidae) is another widespread generalist, with a geographic range covering most of North America, which has been found feeding on *M. guttatus*. We collected this species feeding on multiple *M. guttatus* populations from the northern Cascades to the southern Sierra Nevada Mountains. *Trichoplusia ni* has predilections for members of the Brassicacea but will often feed on any available forb (Powell & Opler 2009;

FIG. 3. **A.** *Herreshoffia gracea* caterpillar from Gila County Arizona. **B.** Freshly emerged adult. Photo by M. Rotter.

FIG. 4. **A.** *Autographa pasiphaeia* caterpillar from Kern county California. **B.** Freshly emerged adult. Photo by M. Rott

Wagner et al. 2011). *Mimulus guttatus* often grows with members of Brassicacea particularly the non-native water cress (*Nasturtium officinale*) (Rotter unpublished data), to which *T. ni* may be initially attracted to and then utilize other plants in the community.

Two generalists that may sequester plant secondary compounds were recorded feeding on *M. guttatus*. The species *Estigmene acrea* (Drury, 1773) (Eribidae) is a widespread generalist throughout North America, and is a pest of many cultivated crops. Late instar caterpillars were collected feeding on *M. guttatus* near Mormon Lake in central Coconino County, Arizona. *Estigmene acrea* was locally abundant at the time of collection (a likely outbreak year in northern Arizona). Although overall a generalist, *E. acrea* does exhibit host plant preferences at different times of its life cycle (Casterjon et al. 2006). As a late-instar caterpillar, *E. acrea* prefer plants that can offer protection from parasitoids via caterpillar sequestration of plant metabolites (Singer et al. 2004). *Grammia incurropta* (Edwards, 1881) (Eribidae) is a common lab species for investigating the evolution of generalist diets (Wagner & Conner 2008), which is also known to sequester and favor plant metabolites in order to "medicate" itself (Smilanich et al. 2011). Records of this species feeding on *M. guttatus* in the field are from Arizona (Michael Singer, personal communication), and the species readily consumes *M. guttatus* in the lab.

There were three records of Lepidoptera feeding on *M. guttatus* outside of its native western North American range, with no records of feeding on *M. guttatus* within its native range. Our records may thus corroborate results of several studies showing that generalist native herbivores may prefer non-native plants (Agrawal & Kotanen 2003, Parker & Hey 2004). The literature record of *Amphipyra tragopoginis* feeding on *M. guttatus* comes from the United Kingdom where *M. guttatus* is a widespread non-native plant (Hancock & Wallace 1986). *Nematocampa resistaria* (Herrich-Schäffer, [1856]) (Geometridae) and *Spodoptera ornithogalli* (Guenée, 1852) (Noctuidae) were recorded feeding on an isolated population of *M. guttatus* (over 1000 km from the next known population of *M. guttatus*) in the Upper Peninsula of Michigan (Ontonagon County). Both of these species are common in eastern North America where they feed on a wide variety of plants (Wagner et al. 2011, Wagner 2005).

Specialist Lepidoptera feeding on *Mimulus guttatus*.

Specialist Lepidoptera that feed on Mimulus guttatus also share other host plant species. For instance the two *Junonia* spp. (Nymphalidae) that feed on *M. guttatus*, as

TABLE 1. Literature references of Lepidoptera feeding on *Mimulus guttatus*.

Family	Species	Geographic Range	Feeding habit	Other Host Plants	Reference
Nymphalidae	*Junonia evarete* (Cramer, 1779)	Southern North America	Leaves	Plantaganacea, Acanthaceae	Stewart et al. (2001)
Nymphalidae	*Junonia coenia* Hübner, [1822]	Southern North America	Leaves	Plantaganacea, Acanthaceae, Verbanacea	Scott (1986), Bowers (1986), Rotter Personal Observation
Nymphalidae	*Euphydryas chalcedona* (Doubleday, 1847)	Western North America	Leaves	Orobanchaceae, Plantaginaceae, Caprifoliaceae, Boraginaceae (some), Rosaceae (some)	Scott (1986), Rotter Personal Observation
Nymphalidae	*Phyciodes mylitta* (Edwards, 1861)	Western North America	Leaves	Asteracea (Cynareae)	Scott (1986), Sewart et al. (2001)
Noctuidae	*Amphipyra tragopoginis* (Clerck, 1759)	Holarctic	Flowers	Generalist	Hancock & Wallace(1986)
Noctuidae	*Annaphila lithosina* Edwards, 1875	California, Oregon	Flowers and leaves	None known	Buckett (1966)
Noctuidae	*Annaphila casta* Edwards, 1890	Northern California, Oregon	Leaves	*Mimulus moschatus*	Powell & Opler (2009), Henne (1967)
Pterophoridae	*Amblyptilia pica* (Walsingham, 1880)	Western North America	Flower buds and seed heads	Scrouphulariaceae, Orobanchaceae, Plantaginaceae, Primulacea, Labiataea, Caprifoliaceae	Matthews & Lott (2005), Rotter Personal Observation
Eribidae	*Grammia incurropta* (Edwards, 1881)	Southwest North America	Leaves	Generalist	M. Singer personal communication

well as *Euphydryas chalcedona* (Doubleday, [1847]) (Nymphalidae), feed on plant species within the Scrophulariacea senso lato as well as other related families (e.g., Plantaginacea, Verbenaceae, and Acanthaceae). We hypothesize that this shared specialization on host plants within and related to the Scrophulariacea senso lato is due to overlap in host plant phytochemical profile. For example, the PPG verbascoside has been reported in many plant species in this group (Jimenez & Riguera 1994; Keefover-Ring et al. 2014). Compounds in another phytochemical group, iridoid glycosides, are present in many of these plants (e.g., Plantaginacea and Verbanaceae) but not *M. guttatus* (M.D. Bowers, personal communication).

The host plants utilized by *E. chalcedona* populations vary by region, as is the case with many Nymphalidae within the tribe Melitanea. For example, coastal California populations of *E. chalcedona* often feed primarily on *Diplacus aurantiacus* (Phrymacea) and populations from the Sonoran Desert feed typically on *Keckiella antirrhinoides* (Plantaginacea) (Kuussaari et al 2004, Rotter personal observation). *Euphydryas chalcedona* use of *M. guttatus* in the wild is likely localized to several populations in California and Nevada, but populations found feeding on other species will oviposit and the larva will feed on *M. guttatus* in the lab. Chemical variation between host plant populations as well as environmental and biotic factors have likely led to local adaptation of *E. chalcedona* populations (Bowers 1986).

The genus *Annaphila* (Noctuidae) contains several species that feed on *M. guttatus*. *Annaphila lithosina* Hy. Edwards, 1875 was documented ovipositing on *M. guttatus*, and its larva feed on flowers of the plant

TABLE 2. Field and laboratory observations of Lepidoptera feeding on *Mimulus guttatus*, not previously recorded.

Family	Species	Location	Date	Feeding Habit	Other recorded host plants	Notes
Erebidae	*Grammia incurropta* (Edwards, 1881)	Captivity		Leaves	Generalist	Laboratory record, M. Singer Personal communication
Erebidae	*Estigmene acrea* (Drury, 1773)	Coconino Co. AZ	9.1.2014	Leaves	Generalist	This was a local outbreak year, reared MR 718
Geometridae	*Nematocampa resistaria* (Herrich-Schäffer, [1856])	Ontonogan Co. MI	6.28.2015	Leaves	Woody plants including, Pinaceae, Sapidanariaceae, Rosaceae, Grossulariaceae	This record is from an introduced population of *M. guttatus*
Geometridae	*Herreshoffia gracea* Sperry, 1949	Gila Co. AZ	4.25.2015	Leaves	None Known	First known larval feeding record, MR 715
Noctuidae	*Spodoptera ornithogalli* (Guenée, 1852)	Ontonogan Co. MI	6.28.2014	Leaves and flowers	Generalist	This record is from an introduced population of *M. guttatus*
Noctuidae	*Spodoptera exigua* (Hübner, [1808])	Captivity		Leaves	Generalist	Laboratory record
Noctuidae	*Trichoplusia ni* (Hübner, [1803])	Fresno Co. CA	5.20.2016	Leaves	Generalist	Common on inland populations in the native range
Noctuidae	*Autographa pasiphaeia* (Grote, 1873)	Kern Co. CA	4.13.2015	Flower buds and leaves	*Stachys ajugoides* and *S.rigida*	MR 716

(Buckett 1966). Prior to 1966 the host plant of *A. lithosina* was unknown, and Buckett speculated that *M. guttatus* may be the only host plant for *A. lithosina*. *Annaphila casta* Hy. Edwards, 1890 (which forms a complex with *A. lithosina* and *A. miona* Smith, 1908) was recorded to readily feed on *M. guttatus* in captivity, and oviposits on *M. moschatus* in the wild (Henne 1967). Further study of the relationship of *Annaphila* spp. and *M. guttatus*, particularly regarding how the moths interact with PPGs could help elucidate the natural history of the beautiful but little-studied *Annaphila*.

Other host records on *Mimulus guttatus*.

During the course of this study two particularly interesting records of Lepidoptera feeding on *M. guttatus* were recorded. The first record is the first documented host plant record for *Herreshoffia gracea* Sperry, 1949 (Geometridae) (Fig. 3). This moth was originally described from Oak Creek Canyon, Coconino County, Arizona (Sperry 1949). We collected several late instar larval geometrid species from *Mimulus guttatus* along Fossil Creek, Gila County, Arizona in April of 2015. Both Oak Creek and Fossil Creek are semi-shaded perennial streams running through canyons on the edge of the Mogollon Rim of northern Arizona. We collected mature caterpillars actively feeding on *M. guttatus*; live caterpillars were also taken back to the lab where they were fed until they pupated. One adult was successfully reared (all other pupae had parasitoid wasps which emerged after 18 days in a chrysalis; Specimen MR 715, Northern Arizona University). *Herreshoffia gracea* has been recorded in Coconino, Cochise, and now Gila Counties in Arizona and Siskyou County in northern California (Moth

Photographers Group, 2016); in every recorded county for this moth *M. guttatus* is a common riparian plant. Although it is not known if *H. gracea* utilizes other plants, many other members of the Geometrid tribe Xanthorhioni are limited in their larval diet breadth (Powell & Opler 2009).

We collected late instar caterpillars of *Autographa pasiphaeia* (Grote, 1873) (Noctuidae) (Fig. 4) from *M. guttatus* along the Kern River, Kern County, California. These were reared in the lab and had adults successfully emerge (Specimen MR 716 Northern Arizona University). This species ranges throughout California and into Oregon (Powell & Opler 2008). Within this range the common host plant is *Stachys* spp. (Lamiacea), which shares a common clade with *M. guttatus* within the order Lamiales (Stevens 2001). Several Stachys species contain verbascoside (Jimenez & Riguera 1994), a PPG that is also present in *Mimulus guttatus*.

Suspected hosts on *Mimulus guttatus*.

There are several records of the Nymphalid *Phyciodes mylitta* (W.H. Edwards, 1861) using *M. guttatus* as a host plant. More often the host plants of *P. mylitta* are listed as members of the Asteracea tribe Cynareae (thistle tribe). *Mimulus guttatus* is thus an odd addition to this group. The first record of *P. mylitta* on *M. guttatus* appears in Scott's work on North American butterflies (1986). It appears that this record may simply have been repeated in later citations. However, *P. mylitta* is common in many areas where *M. guttatus* is present, so further investigation may be warranted.

Spodoptera exigua (Hübner) (Noctuidae) is a widespread generalist that can utilize a wider array of plants as hosts. This species was reared in the lab on *M. guttatus* and could likely utilize it in the wild.

CONCLUSION

The Lepidoptera fauna that utilizes *M. guttatus* is composed of specialists that also feed on relatively limited numbers of other, related plant species, as well as multiple broad generalists. Although several species have been recorded to have *M. guttatus* as their sole host, this may merely be due to limited records for these Lepidoptera. We are currently conducting no-choice feeding trials using several of the species listed here to characterize the relationship between *M. guttatus* defense traits and herbivore performance. Understanding how these defense traits influence herbivores of *M. guttatus* as well as a thorough knowledge of the diversity feeding on *M. guttatus* will contribute greatly to understanding the evolutionary and ecological history of this model organism.

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IS MULTIPLE MATING BY FEMALE PROMETHEA MOTHS (*CALLOSAMIA PROMETHEA*) (DRURY) (LEPIDOPTERA: SATURNIIDAE) FERTILITY INSURANCE?

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ABSTRACT. Multiple mating is apparently rare in female saturniids but male and female *Callosamia promethea* (Drury) (promethea moth) mate multiple times. Previous study showed that polyandrous females laid significantly more eggs than monandrous females, suggesting the hypothesis that yoke proteins from male ejaculates enhanced fecundity. However, multiple mating by females could arise if a single mating with a previously-mated male results in low fertility. To test this, I compared females mated once to either a virgin male or a male that had copulated the previous day. No differences were found in fecundity or fertility in females mated to virgin males vs. nonvirgin males, showing that multiple mating by female promethea moths is not related to lower fertility when mating with nonvirgin males. I discuss this finding with respect to known cases of polyandry in saturniids and suggest a hypothesis to explain the evolution of polyandry in this group.

Additional key words: fecundity, diurnal mating, seminal gifts, sexual conflict, polyandry

Multiple mating is common in insects and often illustrates sexual conflict (Arnqvist & Nilsson 2000, Härdling & Kaitala 2005) but is uncommon in saturniids. Thus I was surprised to discover that the mating system of the promethea moth (*Callosamia promethea*) includes both polyandry and polygyny (Morton 2009). All female *Promethea* (384) from northwestern Pennsylvania have called on multiple days following emergence since I began to study them there in 2004. Furthermore, five females that emerged from wild cocoons collected near Front Royal, Virginia, (430 km SSE of the Pennsylvania site) called multiple times. Females were observed to call daily for up to five days when allowed to do so; but I restricted the comparison to single vs. twice-mated females. These observations show polyandry is the normal mating system for *Promethea* females and probably throughout their range.

Comparing fertility and fecundity in females forced to mate monandrously with those allowed to mate twice showed that both achieved similar egg fertility but the polyandrous group laid 10% more eggs, a significant difference ($P< 0.05$). This fecundity difference should be considered the minimal difference because it compares females mated once or twice, not with females mating several times, which is likely the norm. How many matings a female normally has needs study. But the fecundity difference between polyandrous and monandrous females in my study was not due to differences in body size, duration of copulations, size of eggs laid, or number of days in the laying period. This suggested the hypothesis that seminal gifts (LaMunyon 1997, Gwynne 2008), perhaps involving yolk proteins (Telfer & Rutberg 1960), boosted egg production in the polyandrous females (Morton 2009).

But this previous study did not eliminate fertility as a source of selection favoring polyandry. A virgin female moth might mate with a male that has mated previously. Polyandry might evolve to compensate for low fertility in males that have already mated several times (Svärd & Wiklund 1986, Torres-Vila & Jennions 2005, Lauwers & Van Dyck 2006). Mating only with a depleted male could result in lower fertility and/or fecundity in monandrous compared to polyandrous females, and thus favor multiple mating in females. Here I test this "reduced fertility" hypothesis by comparing the fecundity and fertility of females forced to mate with a male that had mated the previous day to females mated with a virgin male. I then discuss the potential role of sexual conflict to influence the timing of mating in polyandrous saturniids.

METHODS

To produce moths whose mating history was known, I raised *Promethea* larvae derived from 5 females that emerged from wild cocoons collected during the winter of 2013 in northwestern Pennsylvania (41°47'N, 79°57'W). The broods were raised separately within remay cloth sleeves (2.29m long and 1.70m in circumference) tied over branches of the same black cherry tree (*Prunus serotina*, Ehrhart 1784). The resulting cocoons were kept at ambient temperature in a screened porch (2.4m high × 2.8m wide × 6.6m long) attached to a house during the following winter.

In May, 2014, I attached the cocoons individually with duct tape and a staple to the tips of 35 cm twigs held by gravel in open topped quart jars and set the jars on a sill inside the screened porch. Females and males emerged in June–July. After emergence, females remained on their cocoons and called from 1500–1830 h EDT. Calling is a conspicuous behavior involving the protrusion of a yellow scent gland (see Fig. 1 in Tuskes et al. 1996). On days when a male(s) also emerged, I hand paired (after

Peigler 1977) him to a virgin female calling within the screened porch. After the pair separated, I placed the male in a paper bag overnight. If, fortuitously, a female emerged on the following day, I hand paired this virgin female with the male who had mated the previous day. This was accomplished after she began calling in the afternoon of her emergence day. I allowed the mating pair to copulate until separation and include only hand pairings that lasted a normal length of time for a first copulation, generally within 271.2 ± 10.2 min (Morton 2009). The female was then placed in a paper bag and allowed to lay her full clutch of eggs over the following days. After she died, I cut the eggs from the paper bag, counted them, and placed the pieces of paper bag containing the eggs in a plastic container kept at ambient temperature in the screened porch. After the eggs hatched, I considered hatched eggs fertile and dissected those that had not hatched under a compound microscope for the presence of an embryo. Eggs with no development were considered infertile, following the protocol of the earlier study (Morton 2009) (see also Fig. 1 in Collins & Rawlins 2013 for electronmicrograph of this technique).

I obtained data from 9 matings between nonvirgin males and virgin females and compared these females' fecundity and fertility to that of 17 captive-raised females that had copulated once with virgin males. Data were analyzed with the JMP 13 SAS statistical package (Sall et al. 2005) using one-way ANOVA tests. Significance was set at $P < 0.05$ and two-tailed tests were used throughout. Standard error of the mean (SE) was used as a measure of dispersion.

RESULTS

Females mated to nonvirgin males (N = 9) laid an average of 247±10.94 eggs (range = 181–309) compared to an average of 235±7.96 eggs (range = 190–282) for females mated to virgin males (N = 17). There was no significant difference in number of eggs laid by the two groups of females (ANOVA, d.f. = 25, F = 0.8175, $P < 0.38$).

Comparing the percent of fertile eggs between the two mating types, females mated to nonvirgin males averaged 243.8±10.856 fertile eggs (99%) to 230.7±7.899 fertile eggs (98%) for females mated to virgin males; no significant difference (ANOVA, d.f. = 25, F = 0.9565, $P < 0.34$). For both fecundity and fertility rate, the females mated to nonvirgin males had nonsignificant, but higher, values than for females mated to virgin males. Females mated with virgin males averaged 4.12 infertile eggs per clutch whereas females mated to nonvirgin males averaged 3.22 infertile eggs per clutch, an insignificant difference statistically.

DISCUSSION

My reduced fertility hypothesis suggested that females mated to nonvirgin males would have lower fertility. Instead, mating history of males had no effect on females in fecundity or fertility. There was no statistical difference between the two groups. In fact, females mated to nonvirgin males laid more eggs than females mated to virgin males, so the effect found was in the opposite direction of the prediction. A power test showed that a mean difference of 28 eggs (sensitivity) or a sample size of 125 would be needed to show a significant difference between the two groups in fecundity, but it would confirm the null hypothesis not the prediction. I therefore accept the null hypothesis that a female mated to a male that had copulated the previous day does not differ in fertility or fecundity from a female mated to a virgin male.

Therefore, multiple mating is not due to fertility enhancement in *Promethea*. This was found earlier in an arctiine moth (LaMunyon 1997) who found that fertility was not affected by male mating status in *Utetheisa ornatrix* (Linneaus) but multiple mating resulted in increased fecundity, as I found for *Promethea* (Morton 2009). LaMunyon suggested that fecundity was limited by resources needed for egg construction and that male spermatophores contributed these resources. Indeed, nutrients from spermatophores are thought to be the most common benefit of multiple mating in insects (Arnqvist & Nilsson 2000). Thus the question becomes: Why don't more saturniid species, all of which are non-feeding as adults, exhibit multiple mating, given that nutrition from spermatophores is the only way they can obtain more nutrients for egg production than those gained through larval feeding?

I suggest the answer involves tradeoffs that include time and female control of mating. Time is important because copulations take several hours in *Promethea*. Multiple mating would constrain time for oviposition and dispersal if mating overlapped in time with oviposition. Given that females control mating via calling (a non-calling female is invisible to males) they are in control of the timing of mating (e.g., Allison & Cardé 2016). It is unlikely that females would opt to oviposit in the daytime because of the threat of predation by birds. Instead, I suggest saturniid species known to have multiple mating call during the day time and have nocturnal hours reserved for egg laying and dispersal (Table 1).

For example, *Callosamia* species differ in mating time, with *C. securifera* (Maaassen) and *C. promethea* mating diurnally and *C. angulifera* (Walker) mating nocturnally (Tuskes et al. 1996). D. Bayer (pers. com.)

TABLE 1. Multiple mating in saturniids may be predicted by a separation of diurnal mating and nocturnal egg laying. Multiple mating species are listed together with unstudied species showing the diurnal/nocturnal mating/egg laying association.

Species	Multiple mating?	Mating time	Egg laying time	Reference
Callosamia promethea	yes	diurnal	nocturnal	Morton 2009
Callosamia angulifera	no	nocturnal	nocturnal	Morton 2009
Callosamia securifera	yes	diurnal	nocturnal	D. Bayer, pers. comm., Brown 1972
Saturnia pavonia	yes?	diurnal	nocturnal	Tutt 1902
Eupackardia calleta	yes	diurnal	nocturnal	Louwagie & Peigler 2016
Hyalophora cecropia	no	nocturnal	nocturnal	Tuskes et al. 1996
Rothschildia lebeau	no	nocturnal (20h)	nocturnal	Agosta 2008
Automeris phrynon	no data	diurnal	nocturnal	Marquis, R. J. 1984
Saturnia albofasciata	no data	diurnal	nocturnal	Tuskes & Collins 1981
Hemileuca burnsi	no data	diurnal	nocturnal	Tuskes 1984
Anisota ssp.	no data	diurnal	nocturnal	Tuskes et al. 1996

captured female *C. securifera* at lights at night and obtained fertile eggs from them over that same night. He then noticed the caged females called and attracted males the next day, even though they had already been fertilized, and allowed two of these females, several weeks apart, to copulate again. I had predicted, a priori, that *C. securifera*, as well as *Promethea*, would mate multiple times because of their shared characteristic of diurnal mating, while the nocturnal *angulifera* is monandrous (Morton 2009). The day mating *Eupackardia calleta* (Westwood) also mates multiple times (Louwagie & Peigler 2016) as possibly does *Saturnia pavonia* (Linnaeus), also day mating (Tutt 1902, as quoted in Louwagie & Peigler 2016). In all these cases, the females are not dayflying, only the males, suggesting females control whether or not multiple mating occurs and they control the time of mating through their pheromone emission to obtain what is optimum for their reproductive success. I hypothesize that the optimum is to temporally separate mating and oviposition, and the finding here that fertility assurance is not an issue in these cases of polyandry in saturniids, enhances this interpretation.

Female control (Eberhard 1996) is hypothesized to lead to diurnal mating. As a result of female control, females force males to fly in the daytime but can remain nocturnal themselves to avoid predation. Males, on the other hand, are faced with diurnal predators. In response, they traded one form of communication, iconic (crypsis), for indexical (Morton 2017) by mimicking distasteful diurnal butterflies (Jeffords et al. 1979, Louwagie & Peigler 2016).

An alternative neutral hypothesis might be that calling by nonvirgin females is an incidental byproduct of physiological processes affected by circadian rhythms. Females call again due to a non-adaptive activation of calling behavior due to these physiological processes (e.g. Riddiford & Williams 1971). This is not likely because of the proven increase in fecundity due to polyandry that provides a source of selection that favors females that call many times.

We need more information on the timing of mating and oviposition to strengthen the relation between polyandry, diurnal mating, nocturnal ovipositing, and female control. There are suggestions polyandry may be more frequent in saturniids. For example, Tuskes (1984) stated that "certain females" of the dayflying *Hemileuca maia* "mate again" after laying a first clutch of eggs. He also stated that all members of the *Hemileuca* mate once. He describes *H. burnsi* (J. H. Watson) males as flying during the day but females ovipositing at night. If my prediction that mating diurnally and egg laying/dispersing nocturnally are adaptations for multiple mating this species may be polyandrous.

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REMARKABLE LONGEVITY OF THE CHEMICALLY DEFENDED MOTH, *UTETHEISA ORNATRIX* (LEPIDOPTERA: EREBIDAE) AND THE FACTORS THAT AFFECT IT

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ABSTRACT. Most butterflies and moths, with the exception of *Heliconius*, live only a week or two in their non-hibernating state. In the present study, we evaluated the longevity of the chemically defended Ornate Bella Moth, *Utetheisa ornatrix*, using a sample of 214 individuals from two broods. On a diet of 6% sugar water or Gatorade®, a quarter of the moths survived for one month or longer, with a maximum survival of 50 days. A glimpse into the genetic component offered by using two broods suggests that one can expect to find greater variability in longevity between different populations of this species. Males lived on average longer than females, and moths from heavier pupae lived longer than their smaller siblings. The nutrition of caterpillars, translated into pupal weight, appears to have a positive effect on the longevity of resultant adults. While it was not surprising to find that sugar played a positive role in the longevity and fecundity of adult moths, the fact that *Utetheisa ornatrix* can subsist solely on water for up to 36 days and that males tended to live longer than females, which is reverse of most species for which such data is available, were intriguing findings. The chemically defended nature of this species, its high fecundity (251±64 eggs in this study) paired with its habit of laying eggs in small batches, and its propensity to disperse as adults help explain why these moths evolved prolonged life spans.

Additional key words: community ecology, herbivores, trophic interactions, tiger moths

According to mark-recapture studies of temperate butterflies, most live only a week or two in a non-hibernating state, and males tend to live slightly shorter lives than females (e.g., Scott 1973). There are exceptions to the rule, however. In the tropics, *Heliconius* butterflies, which feed not only on nectar but also on pollen, can live as long as five months (Turner 1971, Ehrlich & Gilbert 1973, Boggs 1979). For moths, the longevity data is mostly derived from the laboratory cultures of several economically important species. For instance, females of the European Corn Borer Moth, *Ostrinia nubilalis* (Hübner, 1796) (Pyralidae), live around 13–14 days on 8% sucrose solution (Royer & McNeil 1993), females of the Cotton Bollworm Moth, *Helicoverpa armigera* (Hübner, [1809]) (Noctuidae), live 10–12 days on 10% honey solution (Hou & Sheng 1999), and both males and females of Dolichos Armyworm Moth, *Spodoptera dolichos* (Fabricius, 1794) (Noctuidae), live 12–13 day on a diet of 7% honey/sucrose solution (Montezano et al. 2015). Based on two species, Murphy et al. (2011) found that female limacodids (the group that does not feed as adults), live 8–9 days on average, but some female Saddleback Caterpillar Moths, *Acharia stimulea* (Clemens, 1860), lived as long as three weeks in the lab.

In the present study, we evaluated the longevity of the Bella Moth, *Utetheisa ornatrix bella* (Linnaeus, 1978) (Erebidae) in the lab. The moth has intriguing and intricate relationships with its hostplants in the genus *Crotalaria*, which determine its ecology (Conner 2008). As the primary focus of the second author's interest in *U. ornatrix* are these relationships, we explored here how caterpillar performance, interpreted as greater or smaller pupal weight, may be influencing the longevity of adult moths. As *U. ornatrix* is a nectar-feeder for which the nectar and rain may be scarce in early spring or late fall/winter when this moth flies in north-central Florida (Sourakov 2015), we also investigated the roles that sugar and water play in determining longevity. There exists anecdotal evidence that butterflies live longer on Gatorade® (Daniels, pers. com.), and adult butterflies are routinely maintained in colonies on that diet (e.g., Trager 2009, Saarinen 2009). Because of this and considering the salt-seeking, puddling behavior of many Lepidoptera species in the tropics, we also tested if Gatorade® would have any benefits compared to sugar water of the same concentration. We evaluated longevity separately by sex, as the biological objectives, physiology, and pupal weights of males are all different from those of the females.

MATERIALS AND METHODS

A total of 214 pupae from two broods were obtained in the laboratory of the McGuire Center for Lepidoptera and Biodiversity, by rearing larvae from eggs, which were laid in late April 2016 by two *U. ornatrix* females netted on the University of Florida campus. These females, similar in color pattern and size, flew in close proximity to each other, were collected a few minutes apart, and represented a very isolated population located in the middle of urban development; hence we assume that they are genetically similar. The resultant larvae were fed on the foliage and seeds of *Crotalaria lanceolata* E. Mey. collected from the same location. As it has been demonstrated that adding green seeds of this host to the diet has a positive effect on pupal weight, which in turn corresponds to wing size (Sourakov 2015, Long & Sourakov 2016), we varied the amount of seeds provided to larvae in later instars to achieve a greater variation in pupal weight.

As the larvae grew, they were divided into smaller and smaller groups feeding in 2 oz. clear plastic cups, until they pupated individually in these cups and were assigned voucher numbers. Pupae were weighed using a Mettler Toledo AL104 analytical balance. The mean (±SD) pupal weights of the two broods were more similar in females (154±21mg (N=40) vs. 155±23mg (N=62), P=0.8) than in males (179±27mg (N=38) vs. 166±22mg (N=74), P=0.06)).

Upon emergence, moths were randomly placed in one of the three experimental groups which were provided with water, 6% sugar-water solution, or Gatorade® (fruit punch flavor), which, in addition to 6% sugar solution, contains sodium, potassium, food starch, phosphoric acid, flavors and preservatives. The liquids were delivered via soaked cotton tips (halved Johnson's baby-proof swabs were used as they retained moisture to a much greater degree than the regular kind) inserted into the lid of the cup. The swabs were changed every two days to avoid molding and drying, during which time the mortality was assessed and the swabs were re-wetted with solution. A small control group of moths was kept in similar cups with holes but without any sustenance. Cups were kept at 23°C and organized in a checkerboard manner to reduce potential biases. Females of *U. ornatrix* will lay eggs on the walls and lid of the cups even when they are not mated, and we randomly chose 5 females from each experimental group to compare fecundity. The data analysis (T-test and Ordinary Least Squares (OLS) regression analysis) was conducted using PAST statistical program (Hammer et al. 2001).

RESULTS

The results of correlating pupal weight and longevity are summarized in Table 1. Greater pupal weight appears to have had a positive influence on the longevity of *Utetheisa ornatrix*, regardless of diet or sex, except for one group (females, brood 2, on Gatorade®). The

TABLE 1. Survival rates of *Utetheisa ornatrix* adult moths in the lab in relation to sex and diet

Sex / Diet	**Brood**	**Regression analysis (OLS)**			**50% surv.(days) ± SE, variance & sample size**
		Slope	**P**	**r^2**	
Males / Sugar Water	1	57.8	0.75	0.02	41.5±3.5 (var 99.1) (N=8)
	2	133.6	0.12	0.09	28±1.9 (var 101.2) (N=27)
Females / Sugar Water	1	117.6	0.12	0.20	25±1.5 (var 30.9) (N=13)
	2	29.1	0.71	0.005	22±1.6 (var 71.8) (N=29)
Males / Gatorade®	1	41.9	0.50	0.06	29±1.7 (var 29.8) (N=10)
	2	71.7	0.45	0.03	22±2.0 (var 84.4) (N=21)
Females / Gatorade®	1	91.7	0.37	0.05	24.5±2.1 (var 77) (N=18)
	2	-53.4	0.34	0.003	21±1.3 (var 52.9) (N=30)
Males / Water only	1	108.2	0.13	0.13	21±1.8 (var 58.4) (N=19)
	2	133	0.03	0.24	22.5±1.2 (var 30.1)(N=20)
Females / Water only	1	92.9	0.17	0.41	22±0.8 (var 4.3) (N=6)
	2	-167.2	0.74	0.15	15±3.5 (var 42.2) (N=3)
Control – no sustenance	1&2	9.1	0.36	0.11	6.5±0.3 (var 0.7) (N=10)
All males	1&2	104.6	0.007	0.06	23±0.9 (var 99.8) (N=112)
All females	1&2	49.0	0.18	0.02	22±0.8 (var 64.8) (N=102)

variance is great and hence, this correlation is weakly supported for each group separately. When all groups are combined and analyzed by sex (Table 1, bottom), the increase in sample size strengthens this conclusion. Increase in pupal weight appears to have stronger positive effect on the longevity of males than of females.

As illustrated by 50% survival data (Table 1) and the 25% survival graph (Fig. 1), males lived longer than females in all test groups, with the longest living male surviving 50 days, but with as many as a quarter living over a month on the sugar-containing diet. While males that were provided only water lived shorter lives on average, several also lived past the 30-day mark. Moths fed sugar-water and Gatorade® had similar fecundity (Mean ± SD = 249±70 eggs and 253±63 eggs, respectively, P=0.94), and these two groups differed significantly (P=0.02; P=0.03) from the group maintained on water only, which laid average of 127±30 eggs.

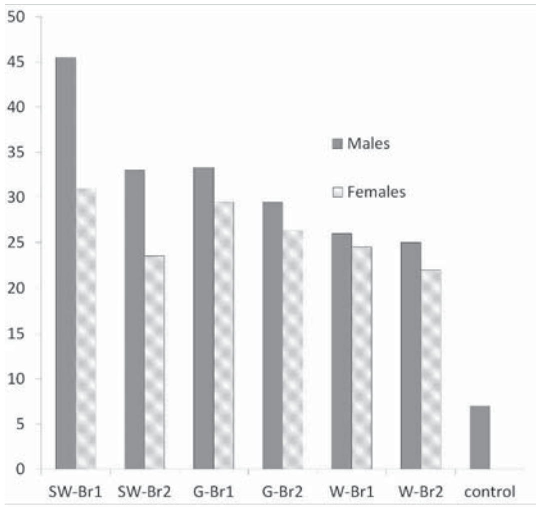

FIG. 1. Survival time (days) of a quarter of the *Utetheisa ornatrix* moths by brood, sex and diet. Males lived longer than females in every instance (SW-Sugar water, G- Gatorade®, W-Water).

DISCUSSION

Our results have demonstrated that *Utetheisa ornatrix* is an exceptionally long-lived moth, approaching *Heliconius* butterflies in longevity, but without the benefits of digesting nitrogen from the pollen—the way in which *Heliconius* are thought to achieve their remarkable longevity and in which they differ from other butterflies. While surviving up to the 20-day mark did not require that *U. ornatrix* adults feed on sugar, beyond approximately 25 days, the availability of sugar became essential for their survival. One can speculate that a freshly emerged *U. ornatrix* must be storing sufficient body-fat resources which it can convert into water, as, even when deprived of water, they lived for 8–9 days—almost as long as the limacodids in the study by Murphy et al. (2011). While limacodids, with their vestigial proboscises and thick bodies, evolved as non-feeders, *U. ornatrix* is a slim, seemingly delicate moth and an active nectar feeder with a well-developed proboscis.

Brood 1 and Brood 2 show slight but statistically significant differences in longevity of males despite their likely genetic similarity. Considering our knowledge of how different geographic populations of this species can be from each other and how widely this species is distributed (e.g., Pease 1968), we can expect to find an even greater variability in longevity when we start considering this species as a whole. The present study however had an objective to estimate how phenotypic plasticity of size induced through variation in caterpillar diet would affect longevity. Hence, it will be up to future studies to characterize the role that overall genetic variation plays in the longevity of this species, the range of which spans two continents. As for our primary objective, if we consider all diet groups and two broods as a subset of natural population, we can conclude that based on a sample of 112 males and 102 females, there exists a positive correlation between the pupal weight, induced through caterpillar diet, and adult moth longevity. Hence, we can speculate that oviposition choice by a maternal female, and subsequent caterpillar feeding and dispersal behaviors will have an effect on the longevity of the next generation of adult moths.

Regardless of diet, the males of this species tended to live longer than the females, which is the reverse of most species, but corresponds to the other ways in which this is an unusual moth. Male *U. ornatrix* are both larger than females and develop longer as caterpillars because they are tasked with sequestering alkaloids from their hostplants, not only for their own defense but also for nuptial gifts and the production of pheromones. For males, in addition to increased dispersal potential, which reduces inbreeding, extended longevity should lead to increase in mating success. In the lab, females of any given brood raised under similar conditions, will always emerge 2–3 days earlier than males because male caterpillars take longer to develop (Sourakov, 2015). However, the development of caterpillars is not synchronized in any given natural *U. ornatrix* population that we observed, and thus the longer a male lives, the higher chance he has to find a receptive mate.

It has been demonstrated previously that *U. ornatrix* females mate preferentially with larger males, and that this mating preference is inherited through the father rather than the mother, and that females with larger fathers have a stronger preference for larger males

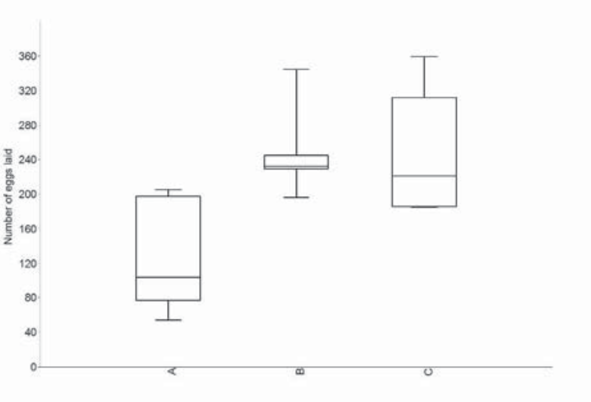

FIG. 2. Fecundity of *Utetheisa ornatrix* females maintained on (**A**)-Water; (**B**)-Sugar-water; (**C**)-Gatorade®).

(Iyengar et al. 2002). The fact that male pupal weight, which is as much a function of a caterpillar's performance and maternal oviposition choice as of the underlying genetics, has, as demonstrated above, a positive correlation with longevity, adds another layer of complexity to the story of how intimately the natural history, sexual selection, and evolution of *U. ornatrix* is intertwined with its hostplants.

We think, however, that the main reasons why *U. ornatrix* evolved the ability to survive 3–4 times longer than other moth species for which data is available is that the predation is less of a factor for *U. ornatrix*, as they are chemically defended (e.g., Eisner & Eisner 1991, Martins et al. 2015, Sourakov, pers. obs.). It is therefore realistic to expect that in nature *U. ornatrix* frequently live out their full physiological potential. While a female *U. ornatrix* can occasionally lay larger clusters of eggs, in natural conditions this is unusual. For observations for which we have the photographic evidence, the common size of egg clusters in nature is around 20, though it is quite variable: Mean±SD = 31±34 eggs, N=8, Sourakov (pers. obs.). Based on the present study, a female that has access to nectar can lay upward of 300 eggs and therefore, in nature, lays numerous egg clusters. The increased longevity combined with this gradual oviposition can be highly adaptive for *U. ornatrix*, which is thought to also have high rates of dispersal (Cogni et al. 2011, Pease, 1968), and therefore can spread its eggs far and wide.

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NOTES ON THE LARVA AND NATURAL HISTORY OF *LACOSOMA ARIZONICUM* DYAR (MIMALLONOIDEA, MIMALLONIDAE) WITH NEW HOST AND PARASITOID RECORDS

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ABSTRACT. The larva of *Lacosoma arizonicum* Dyar is figured for the first time. This species was previously known to feed on oaks (*Quercus*), but no specific *Quercus* species has been reported. We studied the life history of *L. arizonicum* and confirm four larval host plants from field-collected larvae: *Quercus arizonica, Q. emoryi, Q. gambelii,* and *Q. hypoleucoides*. Larvae were successfully reared to pupation in captivity on *Q. virginiana* (in Florida). A tachinid fly in the genus *Lespesia*, was reared from a single *L. arizonicum* larva.

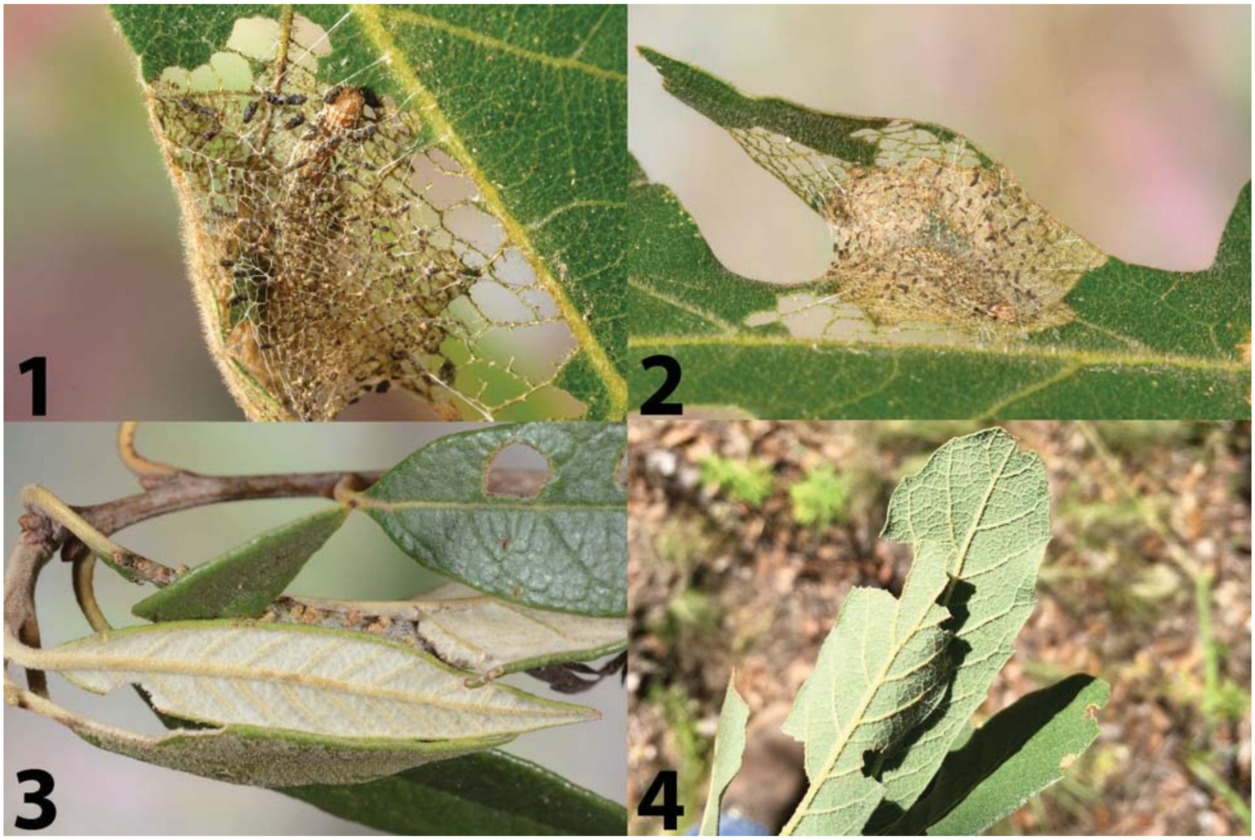
Additional key words: Frass net, larval case, *Lespesia*, *Quercus*, shelter

There are approximately 300 described species of Mimallonidae (RAS unpublished) of which only four occur in America north of Mexico (Franclemont 1973). Species diversity is highest in the tropics; for instance, in the lowland Atlantic tropical rainforests of northeastern Costa Rica, as many as 19 species of mimallonids have been taken at a single station (DLW unpublished). In the western United States (not including southern Texas), two species are reported: *Lacosoma arizonicum* Dyar, 1898 and *Cicinnus melsheimeri* (Harris, 1841) (Franclemont 1973, Powell & Opler 2009). Since the original description of *L. arizonicum* and the works of Franclemont (1973) and Powell and Opler (2009), little has been published on the natural history of *L. arizonicum*. These authors mention oak (*Quercus* L.) (oak species unspecified) as the larval host. Powell and Opler (2009) record the flight season for *L. arizonicum* as June through August, with a late night to early morning adult activity period, approximately 2000–0400 h.

Below we report new observations pertaining to the life history of *L. arizonicum*. We figure the larva for the first time, document four *Quercus* species as hosts in nature, and provide the first record of a parasitoid reared from a wild-collected larva. We also provide new observations and discussion on the larval feeding behavior, pupation, and diapause in *L. arizonicum*.

MATERIALS AND METHODS

Middle instar larvae of *L. arizonicum* were collected by RAS, LER, and AYK at two localities in southern Arizona, USA: Pima Co.: Santa Rita Mts., Coronado Nat. Forest, Box Canyon, 31.80075°, -110.76904°, 1490 m, 16.IX.2016, on *Quercus* arizonica Sarg. [one larva]; and Cochise Co.: Huachuca Mts., Coronado Nat. Forest, Sunnyside Canyon, 31.46495°, -110.38608° [coordinates approximate], ~1985 m, 18.IX.2016, on *Q. arizonica* [one larva] and *Q. emoryi* Torr., *Q. hypoleucoides* Camus [six larvae total from latter two *Quercus* species]. The habitat at both sites where collections were made was Madrean evergreen woodland, dominated by evergreen *Quercus* species. DLW collected young larvae at two localities in southeastern Arizona: Pima Co.: Catalina Hwy, below Incinerator Ridge, 32.40956°, -110.70470°, 2360 m, 1.VIII.2012, on *Q. gambelii* Nutt. [one larva]; Cochise Co.: Carr Canyon Road, 31.42480°, -110.29922°, 2255 m, 24.VIII, 2016, on *Q. hypoleucoides* [three larvae].



FIGS. 1–4. *Lacosoma arizonicum* larval shelters, all from USA, Arizona. **1, 2.** Early instar frass-netting shelter. Pima Co., Catalina Hwy, below Incinerator Ridge, 2360 m, on *Q. gambelii*. **3.** Middle instar shelter, Cochise Co., Carr Canyon Road, 2255 m, on *Q. hypoleucoides*. **4.** Middle to late instar larval shelter being built, Cochise Co., Huachuca Mts., Coronado Nat. Forest, Sunnyside Canyon, ~1985 m.

RAS fed larvae on various *Quercus* sp. native to Florida, with only *Q. virginiana* being consumed for the duration of larval development. DLW reared his collections on natal hosts.

DLW's larval and photographic vouchers have been deposited in the Biological Collections facility at the University of Connecticut, Connecticut, USA. RAS's larval and adult vouchers are temporarily deposited in the research collection of Ryan St Laurent, Gainesville, FL, USA (CRAS), but will be donated to the collection of the McGuire Center for Lepidoptera and Biodiversity, Gainesville, FL, USA.

RESULTS

Natural host plants: We collected larvae on four oak species, *Quercus arizonica, Q. emoryi, Q. gambelii*, and *Q. hypoleucoides* in southern Arizona. Although our sample size is small, *Q. hypoleucoides* appears to be the favored oak species by ovipositing females.

Rearing: From eight larvae collected and reared by RAS on *Q. virginiana*, two individuals pupated. One of the two larvae sealed the anterior opening of the larval shelter prior to pupation in late X.2016, which eclosed as an adult male on 17.XI.2016 without diapause. This exceptional behavior, i.e., a November eclosion, may be a result of this individual being reared indoors with longer than normal photoperiods due to extended indoor lighting, and warm temperatures throughout the night (in Gainesville, Florida). In its natural habitat in mountainous southern Arizona, this species, being a late-season feeder, is exposed to shorter photoperiods and colder nighttime temperatures.

The other larva, however, sealed both openings of the larval shelter and underwent diapause. It is worth noting that this individual was reared under the same conditions as the nondiapausing larva, suggesting variability in the indoor rearing setup or perhaps natural variation in diapausing behavior. Upon opening the overwintering

FIGS. 5–8. *Lacosoma arizonicum* larvae, all from USA, Arizona. **5.** Middle instar, Cochise Co., Carr Canyon Road, 2255 m, on *Q. hypoleucoides.* **6.** Final instar, a more obscurely marked individual, locality as for Fig. 5. **7.** Late instar, a more contrastingly marked individual, Pima Co., Catalina Hwy, below Incinerator Ridge, 2360 m, on *Q. gambelii*. **8.** Late instar head detail, defense posture displaying opened, jetblack mandibles, locality as for Fig. 7.

larval shelter during February/March 2017, a larva was observed, not a pupa, confirming that *L. arizonicum* overwinters as a larva as in other North American Mimallonidae (Dyar 1900, Wagner 2005). The diapausing individual was kept outdoors in a small cylindrical mesh cage, roughly 30 cm tall by 15 cm in diameter.

Larval feeding behavior: Like all Mimallonidae for which the life history is known, *L. arizonicum* builds larval shelters which differ in structure depending on the age of the larva. Early instar larvae feed below a meshwork of silk and frass-netting that spans over the midrib of a leaf, or may be built along the edge of a leaf, curling the edge slightly (Figs 1, 2). Older larvae affix two or more leaves together with silk (Figs 3, 4), forming a shelter, which becomes more rigid and tubular in structure as the larvae develop and feed on the material surrounding the sealed compartment, often killing the incorporated leaves. The final shelter (case) is compact in structure, and has openings on either end, from which the larva extends outwards to feed, retracting inwards at the slightest disturbance. The entire inside surface of the larval shelter is generously lined with silk.

Larval description: Our observations of *L. arizonicum* larvae are based on 12 middle (Fig. 5) and late instar larvae (Figs 6–9, 11), as well as on photographs of the same individuals. We note morphological characters typical of the concealed-feeding Mimallonidae, specifically an enlarged, heavily sclerotized, rugose, anteroventrad projecting head and elongate prothorax; a thick abdomen with the widest segments being A4–A7; and a distinctly flattened, and heavily armored anal plate. There are two L group setae on T1. The crochet arrangement on the anal prolegs is unique: they form a transverse oval with the anterior crochets enlarged and distinctly biordinal (Stehr 1987).

The thick, pitted and verrucose head and flattened anal plate are apomorphic for mimallonoid larvae (Stehr 1987) and are clearly specialized (armored) for blocking the two openings of the larval shelter (Forbes 1923).

FIG. 9. Final instar *Lacosoma arizonicum* feeding on *Quercus virginiana* in captivity, displaying fully extended body, reared ex. wild-collected southern Arizona, USA.

FIG. 10. Prepupal *Lacosoma arizonicum* larva, USA, AZ, Cochise County, Huachuca Mountains, Miller Canyon, 5.X.2012, 4 cm in length. (Photo courtesty of Charles W. Melton, used with permission).

Coloration of the final instar is diagnostic, but with slight variation in the expression of yellow, as well as the degree of development of the broken longitudinal stripes (compare Figs 6 and 7). The thin middorsal stripe is usually continuous (especially anteriorad); there are three splotchy stripes, one subdorsal, and two supraspiracular. The yellow markings contrast with the dirty- to red-brown ground color. The thorax and abdomen are pale and mostly unmarked below the spiracles. The middorsal stripe divides the light red-brown prothoracic shield. The rugose head (Fig. 8) is handsomely patterned with silver-gray protuberances, giving way to black bars and lines above the level of the frons. The primary setae are white, peg-like, and somewhat widened apically. Middle and penultimate instars (Fig. 5) are similar to the final instar, but browner overall, with more subdued markings. Mature larvae are approximately 3–4 cm in length, but may stretch themselves to almost twice in length when extended from their shelters during feeding (Fig. 9).

We have not observed prepupal larvae directly because the larvae that survived to this stage remained within their larval shelters up until and during the pupal stage. However, Charles W. Melton provided photos (one shown in Fig. 10) of what we deduce to be a prepupal larva. This inference is based on the darker than usual coloration, wandering behavior as evident by the observed behavior of this individual being found crawling on the ground in an oak woodland, and the late season record: 5.X.2012 (C. Melton pers. comm.). The photo shows a larva similar to the one that we figure in Fig. 6, but more purple in coloration and fully fed. The purple hue is most obvious on the abdominal segments except A8–A10. Considering that this apparently prepupal larva was found in the absence of its larval shelter, we recognize the possibility that *L. arizonicum* may occasionally pupate outside of a shelter, but have not formally documented this behavior. Our specimen, however, did overwinter within the larval shelter.

Larvae of *L. arizonicum* are similar to those of *L. chiridota* Grote, 1864 (Dyar 1900, Wagner 2005) (Fig. 12), but markings of *L. chiridota* are less distinct due to reduced contrast between yellow dorsal markings and an overall yellowish tan coloration of the larval dorsum. We include images of both species for comparison (Figs 11, 12).

Parasitoid: Of the eight *L. arizonicum* larvae originally collected by RAS, five perished, two resulted in either an eclosed adult or a (presently, at time of writing) diapausing larva, and one penultimate instar produced a single tachinid parasitoid that pupated after

FIGS. 11, 12. Comparison of North American *Lacosoma* species. **11.** *L. arizonicum*, freshly molted final instar, captive reared ex. wild collected southern Arizona, USA. **12.** *L. chiridota*, mature final instar, captive reared ex. female Austin Cary Forest, Alachua County, Florida, USA. Not to scale.

FIG. 13. Adult male *Lacosoma arizonicum*, USA, Arizona, Cochise Co., Ramsey Canyon, Nature Conservancy Preserve. 3.VIII.2012. (Photo courtesy of Margarthe Brummermann, used with permission).

the first week of October 2016. After the adult fly emerged from its puparium, it was killed and deposited in the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (CNC). James O'Hara (CNC) identified the tachinid specimen as a *Lespesia* species, possibly undescribed. This represents the first record of a parasitoid from *L. arizonicum*. The Janzen Costa Rican parasitoid database has records of *Lespesia* parasitizing Mimallonidae in the genera *Druentica* Strand, 1932 and *Trogoptera* Herrich-Schäffer, [1856] (Janzen and Hallwachs 2017).

DISCUSSION

So far as is known, *L. arizonicum* is restricted to *Quercus* hosts, although it seems to be unspecialized with regard to oak species. Its eastern relative, *L. chiridota*, is also an oak specialist (Robinson et al. 2002, Wagner 2005). In the tropics, several woody families are used by *Lacosoma*, such as: Fabaceae, Lauraceae, Melastomataceae, Myrtaceae, Proteaceae, Rosaceae, Sapotaceae, Styracaceae, and Vochysiaceae (Robinson et al. 2010, Janzen & Hallwachs 2017). While it appears that many *Lacosoma* may be dietary specialists (Janzen & Hallwachs 2017, this paper), the alpha taxonomy of the genus is too nascent and knowledge of wild hostplant associations in the Neotropics are too incompletely known to assess to what degree diets might be more generalized.

Very little information is available regarding North American mimallonid parasitoids. Arnaud (1978) lists *Chrysotachina alcedo* (Loew) as a parasitoid of *Cicinnus melsheimeri*, which is interestingly a parasitoid of another concealed shelter former, the hesperiid *Urbanus proteus* (Linneaus). RAS has reared another inconclusively identified tachinid from *C. melsheimeri* sleeve-reared in Tompkins Co., New York, USA. Considering the paucity of parasitoid records for Mimallonidae, this is an area warranting further study. For example, it would be interesting to know if there are tachinid parasitoids specialized on unrelated concealed feeders such as Mimallonidae and Hesperiidae.

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HOST PLANT SUITABILITY AND ALTITUDINAL VARIATION IN COCOON SIZE OF THE INDIAN TARSAR SILK MOTH *ANTHERAEA MYLITTA* DRURY (LEPIDOPTERA: SATURNIIDAE)

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ABSTRACT. An experimental rearing of *Antheraea mylitta* Drury was carried out in the rearing fields at Similipal Biosphere Reserve, Mayurbhanj, Odisha, India, during the rainy season under somewhat natural conditions on live host plants. Female larvae were reared at three elevations utilizing the same eight host plant species, viz. Asan (*Terminalia alata* W. & A.) in family Combretaceae, Arjun (*Terminalia arjuna* W. & A.) (Combretaceae), Sal (*Shorea robusta* Gaertn) (Dipterocarpaceae), Ber (*Ziziphus jujuba* Gaertn) (Rhamnaceae), Sidha (*Lagerstroemia parviflora* Roxb.) (Lythraceae), Dha (*Anogeissus latifolia* Wall.) (Combretaceae), Bahada (*Terminalia belerica* (Gaertn) Roxb.) (Combretaceae) and Jamun (*Syzygium cumini* (L.) Skeels) (Myrtaceae) at each location. The larval growth on various host plants was evaluated in terms of the size, weight, and volume of the resulting cocoons. The host plant that produced the highest quality cocoons at all the elevations is Sal (*Shorea robusta*). The least suitable host plant at all the elevations is Jamun (*Syzygium cumini*). Results for all the eight species of host plants and the influence of parameters related to elevation are presented in detail. The data may be useful for selecting alternate host plants that might aid the sericulture industry in those situations when the "optimal" plant species are not available.

Additional key words: *Ziziphus jujuba* Gaertn, foliovorous, trivoltine, rainy, fifth instar

The present study was carried out during rainy season in order to assess the growth of female larva of "Daba ecorace" of *A. mylitta* in terms of the size, volume, and weight of the resulting cocoon on different food plants at different altitudes for proper gradation of food plants and altitudes. *Antheraea mylitta* Drury (Satuniidae) which produces the traditional Indian tasar silk is a foliovorous semi-domesticated tropical tasar silk moth. It is distributed in the form of about 44 ecoraces over varied geographical tropical zones of our country, particularly in the states of West Bengal, Jharkhand, Bihar, Madhya Pradesh, Odisha, Andhra Pradesh and Maharashtra. The State of Odisha has two ecoraces, viz. Daba and Sukinda (semi domesticated varieties) under *Antheraea mylitta* Drury. In India, it is trivoltine (TV) (three generations produced in a year) at lower altitude (50 – 300 m ASL). However, it is exploited as bivoltine (BV), reared during July–August (first crop or rainy cocoon crop) and September–October (second crop or autumn crop) for the commercial production of tasar silk without utilizing the third generation. Though polyphagous in nature, it is usually reared on primary tasar host plants viz; *Terminalia alata* (Asan), *Terminalia arjuna* (Arjun) and *Shorea robusta* (Sal) by aboriginals during seed crop (July–August) and commercial crop (Sep.–Oct.) seasons. However, nearly two dozen food plants like *Ziziphus jujuba* (Ber), *Lagerstroemia parviflora* (Sidha), *Anogeissus latifolia* (Dha), *Terminalia belerica* (Bahada), *Syzygium cumini* (Jamun), etc. of secondary importance for *A. mylitta* silk worm are also abundant in the natural forests at different altitudes (Sinha & Jolly 1971). The vast availability of these unutilized food plants can be exploited sustainably by the local tribes for the rearing and cocooning of *A. mylitta*. Feeding of nutritionally enriched leaves shows better growth and development of silkworm larvae which directly influences the quality and quantity of silk production. Hence evaluating ecoraces in relation to larval host plant suitability may have a positive impact on the quantity and quality of silk produced by local tribes.

Studies on the cocoon crop performance of *A. mylitta* reared on a few additional food plants like Ber (*Ziziphus jujuba*), Sidha (*Lagerstroemia parviflora*) and Dha (*Anogeissus latifolia*) at lower altitude during different rearing seasons have already been conducted (Dash et al.1992). Information are also available on induction of biomolecules in mature leaves of *Terminalia arjuna* (Abraham et al. 2004), evaluation of Novel Tasar

Silkworm Feed (Kumar et al. 2013), effect of Feeding Trial (Singh et al. 2011), comparative Study of the Effect of Different Food Plants (Deka & Kumari 2013), altitudinal and seasonal Effects on Growth (Jena et al. 2014), evaluation of cocoons preservation (Dinesh et al. 2012), development of fifth instar female larva (Jena et al. 2015), tropical wild silk cocoons (Mohanty 2003), wild silks of the world (Peigler 1993), studies on cocoon characteristics (Nishide 1998), preservation of seed cocoons (Kapila et al.1992), rearing and cocooning of tropical tasar silk worm (Ojha et al.1994), cocoon and post cocoon studies (Rao & Shamitha 2000), but no information is available on the growth performance of larvae in terms of size parameters of resulting cocoons on different primary as well as unutilized secondary host plants at different altitudes during different seasons.

MATERIALS AND METHODS

The rearing of larvae of "Daba ecorace" of *A. mylitta* during rainy season was conducted selecting a number of healthy food plants having identical age and growth at random from each of the eight species at three different altitudes i.e. lower altitude (50–300 m ASL), medium altitude (301–600 m ASL) and higher altitude (601–900 m ASL). The food plants in wild condition were kept under watch and ward activity throughout the rearing process to protect the larvae from predators and parasitoids. The rainy crop experiment was started with 3000 hatchlings of the same age hatched from 5 B.V. (bivoltine) dfls (disease free lyings) of different females of 'Daba ecorace' of *A. mylitta* supplied by Research Extension Centre, Central Silk Board, Bangriposi, Mayurbahnj, Odisha. The larvae were reared as per recommendation of FAO manual and guidelines published by Regional Tasar Research Station, Central Silk Board, Baripada, Mayurbhaj, Odisha, up to mature fifth instar stage when prominent sexual markings appeared. The mature fifth instar female larvae on each food plant were selected and were marked separately by use of level cards bearing serial number. The larval survival rates related to the different host plants and elevations was about 100%.The larvae allotted with different serial numbers were allowed to grow up to cocoon stage. The percentage of healthy vs unhealthy cocoons for each host plant and elevation was about 99%. The healthy cocoons selected on basis of morphological scoring were collected from each type of food plant and were stored in the grainage house according to their serial numbers. The larval growth was measured in terms of length (cm), diameter (cm), volume (cm^3) and weight (g) of the resulting cocoon. The length and diameter of the cocoon were measured by using millimeter scale and slide caliper respectively. The weight of cocoon was determined gravimetrically by using 0.001 mg sensitive digital balance after removing the pupa by cutting open the shell cover of each cocoon. The volume of cocoon was measured by using water displacement technique. The data so obtained were subjected to calculation of mean and standard deviation ($\bar{x}$ ± SD) values for each growth parameter in each type of food plant at different altitudes. Further, the data were analyzed by using standard t-test and analysis of variance (Sokal & Rohlf 1969). The graphical presentation was also prepared by use of the data to study and establish the correlation of growth performance with food plants and altitude. Further, in order to study the effect of environmental parameters on growth, the air temperature (0 C), RH (%) and rain fall (mm) were recorded at each elevation and the mean ($\bar{x}$ ± SD) value of each was calculated.

RESULTS

Lower Altitude. The growth of female larvae of *A. mylitta* was evaluated in terms of length (cm), diameter (cm), volume (cm^3) and weight (g) of the resulting cocoons during rainy season at lower altitude when the mean ($\bar{x}$ ± SD) air temperature, RH and rain fall were 31.83 ± 0.54 °C, 83.47 ± 1.31% and 300.32 ± 41.31mm respectively. The highest values of length (4.98 ± 0.02), diameter (2.97 ± 0.02), volume (28.33 ± 0.12) and weight (10.64 ± 0.06) were observed in case of the cocoon resulting from the Sal grown larva (Table 1). The cocoon from the female larva raised on Jamun showed the lowest values of length (3.98 ± 0.04), diameter (2.01 ± 0.06), volume (25.92 ± 0.14) and weight (8.98 ± 0.07) (Table 1).

Significant ($p < 0.05$) difference in length, diameter, volume and weight of the cocoons resulting from the female larvae raised on different food plants was indicated by the t-test. The ANOVA test also showed significant ($p < 0.01$) interaction between the food plants and the size parameters of the cocoons grown at lower altitude during rainy season. In view of comparatively superior performance of the size parameters of cocoons from female larvae of *A. mylitta* during rainy season at lower altitude the food plants were graded in the order Sal > Asan > Arjun > Ber > Sidha > Dha > Bahada >Jamun.

Medium Altitude. The growth of female larvae in terms of length (cm), diameter (cm), volume (cm^3) and weight (g) of the resulting cocoons during rainy season at medium altitude was also assessed when the mean ($\bar{x}$ ± SD) air temperature, RH and rain fall were 26.08 ± 0.32 °C, 80.15 ± 1.64 % and 407.86 ± 32.64 mm respectively. The cocoon from the female larva grown on Sal showed the highest size parameters in terms of

TABLE 1. Growth ($\bar{x}$ ± SD) of female larva on various host plants measured in terms of size parameters of the resulting cocoon during rainy season at lower altitude

Food plants	Length (cm)	Diameter (cm)	Volume (cm 3)	Weight (g)
Asan	4.83 ± 0.03	2.86 ± 0.03	28.02 ± 0.11	10.41 ± 0.04
Arjun	4.71 ± 0.04	2.74 ± 0.01	27.71 ± 0.13	10.23 ± 0.03
Sal	4.98 ± 0.02	2.97 ± 0.02	28.33 ± 0.12	10.64 ± 0.06
Ber	4.57 ± 0.03	2.62 ± 0.04	27.36 ± 0.09	10.06 ± 0.04
Sidha	4.46 ± 0.02	2.49 ± 0.03	27.07 ± 0.11	9.82 ± 0.07
Dha	4.31 ± 0.04	2.34 ± 0.02	26.74 ± 0.13	9.51 ± 0.06
Bahada	4.12 ± 0.03	2.18 ± 0.04	26.33 ± 0.12	9.27 ± 0.08
Jamun	3.98 ± 0.04	2.01 ± 0.06	25.92 ± 0.14	8.98 ± 0.07

TABLE 2. Growth ($\bar{x}$ ± SD) of female larva on various host plants measured in terms of size parameters of the resulting cocoon during rainy season at medium altitude

Food plants	Length (cm)	Diameter (cm)	Volume (cm 3)	Weight (g)
Asan	5.26 ± 0.02	3.02 ± 0.02	33.01 ± 0.13	10.57 ± 0.06
Arjun	5.13 ± 0.02	2.91 ± 0.03	32.67 ± 0.11	10.26 ± 0.04
Sal	5.42 ± 0.03	3.13 ± 0.02	33.41 ± 0.16	11.84 ± 0.07
Ber	5.01 ± 0.04	2.77 ± 0.04	32.29 ± 0.14	10.01 ± 0.03
Sidha	4.88 ± 0.03	2.64 ± 0.02	31.92 ± 0.13	9.72 ± 0.06
Dha	4.76 ± 0.02	2.49 ± 0.03	31.53 ± 0.12	9.43 ± 0.07
Bahada	4.61 ± 0.03	2.32 ± 0.04	31.16 ± 0.11	9.11 ± 0.04
Jamun	4.46 ± 0.04	2.18 ± 0.03	30.74 ± 0.11	8.78 ± 0.08

TABLE 3. Growth ($\bar{x}$ ± SD) of female larva on various host plants measured in terms of size parameters of the resulting cocoon during rainy season at higher altitude

Food plants	Length (cm)	Diameter (cm)	Volume (cm 3)	Weight (g)
Asan	5.68 ± 0.04	3.18 ± 0.03	38.16 ± 0.14	12.49 ± 0.07
Arjun	5.56 ± 0.02	3.06 ± 0.02	37.77 ± 0.12	12.22 ± 0.04
Sal	5.84 ± 0.04	3.31 ± 0.04	38.56 ± 0.11	12.81 ± 0.06
Ber	5.43 ± 0.03	2.93 ± 0.03	37.41 ± 0.13	11.94 ± 0.03
Sidha	5.31 ± 0.02	2.78 ± 0.02	36.96 ± 0.16	11.67 ± 0.08
Dha	5.17 ± 0.03	2.64 ± 0.03	36.42 ± 0.12	11.43 ± 0.04
Bahada	4.98 ± 0.04	2.47 ± 0.04	36.08 ± 0.11	11.15 ± 0.07
Jamun	4.79 ± 0.03	2.29 ± 0.06	35.63 ± 0.13	10.78 ± 0.09

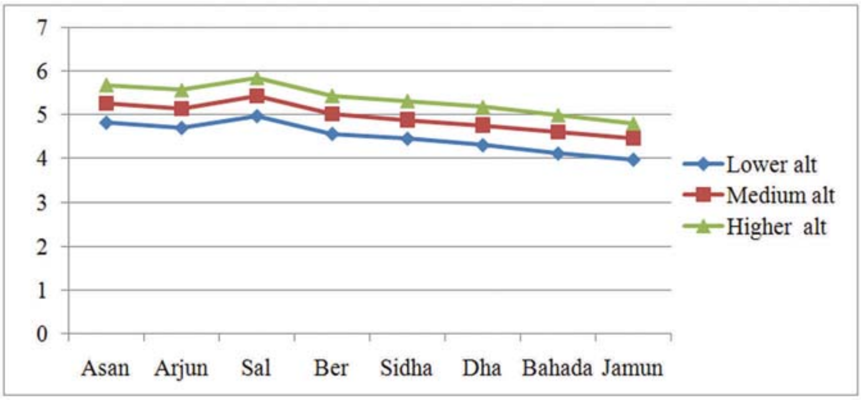

FIG. 1. Growth in length (cm) of cocoon resulting from female larva on various host plants during rainy season at different altitudes.

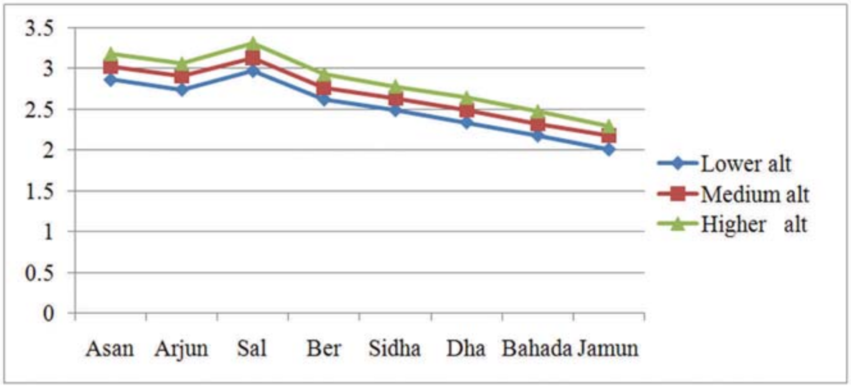

FIG. 2. Growth in diameter (cm) of cocoon resulting from female larva on various host plants during rainy season at different altitudes

length (5.42 ± 0.03), diameter (3.13 ± 0.02), volume (33.41 ± 0.16) and weight (11.84 ± 0.07) (Table 2). The lowest values of length (4.46 ± 0.04), diameter (2.18 ± 0.03), volume (30.74 ± 0.11) and weight (8.78 ± 0.08) were observed in case of the cocoon procured from Jamun (Table 2).

The t-test showed significant ($p < 0.05$) difference in length, diameter, volume and weight of cocoons from female larvae raised on various host plants. Significant ($p < 0.01$) interaction between the different food plants and the size parameters of cocoons spun by female larvae grown at medium altitude during rainy season was also observed from ANOVA test. On the basis of comparatively higher values of growth indices of cocoons resulting from female larvae during rainy season at medium altitude, the experimental food plants were ranked as Sal followed by Asan, Arjun, Ber, Sidha, Dha, Bahada and Jamun.

Higher Altitude. Likewise, at higher altitude during rainy season, the growth of female larvae in terms of length (cm), diameter (cm), volume (cm3) and weight (g) of the resulting cocoons was also evaluated when the mean ($\bar{x}$ ± SD) air temperature, RH and rain fall were 22.86 ± 0.47 °C, 86.67 ± 1.62 % and 608.38 ± 49.91 mm respectively. The highest values of length (5.84 ± 0.04), diameter (3.31 ± 0.04), volume (38.56 ± 0.11) and weight

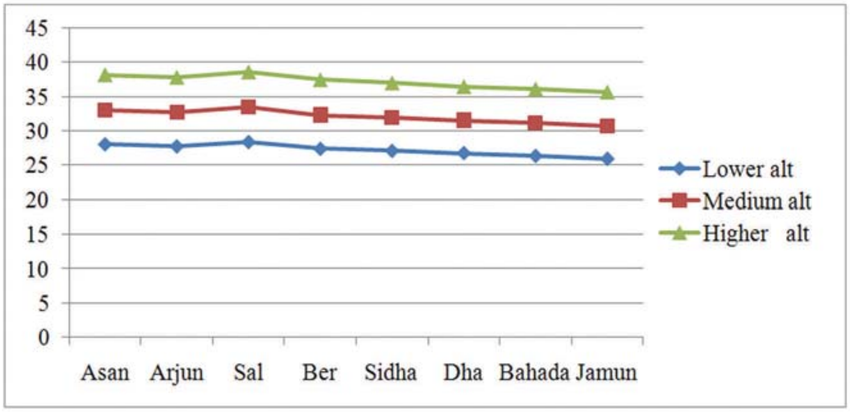

FIG. 3. Growth in volume (cm^3) of cocoon resulting from female larva on various host plants during rainy season at different altitudes.

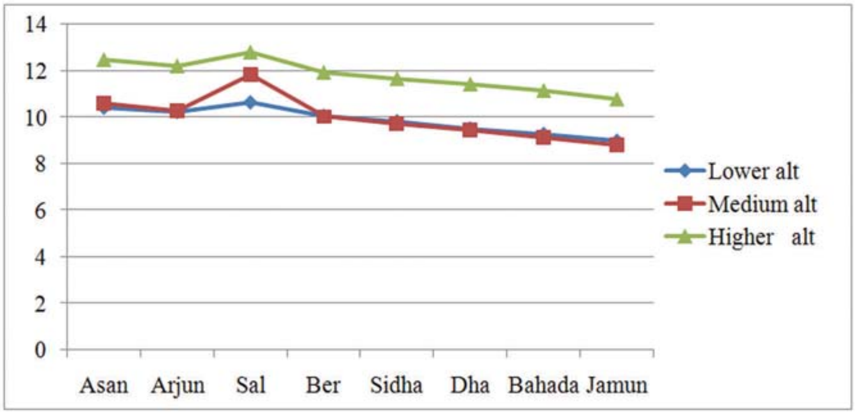

FIG. 4. Growth in weight (g) of cocoon resulting from female larva on various host plants during rainy season at different altitudes.

(12.81 ± 0.06) were observed in case of cocoon from female larva grown on Sal (Table 3).The cocoon procured from Jamun exhibited the lowest length (4.79 ± 0.03), diameter (2.29 ± 0.06), volume (35.63 ± 0.13) and weight (10.78 ± 0.09) at higher altitude during rainy season (Table 3).

The t-test indicated significant ($p < 0.05$) difference in all the size parameters of the cocoons resulting from female larvae grown on different food plants. The ANOVA test also showed significant ($p < 0.01$) interaction between the food plants and the growth indicators of cocoons from female larvae at higher altitude during rainy season. Considering the overall performances of size parameters of cocoons during rainy season at higher altitude, the food plants were graded in the order Sal > Asan > Arjun > Ber > Sidha > Dha > Bahada >Jamun.

At all the three altitudes, during the rainy season, larval growth was superior on Sal and lowest on Jamun hosts, as judged by cocoon length, diameter, volume and weight. (Fig. 1, Fig. 2, Fig. 3 & Fig. 4).

DISCUSSION

Production of better quality cocoons spun by larva of *A. mylitta* during winter season was earlier reported (Sengupta 1986, Dash et al. 1992). Jolly et al. (1974)

reported a superior cocoon crop when larvae raised on Sal. Dash et al. (1992) recorded superiority of Sal for cocoon crop parameters (weight of cocoon, pupa and shell) at lower altitude during rainy season only; whereas superiority of Asan was observed during autumn and winter season in the same altitude. In the present investigation the growth of female larva in terms of length, diameter, volume and weight of resulting cocoons during rainy season at lower, medium and higher altitudes was observed to be the highest in Sal. This shows the superiority of Sal among all the host plants for cocoon crop performance of *A. mylitta*. This might be due to better nutritional supplement obtained from Sal leaf for growth of cocoon favoured by prevailing optimum climatic conditions which can be ascertained by further biochemical investigation.

Nayak et al. (1992) reported that voltinism in wild silk moth *Antheraea paphia* L. is primarily governed by altitudinal gradient as well as the changing environmental factors. Change of attitude also influences the change of body size in many lepidopterans and there is a positive size to altitude relation (Sullivan & Miller 2007). The life span and growth of different stages of *Antheraea paphia* L. varies at different altitudes (Dey et al. 2010).The present findings reflect the highest growth of female larvae in terms of size parameters of resulting cocoons in all respect at higher altitude during rainy season when the mean ($\bar{x}$ ± SD) air temperature, RH and rainfall were 22.86 ± 0.47 °C, 86.67 ± 1.62 % and 608.38 ± 49.91 mm respectively irrespective of the species of host plants. The lowest larval growth in terms of size parameters of cocoons spun was recorded at lower altitude during rainy season when the mean ($\bar{x}$ ± SD) air temperature, RH and rainfall were 31.83 ± 0.54 °C, 83.47 ± 1.31% and 300.32 ± 41.31mm respectively. The probable reasons for the greater performance of growth parameters of cocoons from female larvae of *A. mylitta* with increase in altitude might be due to decrease in temperature but increase in RH as reported by many earlier workers in different Antheraea species.

Jolly (1966) reported that Asan, Arjun and Sal host plants are of primary importance and are most often used for cocoon crop performance. Dash et al. (1992) reported acceptable cocoon crop performance on the food plants like Asan, Arjun, Sal, Ber, Sidha and Dha only. But the present study indicates consideration of Ber, Sidha and Dha as alternate hosts for rearing activities of *A. mylitta* when there is inadequacy of primary host plants in the rearing field without significantly reducing the cocoon crop yield, although they are graded as secondary food plants by Jolly (1966). The present finding further indicates encouraging results on Ber which can be also included as a primary food plant of *A. mylitta*, since the overall performance on it remains very much at par with Sal and Arjun. However, in case of acute shortage of food plants during peak period of rearing seasons, the consideration of food plants like Sidha and Dha for rearing purpose is suggested here. In the present study the growth performance of female larvae in terms of size parameters of resulting cocoons on Bahada food plant indicates acceptable results for its utilization at the time of severe scarcity of food plants. The growth performance was unsuitable on Jamun (*Syzygium cumini*) at all the three altitudes during rainy season which indicates the commercial non viability of this food plant for rearing activities. In order to draw a concrete conclusion, further investigation on the above growth parameters at the other stages of life cycle of the silk worm during different rearing seasons may be carried out.

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A NEW HOST PLANT AND NOTES ON THE LAST LARVAL INSTAR OF *COLOBURA ANNULATA* (NYMPHALIDAE: NYMPHALINAE) IN SURINAME

Additional key words: *Cecropia*, *sciadophylla*, Urticaceae, Neotropics

The genus *Colobura* Bilberg, 1820 (Lepidoptera: Nymphalidae: Nymphalinae) has two species (Lamas 2004). *C. dirce* (Linnaeus, 1758) is distributed from Mexico and the Greater Antilles to Argentina and SE Brazil in two subspecies (Smith et al. 1994, Willmott et al. 2001). Six species of *Cecropia* (*C. hololeuca*, *C. insignis, C. obtusa, C. obtusifolia, C. pachystachya, C. peltata*) and one species of *Coussapoa* (*C. nymphaeifolia*) have been reported as host plants, all Urticariaceae (Wilmott et al. 2001, Beccaloni et al. 2008, Robinson et al. 2017, Janzen & Hallwachs 2017). In Suriname, there are numerous records for *Colobura dirce* from the northern part of the country, from primary and secondary forests as well as cultivated areas (Gernaat et al. 2012). The larvae feed on *Cecropia obtusa* (second author, pers. obs.).

Colobura annulata Willmott, Constantino & J. Hall 2001 (Fig. 1) ranges from S Mexico to W Ecuador and from Venezuela, Trinidad and the Guianas to Bolivia (Willmott et al. 2001). Host plants records known from Costa Rica and Colombia are *Cecropia insignis, C. longipes, C. obtusifolia, C. peltata, C. virgusa* and *Pourouma cecropiifolia* (Wilmott et al. 2001, Beccaloni et al. 2008, Janzen & Hallwachs 2017). In Suriname, there are scattered records of *Colobura annulata* from northern (Rama (Fig. 1), Brownsberg) and central (Raleigh falls) primary forests.

We describe a new host plant for *C. annulata* from Suriname, add descriptive detail to the last instar larva and mention some aspects of late larval variation. On 25 October 2015, at Plantage Katwijk, Suriname (05º 51' 50" N, 54º 59' 45" W, 5 m asl; about 22 km NEE of Paramaribo), two *C. annulata* larvae were found feeding on the foliage of a *Cecropia* tree at a height of about 2.5 m. They were collected and reared according to standard methods in Paramaribo. On 25 October 2015, one larva was 41 mm long. The next day, it had stopped eating and was 31 mm. It pupated in the afternoon of 27 October. The pupa was 30 mm long. A male *C. annulata* eclosed on 6 November 2015, it was deposited in the collection of Naturalis Biodiversity Center. The length of the second larva was 26 mm on 25 October and 37 mm on 29 October. It pupated on 1 November. The next day, it had died of unknown causes. The larvae were compared with a larva collected in the field and reared in the Lelydorp butterfly garden in Suriname in 2010. Photographs were made with Nikon D300s and D700 cameras, an AF Micro Nikkor 105 mm 1: 2.8 D lens and a SB-800 flash. Photographs were made in NEF-format and with minor adjustments of exposure, contrast and sharpening converted to TIF-files in the same color space.

Host plant (Fig. 2). The host plant was identified as *Cecropia sciadophylla* Mart. Description (Mori et al.

FIG. 1. *Colobura annulata*, female, forewing length 42 mm, Rama, Suriname, 22-VII-1972, leg. D. Schilder, coll. Naturalis Biodiversity Center; dorsal (left) and ventral (right) view (from Gernaat et al. 2012).

FIG. 2. *Cecropia sciadophylla* Mart., host plant of *Colobura annulata* in Suriname. **A.** pistillate inflorescence. **B.** staminate inflorescence eclosed by spathe. **C.** staminate inflorescence. **D.** palmately compound leaf with leaflets. **E1**: pistillate flower with tubular perianth. **E2**: tuberculate achene. **E3**: seed. **E4**: apical view of pistillate flowers. **F1**: apical view of staminate flowers. **F2**: lateral view of staminate flowers with tubular perianth. **F3**: lateral view of staminate flowers with anthers exerted. **F4**: detail of stamens. Drawing by W. Hekking, Naturalis Biodiversity Center.

2002, van Andel & Ruysschaert 2011): Tree, up to 30 m, unisexual, with stilt roots. Stem and branches with circular scars, hollow with septa, cavities not inhabited by ants, trichilia absent. Stipules envelop the bud, sparsely hirsute, up to 50 cm. Leaves alternate, long petiolate, palmately compound; leaflets 11–15, petiolate, up to 60 × 10 cm; adaxially glabrous, abaxially sparsely hirsute. Inflorescence a unisexual, axillary, petiolate bundle of spikes, surrounded by densely hirsute bract. Male inflorescence with 8–15 spikes 0.4 – 0.8 cm thick; female inflorescence slightly larger, with 4–6 spikes, in fruit up to 20 × 2 cm. Fruit small, tubular. Seed 1, small.

Last instar larva (Fig. 3). Overall appearance of a black caterpillar with two orange head horns, white transverse rings and yellow scoli. **Head**: Vertices, lobes, and frontoclypeus shiny black. Epicranial notch shallow. On both sides of the epicranium a long (about 1.4 times the distance between the epicranial notch and the mid-labrum) orange scolus with multiple, dark red-tipped spines of various length, each ending in a seta. Laterally on the head capsule, multiple, prominent chalazae. Epicranial suture and ecdysial lines gray. Anteclypeus gray-brown, labrum dark gray.

Thorax: Ground color velvet black, intersegmental membranes gray. T1 with a black prothoracic shield with a narrow, interrupted, middorsal light gray stripe (continuous with epicranial suture) and, depending on light conditions, an irregular pattern of transverse gray bands from the middorsal stripe to the subdorsal area; subdorsally, a white-based, brown-tipped, yellow-orange spine on either side of the gray bands; length of prothoracic shield about 60% of the segment; spiracle black. T2 and T3 with paired midsegmental subdorsal and lateral scoli. The scoli are dark yellow, some with a creamy-white basal part, and have five to seven spines, the tip of which may be dark brown. The lateral scolus on T2 is located just above the T1 spiracle, the one on T3 at the same level as the A1 spiracle.

Abdomen: Ground color velvet black, including spiracular area. Intersegmental membranes brown-gray. Prolegs on A3–A6 and A10, with multiple setae, a rather short, black base and gray planta. A1–A8 with subdorsal, lateral and subventral scoli, A9 with lateral scolus only. Scoli as on thorax. Abdomen with creamy-white rings, encircling the body between each segment, anteriorly and posteriorly on the segment and adjacent to the intersegmental membranes, running transversely with their lowest point on either side just below the lateral scoli or about midway between the lateral and subventral scoli. Caudoventrally to the anterior tranverse band, there is an oval, teardrop-shaped, triangular or almost rectangular creamy-white spot of varying size, its upper edge at, just above or below the lateral scoli, its lower end about midway between the lateral and subventral scoli. Anal plate black with multiple setae.

FIG. 3: Last instar larvae of *Colobura annulata* in Suriname. **A**: last instar larva, Suriname, Plantage Katwijk, 25 October 2015; dorsolateral view, note one two-spined subdorsal scolus on A6. **B**: second last instar larva, Suriname, Plantage Katwijk, 25 October 2015; lateral view, note lateral scolus on A4 lacking and rectangular/oval white lateral spots. **C**: last instar field-collected larva, butterfly farm Lelydorp, 24 November 2010; note teardrop-shaped white lateral spots. Photographs: A–B: second author, C: Borgesius G. Beckles.

Cecropia sciadophylla is a new host plant record for *Colobura annulata*. The tree is distributed throughout the Guianas and the Amazon region. In Suriname, it is common in abandoned plots, secondary forest and open areas in primary forest (Mori et al. 2002, Van Andel & Ruysschaert 2011). In view of its wide distribution, partly overlapping the range of *C. annulata*, it is likely to be found as a larval host plant in several other countries.

The last instar *C. annulata* larvae showed variation in the number of spines of the scoli (Fig. 3a, b), in the shape and size of the abdominal lateral creamy-white spots (Fig. 3b, c) and one individual had the A4 lateral scolus on one side missing (Fig. 3b). Further research is required to further document larval variation, defence mechanisms, pathogens, predators and parasitoids.

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TWO RECORDS OF *PHEREOECA PRAECOX* (TINEIDAE) IN SOUTH CAROLINA AND OBSERVATIONS ON ITS BIOLOGY

Additional key words: feeding, bagworm, household casebearer, distribution, barcode

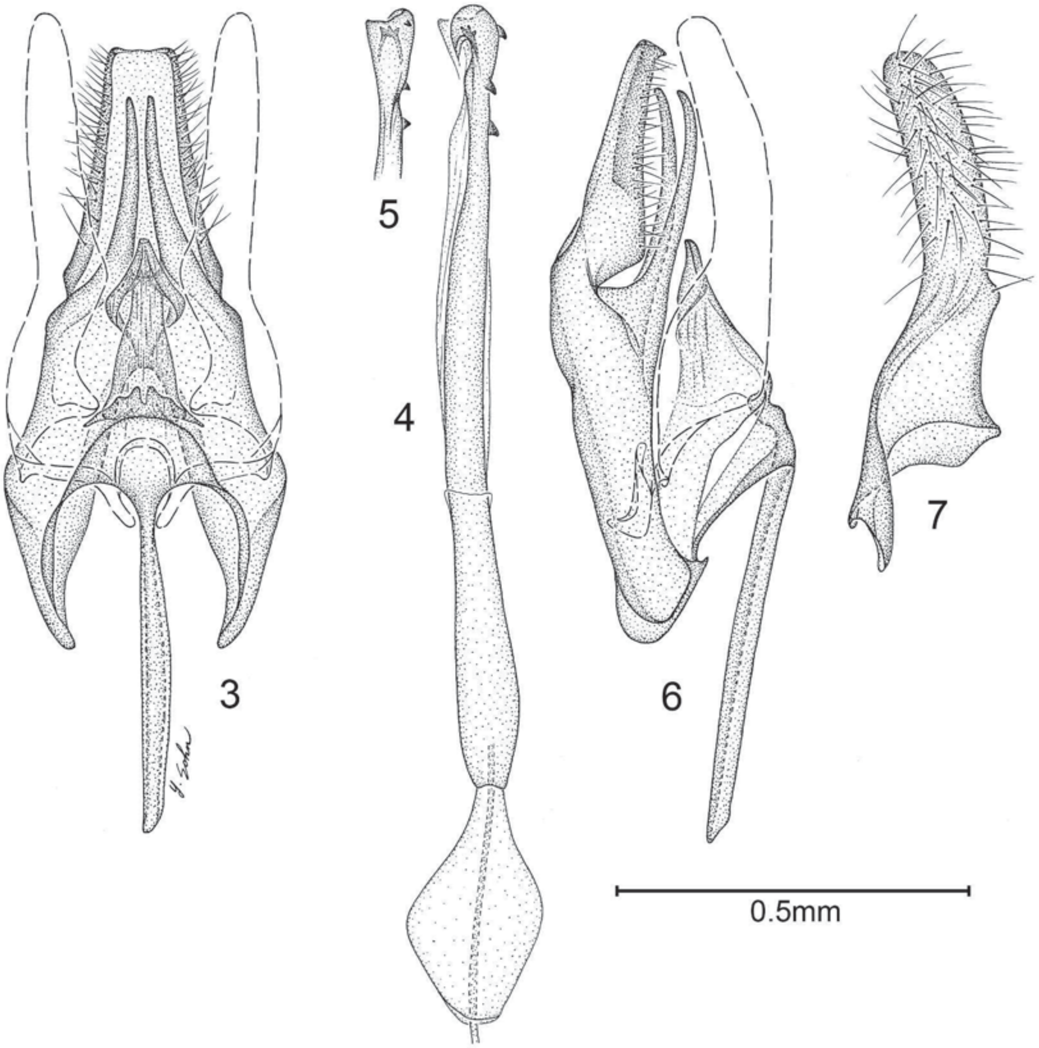
The genus *Phereoeca* contains several micro-moths that typically occur in tropical and neotropical locations. To date, two *Phereoeca* species have been found in the United States: *Phereoeca praecox* (Gozmany and Vari, 1973) (Fig. 1) and *Phereoeca uterella* (Walsingham, 1897). *P. praecox* occurs in the western United States (Gulmahamad 1999; Powell and Opler 2009), while *P. uterella* occurs in Florida, Louisiana, Mississippi and North Carolina, and is predicted to be present throughout most of the coastal regions of the South (Kimball 1953, Villanueva-Jimenez et al. 2010).

Both of these species spend their entire larval stage in a case (Fig. 2) that they spin out of silk and cover with sand and other debris. The cases are flat, 8 to 14 mm long and 3 to 5 mm wide. The larvae have a brownish fuscous head and sclerites on the dorsal thorax, and reach a length of about 7 mm when fully developed. Adult females are larger than males, with wingspans of 10 to 13 mm and 7 to 9 mm respectively (Walsingham 1897, Hinton 1956, Gozmany & Vari 1973).

P. praecox and *P. uterella* reportedly feed on wool carpets, clothing, and other fabrics, making them minor pests, though they are not known to be of major economic concern (Heppner 2005). *Phereoeca* are most often found in bathrooms and other humid places in homes when encountered by humans (Kea 1933), but little is known about specific habitat requirements.

While the two moth species have a similar overall appearance, *P. praecox* and *P. uterella* can be distinguished by differences in the COI barcoding sequences and by the morphology of the male genitalia (Figs. 3–7). In particular, the male valvae of *P. praecox* (Fig. 7) are broader (more than twice the width) than the valvae of *P. uterella*.

FIGS. 1–2. *Phereoeca praecox*. **1.** (♀), Clairmont, San Diego County, California. Wingspan 12.8 mm. **2.** Larval case of adult in Figure 1. Length of case 10 mm.



FIGS. 3–7. *Phereoeca praecox*. Male genitalia: **3.** Ventral view of genital capsule. **4.** Phallus. **5.** Apex of phallus. **6.** Lateral view of genital capsule. **7.** Valva, mesal lateral view.

FIG. 8. *Phereoeca praecox* larvae feeding on feces from Aruba Island rattlesnake (*Crotalus unicolor*) in exhibit in the Aquarium Reptile Complex at Riverbanks Zoo and Garden.

We collected larval and adult *P. praecox* specimens from two separate locations in South Carolina, representing the first documented occurrences of this species in the state. We collected a single larval specimen from the wall in the bathroom of a private residence in Florence County, South Carolina, USA in April 2014 near Second Loop Road and Poinsett Drive (34°09'58" N, 79°47'07" W). We also collected several larval and adult specimens for examination from exhibits and a service area in the climate-controlled Aquarium Reptile Complex at the Riverbanks Zoo and Garden in Richland County, South Carolina in August and November 2016 respectively (34°00'37" N, 81°04'27" W), where a breeding population has been established for at least seven years. We collected all specimens by hand and preserved them in 95% ethanol. We identified specimens morphologically (e.g., Hinton 1956) and genetically using the barcoding region of the COI gene (Folmer et al. 1994, Hebert et al. 2003). We used four Riverbanks Zoo specimens for DNA analysis which all yielded an identical haplotype (Genbank Accession No.: KY575118) of 658 nucleotides. While *P. praecox* occurs in the western United States, these are the first documented findings of the moth in South Carolina, which expands the moth's known range in the United States.

Larvae at Riverbanks Zoo occur in a wide range of reptile habitats, including temperate rainforest, tropical rainforest and xeric conditions, and larvae do not appear to show a preference for moisture levels. Our observation of larvae in xeric exhibits suggests they may not be dependent on high humidity. Pupae are commonly observed on the sides of the exhibit or under rocks, logs and other enclosure furniture and on the concrete walls in the service area behind the exhibits. Adults are also commonly observed in exhibits and on the service area walls.

Larvae are active throughout the day, especially when organic matter is present. Larvae feed on fecal material (Fig. 8) and the molted skins from snakes and lizards as well as on dead rodents offered to reptiles for food. Larvae found on dead rodents aggregate around the rodents' faces or near openings where cockroaches have chewed through the carcasses, though we have not observed if larvae feed on hair or flesh.

While it is generally accepted that *Phereoeca* species feed on proteinaceous materials, specific larval feeding habits are the subject of contention. It has been widely reported that the larvae feed on woolens and furs (e.g., Kea 1933, Mallis 1990); however, some authors (Robinson & Nielson 1993, Heppner 2005) suggest that these claims are false and based on misidentification of the larvae. Hetrick (1957) suggested that larvae most commonly feed on the silk of spiders, psocopterous and embiopterous insects based on his observations of wild and caged larvae. He also speculated that the silk of other insects might provide food for the larvae. More recently, larvae were observed feeding on cotton window shades (Heppner 2005), though this seems atypical since cotton is largely cellulosic, rather than proteinaceous. Moreover, larvae have refused cotton fibers when held in captivity (Kea 1933). Our observation of carcass feeding is novel, though we cannot say if it is common outside of the confines of the Riverbanks Zoo. However, it does show that larvae are able to exploit a greater range of food sources than previously reported. A more thorough understanding of feeding requirements awaits additional study.

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Send ***Journal*** submissions electronically to: **ksummerville@drake.edu** or to the editor at the above address. Contributors should feel free to recommend one or two reviewers upon submission of their manuscript.

Before submitting manuscripts or electronic files, consult instructions on the Society's web site at **http://www.lepsoc.org/**.

Authors should provide an electronic copy of the manuscript in a standard PC or Macintosh-based word processing format.

Abstract: Include an informative abstract for Articles, Profiles, and Technical Comments.

Additional key words: Up to five key words or terms not in the title should accompany Articles, Profiles, General Notes, and Technical Comments.

Text: Write with precision, clarity and economy, and use the active voice and the first person whenever appropriate. Make title explicit, descriptive, and as short as possible. The first mention of a plant or animal in the text should include the *full scientific name* with *author,* and *family.* Use metric units for measurements, and a 24-h clock (0930 h, not 9:30 AM) to express time. Underline only where *italics* are intended.

Literature cited: References to Articles, Profiles, General Notes, and Technical Comments should be given as Sheppard (1959) or (Sheppard 1959, 1961a,b) and listed alphabetically under the heading Literature Cited, in plain text (**do not capitalize author names**) and without underlining as follows:

Sheppard, P. M. 1959. Natural selection and heredity. 2nd ed. Hutchinson, London. 209 pp.

—— 1961a. Some contributions to population genetics resulting from the study of the Lepidoptera. Adv. Genet. 10:165–216.

Illustrations: Electronic submissions are expected. Initial submissions may be low-resolution *.jpg format, but final illustrations should be *.tif format with image layers flattened, saved at a resolution of at least 350 ppi (137 pixels per cm). Illustrate only half of symmetrical objects such as adults with wings spread, unless whole illustration is crucial. Bear in mind that your illustrations will be sized to fit a *Journal* page (plan to make lettering sufficiently large) and that the figure legend will appear at the bottom of the page (*Journal* page: 16.5 cm width, 22.5 cm height). One-column illustrations should be a minimum of 1000 pixels wide; two-column illustrations should be at least 2200 pixels wide. Figures, both line drawings and photographs, should be numbered consecutively in Arabic numerals; do not use "plate." Type figure legends double-spaced at the end of the manuscript following Literature Cited. Use a separate paragraph for each legend. Color illustrations are encouraged; contact editor or consult our web site for submission requirements and cost.

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