Full Issue

Source: The Journal of the Lepidopterists' Society, 70(3)

Published By: The Lepidopterists' Society

URL: https://doi.org/10.18473/lepi.v70i3.a12

Volume 70 Number 3
2016
ISSN 0024-0966

Journal of the Lepidopterists' Society

Published quarterly by The Lepidopterists' Society

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Journal of The Lepidopterists' Society (ISSN 0024-0966) is published quarterly by The Lepidopterists' Society, *c/o* Chris Grinter, Asst. Secretary, Illinois Natural History Survey, 1816 South Oak St., Champaign, IL 61820-0904. Periodicals postage paid at Champaign, IL and at additional mailing offices. POSTMASTER: Send address changes to THE LEPIDOPTERISTS' SOCIETY, *c/o* Chris Grinter, Illinois Natural History Survey, 1816 South Oak St., Champaign, IL 61820-0904.

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Cover illustration: A monarch butterfly chrysalis. Although monarch larvae are highly conspicuous, due to their warning coloration, when the butterfly larva undergoes metamorphosis and becomes a pupa, the insect becomes very hard to find due to the cryptic coloration of the chrysalis in which the pupa resides. Photo courtesy of John Alcock.. See article on page 177.

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70(3), 2016, 177–181

MONARCH BUTTERFLIES USE REGENERATING MILKWEEDS FOR REPRODUCTION IN MOWED HAYFIELDS IN NORTHERN VIRGINIA

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ABSTRACT. The effects of mowing milkweeds in areas visited by monarch butterflies (*Danaus plexippus* L., Nymphalidae) were studied by counting the eggs and larvae on regenerating common milkweeds (*Asclepias syriaca* L., Apocyanaceae) in five adjacent mowed hayfields in northern Virginia in late summer 2015. At the same time monarch larvae were counted on mature senescent common milkweeds in unmowed areas adjacent or near to the mowed hayfields. Milkweeds supported populations of immature monarchs in both habitat types with initially many eggs and early instars found on regenerating plants in the mowed hayfields while late instars dominated the unmowed older milkweeds. As September proceeded, the censuses revealed an increase in the numbers of late instars on the mowed regenerating milkweeds whereas the abundance of larvae declined sharply on the older senescing milkweeds, many of which had lost all or most of their leaves. The study showed that late season mowing of hayfields provided adult female monarch butterflies with rejuvenated resources for reproduction during a time when senescent milkweeds were becoming unsuitable for the monarch larvae. Our findings have implications for managing land in ways to benefit monarchs and for mitigating the widespread decline of milkweeds, although the research raises several caveats and more needs to done to measure the fitness of monarch adults that are produced late in the flight season of the butterfly.

Additional key words: extension of breeding season by mowing, milkweed regeneration, monarch butterfly conservation

Monarch butterflies (*Danaus plexippus*) have experienced a dramatic population decline over the past two decades and various factors have been proposed as causes of this decline. Among the possible factors is (1) degradation of the Mexican high elevation forests of Oyamel fir (*Abies religiosa*, H.B.K., Pinaceae) where the monarchs overwinter (Brower et al. 2012, Vidal & Rendon-Salinas 2014, Brower et al. 2016) despite some success in protecting and reforesting critical overwintering habitat (Vidal et al. 2014). Another major contributor to monarch declines is (2) the widespread application of herbicides to herbicide-tolerant agricultural plants in the United States with the consequent loss of farm field milkweeds (Pleasants & Oberhauser 2012). Moreover, monarch butterflies are also affected by (3) the loss of extensive milkweed habitats in the United States to housing developments, industrial expansion, and the reduction of the acreage in the USDA Conservation Reserve Program (Taylor 2014). Flockhart et al. (2015) considered the losses of milkweed breeding habitat in the United States to be the key to understanding the population collapse of the monarch. As Taylor emphasized, government entities, conservationists, and the general public in the United States should try to restore milkweed populations so that the phenomenon of monarch butterfly migration will persist.

Increased awareness of monarch decline, highlighted by submission of a petition to the US Fish and Wildlife Service to designate the monarch as a threatened species (Crouch et al. 2015), has contributed to a focus on the possible causes of and suggested mitigations of the species' collapse. For example, Freese & Crouch (2015) and Mirocha (2015) have documented the massive increase over the past two decades in acreage planted with "Roundup Ready" corn and soybean crops that are genetically modified to resist the herbicide glyphosate that kills milkweeds and nectar source plants. The loss of milkweed has led several organizations (e.g., Journey North, Monarch Joint Venture, Monarch Watch, The Xerces Society) to encourage the planting of milkweeds in home yards and gardens, along right-of-ways, as well as to question the frequent mowing of roadside verges that often support milkweed populations.

In addition, Fischer et al. (2015) and Baum & Mueller (2015) have shown that appropriately timed mowing during the growing season can lead to the

regeneration of milkweeds, which provides a fresh supply of food for larval monarchs later in the season when most of the naturally growing milkweeds have senesced. It is this last possibility that we explore in northern Virginia.

MATERIALS AND METHODS

The abundance of monarch butterfly eggs and caterpillars were monitored by the first author in five large mowed hayfields at Monterey Farm, a 200+ acre farm in Fauquier County, VA, near 38°52'26"N, 77°54'21"W at approximately 165 m elevation. Most of the acreage of the farm is hayfield, and haying of the fields took place from 8 to 16 August 2015. The farm owners have an arrangement with a hay cutter that specifies a single cutting per summer and the retention of unmowed buffer strips between wooded areas and the hayfields.

On 30-31 August, regenerating common milkweeds, *Asclepias syriaca* L., in mowed areas were searched for monarch eggs and larvae, and twelve patches of unmowed mature milkweeds were also examined. Counts were made every five days from 30 August to 23 September, giving a total of six censuses, each requiring 90–120 min. The unmowed patches in the buffer strips were close to or adjacent to the mowed areas that were inspected and so could be readily monitored. No attempt was made to check the same plants in the hayfields on successive censuses. The main purpose of the study was to document whether the regenerating milkweeds attracted reproductive females by observing ovipositing females and larvae in this habitat. The hayfield milkweeds were largely *A. syriaca* but also present was the significantly less common honeyvine milkweed (*Cynanchum* leave (Michx.) Pers.) as well as very uncommon (in the hayfields) butterfly weed (*A. tuberosa* L.). In addition, the censuses were designed to reveal if and when the unmowed common milkweeds near the hayfields were simultaneously utilized by monarchs. Observations made as the season progressed were done to establish whether any caterpillars in the hayfields developed into mature larvae when the larvae were no longer present on the mature milkweeds in the buffer zones. This would demonstrate that the reproductive season of the butterfly had been extended by cutting the hayfield grasses and weeds.

RESULTS

Common milkweeds had begun regenerating in large numbers in the mowed hayfields by 24 August, 16 days after haying had begun and just a few days after rains on 20 and 21 August had drenched the fields. By 1 September, the milkweeds had grown substantially so that many hundreds were already between 25 cm and 50 cm in height, much taller than the grasses, which were slower to regrow (Fig. 1).

Between 3 and 16 September, observations of the mowed hayfields yielded 15 records of ovipositing females, three of which were laying on the leaves of *C. leave*, demonstrating that both regenerating milkweeds

FIG. 1. (**a**) Young regenerating milkweeds, *Asclepias syriaca*, in the mowed hayfields (photographed on 24 August 2015). (**b**) Plants of *A. syriaca* that have regrown rapidly (photographed on 1 September 2015). (**c**) A monarch butterfly egg laid on a leaf of the honeyvine milkweed (*Cynanchum laeve*) (photographed on 3 September 2015). Photos: J. Alcock.

did attract adult monarch females. On 17 September two females perched on larval foodplants and bent their abdomens into the egg-laying position but did not oviposit on the selected plants. On 18 September and afterwards, no females were seen exhibiting oviposition behavior in the mowed areas; however, one egg was found in the mowed hayfields on 23 September.

Fig. 2 presents the results of the six censuses of the mowed and the adjacent patches of unmowed milkweeds. Although the milkweeds in the unmowed patches were senescent in early September, their yellowed, tattered leaves were nevertheless being eaten by substantial numbers of late instar caterpillars at the outset of the study (Fig. 3). By the later censuses the unmowed milkweeds were occupied by only a few larvae of any size. In contrast, the regenerating milkweeds in the mowed field soon became populated by early instar larvae, so that late instar caterpillars were present on the regenerating milkweeds through 23 September. The significant comparisons in these data are between the numbers of juvenile stages in the two habitat types on the same date.

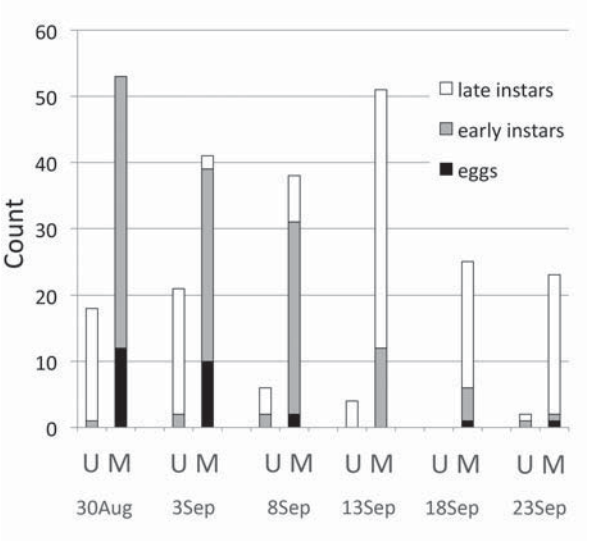

Fig. 2. The number of eggs, small larvae (first, second and third instars), and large larvae (fourth and fifth instars) found on senescent *Asclepias syriaca* in unmowed borders to the hayfields in comparison to the numbers of those found on regenerating *A. syriaca* and honeyvine (*Cynanchum laeve*) in the mowed hayfields. Six censuses were conducted from 30 August to 18 September 2015.

Discussion

The consequences of using mowing to promote regeneration by the common milkweed were examined by Fischer et al. (2015) in upstate New York during an experiment in which plots of milkweed-rich fields were mowed at three different times while control strips were left unmowed. The experimental and control strips were then monitored for milkweed regeneration and their use by monarch butterflies. The authors reported that milkweeds in the mowed strips did indeed regenerate and attract adult female monarchs but only if the mowing was done in July, not in August, to give time for the plants to regrow at this more northerly latitude. Therefore, the timing of mowing was critical to monarch reproduction. As the authors noted, the effectiveness of mowing as a conservation measure almost certainly depends not only on when the mowing occurs but on local phenology at that geographic location and the species of milkweeds involved.

The conclusion of Fischer and co-authors is supported by the current study in northern Virginia in which mowing of hayfields was not completed until mid-August and yet the milkweeds did regenerate sufficiently at this lower latitude to be used by ovipositing female monarchs well into September. The young regenerating milkweeds were quickly discovered in mid-August by the butterflies, and relatively large numbers of eggs and early instar larvae were quickly produced. In addition, females also had access to large numbers of the honeyvine, another perennial milkweed that regenerated strongly in the mowed fields. In contrast, the unmowed areas provided acceptable food for monarch larvae only early in the study even though the patches of *A. syriaca* at this time (mid-August) were dominated by senescent plants with yellowed and damaged leaves. By mid- to late September these senescent milkweeds often were leafless, and as a result, few caterpillars were found on them. At this time, surviving caterpillars in the mowed hayfields were primarily large, late-instar larvae. These results suggest that monarch reproduction in northern Virginia may benefit from mowing portions of overgrown fields with common milkweeds fairly late in the season while also maintaining the senescent common milkweeds in the borders to these fields or in places nearby. In the unmowed patches, mature monarch larvae were present in substantial numbers through 3 September whereas those in the mowed field were present through 23 September; that is, the breeding period was extended by nearly three weeks. Mowing in New York, about 3° latitude farther north than this Virginia site, extended the breeding period for at least two weeks, a similar length of time. A primary difference between the results in the NY and VA sites was that mowing in NY on 17 August produced very little milkweed regrowth, whereas mowing on the same date in the more southerly Virginia site did result in substantial new

FIG. 3. An unmowed patch of the common milkweed (*Asclepias syriaca*) photographed on 23 August 2015 at a time when the senescent plants still possessed many yellow, damaged leaves most of which had fallen off by mid-September. (INSET) A fifth instar monarch caterpillar that was feeding on the damaged leaves of an old milkweed growing in an unmowed patch photographed in September 2015. Photos: J. Alcock.

growth. In the future it would be valuable to conduct mowings at similar times across several latitudinally different sites to determine the degree to which the timing of one or more cuts can extend the geographic breeding windows. Much more work, however, remains to be done to determine the best mowing regimes at different latitudes.

Nevertheless, there are now two studies spanning 500 km (approximately 3°) of latitude indicating that mowing of late season milkweeds has the potential to provide fresh plants for ovipositing females, resulting in an extended growing season for the larvae that feed upon the regenerated milkweeds. If properly timed mowing were instituted on a broad scale, the current widespread loss of milkweeds suitable for monarch caterpillars caused by the increasing use of herbicides on household landscapes, commercial agricultural crops, roadsides, and power and gas line right-of-ways might be ameliorated to some degree. As seen in this study, however, management of land for increased use by monarchs may effectively combine regenerating milkweeds in mowed areas with uncut milkweeds in nearby unmowed habitat, thereby providing continuously available larval hostplants during mid to late summer extending the window of breeding.

There are, however, several caveats raised by the present study. The first is whether the loss of eggs and caterpillars during the mowing of the fields is compensated by reproduction on the regenerating milkweeds. As we have shown, the breeding season is extended but we do not know whether the offspring of monarchs produced during the lengthened reproductive can migrate successfully to Mexico. As Batalden and Oberhauser (2015) have demonstrated, senescing milkweed leaves are one of a constellation of migratory stimuli and their replacement by regenerating young milkweeds could slow adults from entering reproductive diapause and migrating. Another caveat is

that an extended breeding season could increase the possibility of infection by the protozoan parasite *Ophryocystis elektroscirrha* (Bartel et al. 2011, Satterfield et al. 2015). The use of regenerating milkweeds could in other words be an ecological trap for migrant adults that are induced by the availability of fresh milkweed plants to reproduce at a time when they normally would have little or no chance of increasing their production of viable young. Resolving these points is essential before we can say with certainty that the late season haying of fields with mature milkweeds provides a net benefit for monarch butterflies. However, this study and that of Fischer et al. (2015) effectively document that properly timed mowing can extend the time available for monarchs to reproduce.

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Submitted for publication 30 October 2015; revised and accepted 11 January 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 182–193

POPULATION BIOLOGY OF MONARCH BUTTERFLIES, *DANAUS PLEXIPPUS* (L.) (LEPIDOPTERA: NYMPHALIDAE), AT A MILKWEED-RICH SUMMER BREEDING SITE IN CENTRAL WASHINGTON

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ABSTRACT. The population biology of adult Monarch butterflies (*Danaus plexippus*) was studied during regular visits over three years (June–September 2013–15) at a milkweed (*Asclepias speciosa*)-rich site in central Washington. Small numbers of spring migrants colonized the site during June 5–17 each year and produced two adult generations one in early July and the other in late July–August, increasing the population at the site until mid–late August in 2013 and 2014. Greatest numbers of adults occurred in late July and August (20–24 per hour). In 2015 the population fell substantially in early August apparently as a consequence of heat wave conditions in late June–early July adversely affecting survival of second generation immature stages. Mark, release and recapture provided maximum population estimates at the site of 160–190 males, a recapture rate of 25–32% and intervals between tagging and recapture of 5–39 days. Sex ratio was imbalanced in favor of males on all dates ranging from 57–100%. Males patrolling milkweed patches was the most common behavior observed. Nectaring on *A. speciosa* and the exotic Purple Loosestrife (*Lythrum salicaria*) was frequently observed and the introduced Russian Olive (*Elaeagnus angustifolia*) was used for resting during the day. Dispersal from the site occurred in late August or early September. The use of large, dense areas of milkweed in relatively moist locations with some shade may be an important component of summer breeding of *D. plexippus* in the arid western United States. Expanding and/or creating additional such sites may be a useful conservation strategy for *D. plexippus* in the arid west.

Additional key words: Western North American population, *Asclepias speciosa*, population estimates, sex ratio, wing condition, generations, nectaring, heat wave, conservation

The Monarch butterfly, *Danaus plexippus* (L.) is a summer resident of the Pacific Northwest (Oregon, Washington, Idaho, British Columbia) arriving in May or June from areas further south and producing one or two generations before adults begin moving southward during September and October for overwintering (Pyle 2002, Dingle et al. 2005, James and Nunnallee 2011, Yang et al. 2015). Summer populations in the Pacific Northwest over the past two decades have declined (James and Nunnallee 2011, Pyle 2015) similar to the recent steep declines reported for the western and eastern North American populations (Stevens and Frey 2004, Brower et al. 2012, Flockhart et al. 2014, Griffiths and Villablanca 2015) but see Davis and Dyer (2015) for a dissenting view. In both the east and the west, it is believed that populations of *D. plexippus* have declined as a result of sub-optimal summer breeding caused by a reduction in milkweed incidence and abundance (e.g. Hartzler 2010, Pleasants and Oberhauser 2013) and periodic extreme weather (Brower et al. 2012). Degradation of overwintering sites in Mexico and California is also considered to be a factor in the decline (Brower et al. 2012, Griffiths and Villablanca 2015). Measures currently being adopted to reverse the decline in *D. plexippus* populations are focused on milkweed restoration replenishing and expanding the habitats needed by summer breeding Monarchs. Surprisingly, little has been published on the breeding ecology of site-specific *D. plexippus* populations in North America. Some studies on the ecology of year-round breeding populations in Florida and southern California have been reported (Brower 1961, Urquhart et al. 1970, Urquhart and Urquhart 1976, Knight and Brower 2009). Studies have also been published on regional aspects of summer breeding in more northerly areas like voltinism (Malcolm et al. 1987, Cockrell et al. 1993, Brower 1996, Flockhart et al. 2013), larval survival (Borkin 1982, Oberhauser et al. 2001, Nail et al. 2015a), larval competition (Flockhart et al. 2012) and geographic/temporal variation in egg and larval abundance (Prysby and Oberhauser 2004, Ries et al. 2015, Stenoien et al. 2015), but none have focused on the biology and ecology of site-specific summer breeding populations of *D. plexippus* in North America. Such studies are important in developing a more complete understanding of monarch population biology which will better inform development of optimal conservation strategies. In contrast, a number of such studies have been published for Monarch populations in Australia (Smithers 1972, James 1981, Zalucki and Kitching 1985, Zalucki and Suzuki 1987, Zalucki and Rochester 2004) and Bermuda (Hilburn 1989). The lack of site-specific ecological studies on North American *D. plexippus* may be a consequence of breeding in mid-high latitudes often occurring on small patches of widely separated host plants (Brower 1996, Prysby and Oberhauser 2004, Hartzler 2010, Flockhart et al. 2012). Thus, Monarchs are often not necessarily resident for more than one generation in one location, unlike the situation in Australia where milkweed (primarily *Gomphocarpus fruticosus* (L.) W. T. Alton) often occurs in large patches of up to 5000 m² (James 1981, Bull et al.

FIG. 1. Monarch butterfly (*Danaus plexippus*) summer breeding site (outlined in red) at Lower Crab Creek east of Beverly and south of Vantage in central Washington.

FIG. 2. High density, large, contiguous patches of showy milkweed (*Asclepias speciosa*), characterize the Lower Crab Creek Monarch butterfly breeding site.

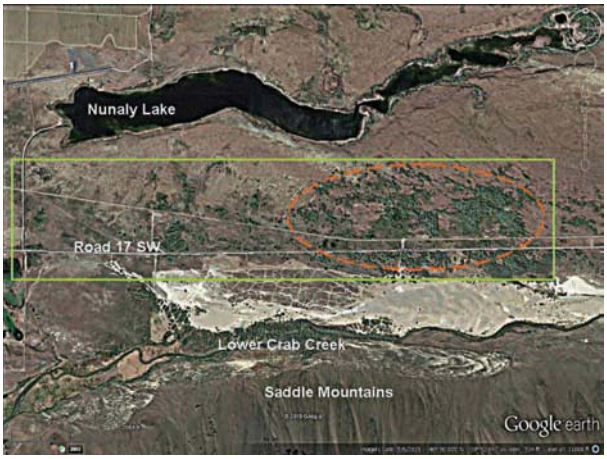

FIG. 3. Close up view of the 4.1 X 0.6 km area at Lower Crab Creek supporting large populations of *Asclepias speciosa* and *Danaus plexippus* during 2013–15. The area bounded by the dotted orange ellipse had the highest density of *A. speciosa*. The meandering walk used in this study to monitor *D. plexippus* was within this area.

1985), supporting multiple generations of *D. plexippus* continuously.

In order to develop optimal strategies for milkweed habitat restoration, more information is needed on the choice and use of milkweed habitats by *D. plexippus* as well as species, population and distribution characteristics of milkweed populations themselves. This information is particularly critical for the largely arid and mountainous western United States where milkweeds are more patchily distributed (Nelson et al. 1981, Brower, et al. 1982, Stevens and Frey 2010, Pyle 2015) than in the eastern United States and where we have very little information on the ecology of *D. plexippus* breeding in inland areas. Morris et al. (2015) reported summer breeding of two to three generations of monarchs at higher elevations (1067–1829 m) in Arizona but did not provide details of population ecology. The Pacific Northwest is occupied annually by summer breeding populations of *D. plexippus* at the greatest distance from Californian overwintering areas (Yang et al. 2015). Breeding is known to occur primarily in inland areas of Oregon, Washington, Idaho and British Columbia with limited or no incidence of milkweed in coastal areas (Pyle 2002, Stevens and Frey 2010, James and Nunnallee 2011). Much of central Washington is hot and dry during summer and milkweed occurrence is restricted to moister valleys, marshes, riversides, roadsides and irrigated areas. Most milkweed patches are small and scattered but a large concentration occurs near the Columbia River 210 km southeast of Seattle. The site was first noted as a potential Monarch breeding habitat in 1996 by R. M. Pyle during field work for his book Chasing Monarchs (Pyle 1999). Pyle observed 'acres and acres' of Showy Milkweed and tagged a Monarch on-site. The stand was visited in 2012 and found to contain area ~ 2.4 km² of almost contiguous high density milkweed. This study reports the results of a three year survey of summer breeding populations of *D. plexippus* at this site. The aim was to determine and document the existence of summer breeding of Monarchs at the site, adult population dynamics, behavior and the impact of climate and plant resources on persistence and survival.

MATERIALS AND METHODS

Populations of adult *D. plexippus* were monitored at a single site (Lower Crab Creek LCC) 15 km south east of Vantage in central Washington from June-September 2013–15. The site is a 4.1 by 0.6 km, east-west oriented tract of largely open land (elevation 157 m) bisected by Road 17 SW (46° 49.997' N, 119° 52.915' W) (Fig. 1). A small creek (Lower Crab Creek) lies 0.5 km to the south and drains into the Columbia River, and a small natural

TABLE 1. Numbers and wing condition of male and female *Danaus plexippus* seen at the Lower Crab Creek site during each three hour survey during June-September 2013–15

Date	Temp° C	Number of Monarchs	Male	Female	Worn	Good	Fresh
2013							
June 6	29-33	0					
June 14	22-26	2	2	0	0	2	0
June 27	20-36	2	2	0	0	1	1
July 10	33-38	2	2	0	0	1	1
July 19	25-31	3	2	1	1	2	0
July 26	26-33	1	1	0	0	0	1
Aug 1	26-28	23	13	10	1	1	21
Aug 9	21-30	35	23	12	2	18	15
Aug 16	21-28	29	22	7	0	23	6
Aug 22	18-28	17	13	4	0	12	5
Aug 27	21-27	21	13	8	0	12	9
Aug 30	21-28	0					
2014							
June 6	27-29	0					
June 17	20-23	9	7	2	1	8	0
June 25	23-27	7	7	0	3	4	0
July 2	26-28	2	2	0	2	0	0
July 11	27-31	21	18	3	2	1	18
July 25	19-26	64	58	6	4	56	2
Aug 5	27-32	51	43	8	4	44	1
Aug 11	16-30	48	38	10	2	16	30
Aug 18	21-27	73	49	24	3	15	55
Aug 26	16-28	45	21	24	0	13	32
Sep 1	18-25	23	13	10	1	5	17
Sep 9	18-27	1	1	0	0	1	0
2015							
May 29	28-31	0					
June 5	28-30	1	1	0	0	1	0
June 12	22-27	2	1	1	0	2	0
June 23	26-29	6	4	2	2	4	0
July 2	22-32	14	8	6	3	7	4
July 14	28-32	38	28	10	4	13	21
July 22	22-27	61	49	12	4	52	5
July 29	18-27	58	46	12	12	38	8
Aug 6	20-25	16	12	4	4	11	1
Aug 12	28-32	29	24	5	5	13	11
Aug 18	19-28	25	20	5	7	5	13
Aug 26	18-22	6	4	2	1	2	3
Sep 1	25-26	0					

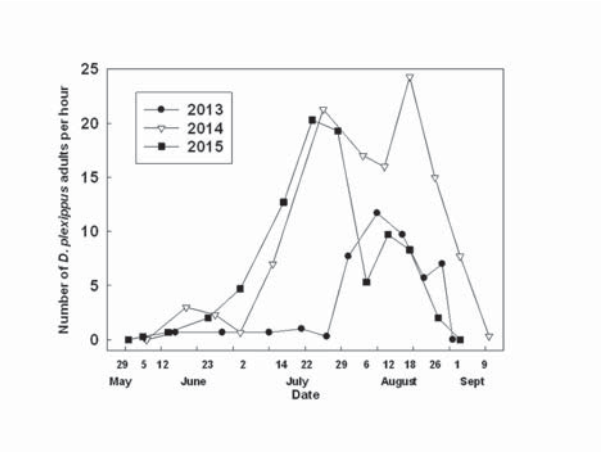

FIG. 4. Mean number per hour of *Danaus plexippus* adults seen during three hour meandering transects conducted at the Lower Crab Creek summer breeding site during June-August 2013–15.

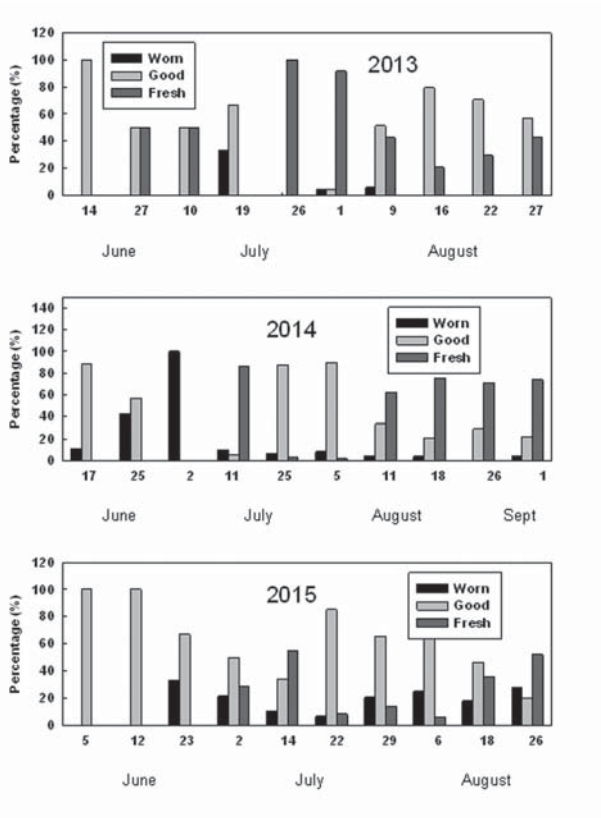

FIG. 5. Wing condition of *Danaus plexippus* adults at the Lower Crab Creek summer breeding site during June-August 2013–15.

lake (Nunaly Lake) lies 0.5 km to the north. Throughout most of the approximately 2.4 km² area, milkweed (*Asclepias speciosa* Torr.) is almost continuous and dominates the landscape occupying an estimated 70% of the land area (Fig. 2). In large areas of the site milkweed forms 100% of the ground cover with the two largest patches occupying approximately 78,000 and 144,000 m². *Asclepias speciosa* propagates effectively by underground rhizomes enabling large areas to be colonized when conditions are suitable (Borders & Mader 2014). Small trees and bushes are scattered throughout the site sometimes forming denser groups (Fig. 3). The dominant species is the introduced and declared noxious plant in Washington, Russian Olive (*Elaeagnus angustifolia* L.). Some native trees, Poplars (*Populus* spp.), bushes, Willows (*Salix* spp.) and shrubs (Buckwheats *Eriogonum* spp., Sagebrushes *Artemisia* spp., Rabbitbrushes *Ericameria nauseosa* (Pall. ex Pursh) G. L. Nesom & G. I. Baird, Purple Loosestrife *Lythrum salicaria* L., Catstails *Typha* spp.) are also present but are relatively limited within the site. The Lower Crab Creek State Wildlife area which encompasses the monitoring site is managed by the Washington Department of Fish and Wildlife. It is bounded to the south by the Saddle Mountains (700 m elevation) and to the north by irrigated agricultural land (350 m elevation). The site was first noted as a potential Monarch breeding site by Bob Pyle in his book '*Chasing Monarchs*' (Pyle 1999) who observed 'acres and acres' of Showy milkweed.

Twelve-thirteen visits were made annually to the site at 7-14 day intervals during May 29–September 9 from before the first migrant arrived until the fall migration emptied the area. At each visit an observational survey of the *D. plexippus* adult population at the site was conducted during a three hour period between 0700 and 1400 h when data on numbers seen, sex ratio, wing condition and behavior were obtained. Some individuals were captured (see below) enabling close-up assessment of sex and wing condition but many were assessed by observation as they nectared, rested or flew in close proximity. Weather conditions at each visit were uniformly sunny, wind ranged from 0–25 km/h and temperatures ranged between 16–38 °C. However, on most visits temperatures were 25–30 °C and periods of temperatures above 38 °C were avoided because of consequent inhibition of *D. plexippus* activity (James unpubl obs.). The same meandering route (~ 3 km) through the site was taken at each visit. The walk traversed all major patches and areas of milkweed (*A. speciosa*) and nectar sources (primarily *L. salicaria*) in a 0.5 km² section of the site (Fig. 2). The number of adult *D. plexippus* encountered was recorded along with

details of the behavior, sex and wing condition of each individual. Steady progression through the site minimized 'double-counting'. Wing condition was qualitatively assessed as 'fresh' (bright colors, immaculate), 'good' (very slight (< 5%) wing wear, good colors), or worn (> 25% wing wear, duller colors). The behavior of each individual seen was assigned to one of eight categories (1. in flight, 2. male patrolling *A. speciosa*, 3. nectaring on *A. speciosa*, 4. nectaring on *L. salicaria*, 5. resting on ground or vegetation, 6. resting on *A. speciosa*, 7. mating/courtship, 8. oviposition). Data on sex ratio and wing condition were analyzed using one way ANOVA. From early July to mid-August male *D. plexippus* were marked, released and recaptured (MRR) to provide data for estimates of male population size. No evidence was seen of post-tagging 'escape flight'. In most instances tagged butterflies were observed to resume normal behaviors shortly after release. Butterflies were marked using a white circular tag (obtained from MonarchWatch.org) with a serial number and email address affixed dorsally to a forewing. MRR data in 2014 and 2015 were analyzed using Jolly's stochastic method (Jolly 1965). Insufficient numbers of butterflies were tagged and recaptured in 2013 to justify analysis. After mid-August, when the population commenced fall migration and began to leave the site, both sexes were instead tagged ventrally on a hindwing for a migration study. During mid-July to late August in all years 10–20 females were removed from the site to produce progeny for a separate study on migration (James, in prep.). Eggs and larvae of *D. plexippus* were not searched for and not observed except for eggs laid by ovipositing females. The dense stands and large areas of *A. speciosa* made detection of eggs and larvae unlikely (Fig. 3). Climate data for June-August 2013-2015 were obtained from WSU Agweathernet (http://weather.wsu.edu) for meteorological recording stations at Desert Aire [13.8 km] south and Vantage [4.0 km] west of Lower Crab Creek. Both sites were at similar elevations (160–180 m) to the study site (157 m). The Vantage station was commissioned in September 2014, thus data from this (closer) site were only available for 2015. Weather data were used to help explain and interpret observed population fluctuations.

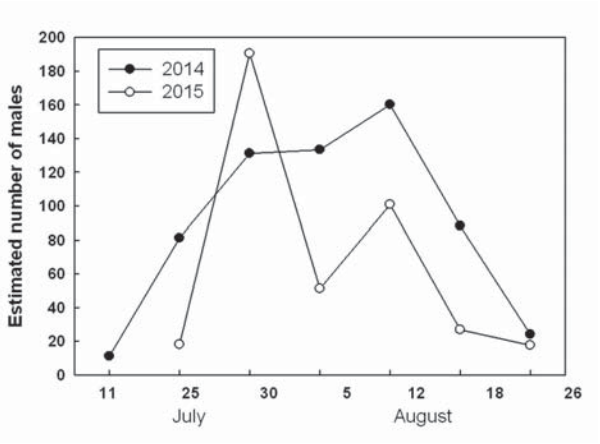

FIG. 6. Population estimates of male *Danaus plexippus* at the Lower Crab Creek site during July-August 2014 and 2015. Estimates derived from Jolly's stochastic analysis of mark, release and recapture data (Jolly 1965).

RESULTS

No Monarchs were seen at the site on June 6 in 2013 and 2014 and May 29 2015. *Danaus plexippus* was first seen at the site on June 5 (2015), June 14 (2013) and June 17 (2014) (Table 1, Figure 4). Arrival numbers ranged from one to nine individuals seen during the three hour survey with good condition males predominating. The population remained low during June in all three years (totals of 2–9 individuals per visit, Table 1) but increased substantially during the first half of July in 2014 and 2015 (7.0–12.7/hr), suggesting emergence of a new generation. The first occurrence of fresh condition individuals at this time also indicated new generation emergence (Fig. 5). In 2013 the numbers remained low throughout July (0.3–1.0/hr)(Fig. 4), but a high proportion of fresh condition individuals at this time indicated emergence of a new generation (Fig. 5). Numbers increased further in late July in 2014 (21.3/hr) and 2015 (20.3/hr) but did not increase until August 1 (7.7/hr) in 2013. The population increased further in August 2013 (up to 11.7/hr). In 2014 the population remained high during August (15.0-24.3/hr) but in 2015 the population fell to 5.3/hr on August 6 and remained below 10.0/hr for the rest of the month (Fig. 4). Increased proportions of fresh condition individuals in late July–early August 2013 and early–late August in 2014 and 2015 provided evidence for a second generation of adults (Fig. 5). The population dispersed from the site, presumably as migrants heading south, during the last few days of August (none seen on 8/30/13 or 9/1/15) or first few days of September (none seen on 9/9/14) (Fig. 4). Population estimates of the male population derived from MRR data in 2014 and 2015 (Fig. 6) concurred with the observational data on population size showing greatest numbers in late July in both years with an earlier decline in 2015. The maximum population at the site was estimated at 160 males in 2014 and 190 in 2015 (Fig. 6). A total of 26 males was tagged in 2013 with 7 (26.9%) recaptures. In 2014 and 2015, 76 and 50 males were tagged with 19 (25%) and 16 (32%) recaptures, respectively. Intervals between tagging and recapture

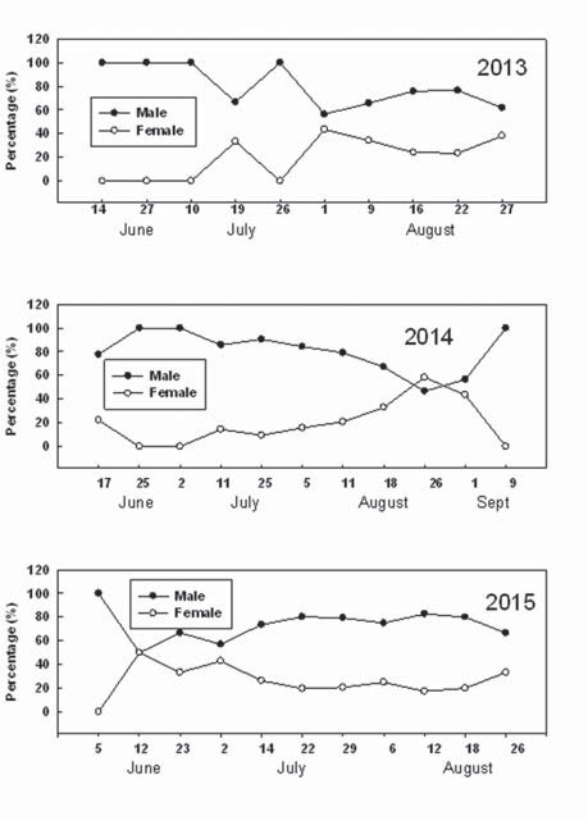

FIG. 7. Sex ratio of *Danaus plexippus* adults seen at the Lower Crab Creek summer breeding site during June-August 2013–15.

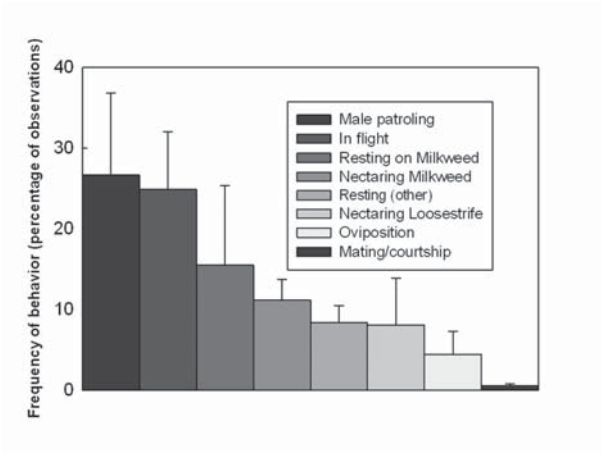

FIG. 8. Percentage frequency of adult *Danaus plexippus* behaviors at the Lower Crab Creek summer breeding site during 2013–15.

ranged from 5–27 (mean 12.1) days in 2013, 6–39 (mean 13.2) days in 2014 and 1-27 (mean 14.6) days in 2015.

Sex ratio was imbalanced in favor of males on all dates in all years ranging from 57.1–100% (Fig. 7). Overall, males accounted for 68.9% of butterflies seen in 2013, 74.7% in 2014 and 76.9% in 2015 and the observed sex ratios did not vary significantly between years ($F = 0.605$; $df = 2, 29$; $P > 0.553$) (Table 2). The wing condition of butterflies in 2013 and 2014 was largely good or fresh with significantly fewer worn individuals (2013: $F = 9.022$; $df = 2, 27$; $P < 0.001$, 2014: $F = 10.823$; $df = 2, 30$; $P < 0.001$) (Table 2). Butterflies in good condition were significantly more numerous in 2015 than either worn or fresh individuals ($F = 11.765$; $df = 2, 30$; $P < 0.001$) (Table 2). Fresh individuals accounted for 28–85.7% of the population in early to mid-July of each year (Fig. 5). A second increase in fresh individuals (indicating new generation emergence) occurred in late July and August in 2013 and 2014. This did not occur in 2015 until mid-late August (Fig. 5).

The relative frequency of different behaviors of *D. plexippus* adults during 2013-15 is shown in Fig. 8. Males patrolling milkweed patches guarding territory or seeking females was the most common behavior closely followed by non-patrolling flight (both sexes). Resting on milkweed and nectaring on milkweed were the next most frequently observed behaviors (Fig. 8). Nectaring on purple loosestrife accounted for just over 8% of behavior observed but reached 19.7% in 2014. Oviposition and mating/courtship activities were rarely observed (0.5–4.4%).

Temperature data for the nearby weather station (Desert Aire) during June to August 2013–15 are shown in Table 3. Temperature values (daily mean, daily mean minimum, daily mean maximum) varied by only 0.1–0.2 °C between 2013 and 2014 and were 0.8-1.0 °C higher than the averages for the previous eight years. However, the corresponding values for 2015 were 1.3–1.5 °C higher than the other two years and 2.3–2.4 °C higher than the eight year averages (Table 3). 2015 had a maximum high temperature of 43 °C compared to 41.2 and 42.2 °C in 2013 and 2014, respectively (Table 3). The longest period of consecutive days with maxima greater than 38 °C occurred in 2015 (15 days) compared to only 3 and 5 days in 2013 and 2014, respectively (Table 3). Daily maximum temperatures during the 2015 'heatwave' are shown for the Vantage weather station, 4 km west of the breeding site (Fig. 9). Total rainfall during June-August was sparse in 2013 (26.0 mm) and very low in 2014 (13.7 mm) and 2015 (8.8 mm).

TABLE 2. Overall sex ratio and wing condition categories (%) recorded for adult *Danaus plexippus* at the Lower Crab Creek breeding site during June-August 2013-2015. * Significantly greater than worn in 2013 and 2014. ** Significantly greater than worn and fresh in 2015 (ANOVA, $P < 0.001$)

Year	Male % (n)	Female % (n)	Worn % (n)	Good % (n)	Fresh % (n)
2013	68.9 (93)	31.1 (42)	3.0 (4)	53.3 (72) *	43.7 (59)*
2014	74.7 (257)	25.3 (87)	6.5 (22)	47.9 (163)*	45.6 (155)*
2015	76.9 (197)	23.1 (59)	16.4 (42)	57.8 (148)**	25.8 (66)

DISCUSSION

The summer breeding ecology of *D. plexippus* in central Washington State is reported for the first time. Surprisingly, this study also appears to be the first multi-year analysis of an annually occurring resident summer breeding population of *D. plexippus* in the northern tier of North America. Brower (1961) and Urquhart et al. (1976) presented some information on resident continuously breeding populations of *D. plexippus* in Florida but focused on immature stages. Borkin (1982) studied distribution and survival of immature *D. plexippus* at a site in southeastern Wisconsin but did not report on adult dynamics and ecology. More recently, there have been a number of regional-based studies on the distribution, incidence and abundance of spring and summer breeding populations in the eastern United States (Riley 1993, Prysby and Oberhauser 1999, 2004, Flockhart et al. 2013, Ries et al. 2015, Stenoien et al. 2015) but none of these have focused on the dynamics and ecology of site-specific resident summer populations within and across seasons. In contrast, several Australian studies have provided information on adult population dynamics and ecology of *D. plexippus* breeding populations. Observations on a summer resident breeding population of *D. plexippus* were made over 4 years by Smithers (1965) south of Sydney. A similar study north of Sydney focused on winter breeding of *D. plexippus* (James 1981). A number of studies on adult population dynamics and ecology of *D. plexippus* in sub-tropical Queensland have been reported (Bull et al. 1985, Zalucki 1983, Zalucki and Kitching 1982, 1984, Zalucki and Suzuki 1987, Suzuki and Zalucki 1986).

This study indicates that a large population of *A. speciosa* (~ 70% coverage of a 2.4 km² area containing an estimated tens of thousands of plants) in the hot, dry summer climate of central Washington can annually attract and sustain a large resident breeding population of *D. plexippus* during June–August. Although it is possible that a small part of the population development observed at Lower Crab Creek during July-August was derived from itinerant *D. plexippus* colonizing the site, it is likely that most of the population increase was a result of breeding at the site. Few significant patches of Showy Milkweed are known to occur within a 50 km radius of Lower Crab Creek and it is unlikely that there is a large itinerant population of *D. plexippus* in this region during summer. Few Monarchs are reported or seen in this area during the summer (James, unpubl. obs.). Little is known about the incidence and characteristics of summer breeding sites of *D. plexippus* in the Pacific Northwest (Jepsen and Hoffman Black 2015, Pyle 2015), but anecdotal evidence suggests that breeding often occurs on small, scattered, localized patches of milkweed along roadsides, watercourses and in other moister areas. A survey of approximately 100 patches of milkweed (mostly *A. speciosa*, few *Asclepias fascicularis* Decne.) in central and eastern Oregon conducted in 2015, showed that 70% were comprised of less than 100 plants. The maximum number of plants in a patch was ~ 300 (Matt Horning USFS, pers. comm.). It is likely that the majority of milkweed patches in eastern Washington are similarly sized, except at sites like Lower Crab Creek where presumably a combination of good climate, soil moisture, companion vegetation and suitable soil types allow patches to occupy much larger areas. It is possible that the Lower Crab Creek site is unusual in supporting such a large population of *A. speciosa* but further studies on milkweed distribution and abundance in Washington and Oregon are needed to determine this.

During the three years of this study, small numbers of *D. plexippus* adults arrived at the breeding site between June 5 and 17. These were in worn to good condition and likely were migrants from further south. Records kept since 2002 show that *D. plexippus* is invariably first recorded in Washington in early-mid June (James, unpubl.). In 2014 and 2015 these early colonizers

TABLE 3. Temperature (° C) data from an Agweathernet station at Desert Aire 13.8 km south of Lower Crab Creek during June–August 2013-15. Values in parentheses represent the eight year (2005–12) mean for the site. Above average temperatures featured in all survey years especially 2015 when an extended period of > 38 °C temperatures occurred.

	2013					2014					2015				
Month	**Min (° C)**	**Mean (° C)**	**Max (° C)**	**Extreme (° C)**	**Consec. Days > 38.0**	**Min (° C)**	**Mean (° C)**	**Max (° C)**	**Extreme (° C)**	**Consec Days > 38.0**	**Min (° C)**	**Mean (° C)**	**Max (° C)**	**Extreme (° C)**	**Consec Days > 38.0**
June	14.4 (14.0)	20.9 (20.9)	27.6 (28.0)	36.2	0	14.2 (14.0)	21.2 (20.9)	28.2 (28.0)	34.3	0	18.1 (14.0)	26.0 (20.9)	33.5 (28.0)	43.0	5
July	19.1 (17.7)	27.1 (25.7)	35.3 (33.6)	41.2	3	19.4 (17.7)	27.4 (25.7)	35.6 (33.6)	42.2	5	19.9 (17.7)	27.3 (25.7)	34.9 (33.6)	41.4	10
August	18.2 (17.2)	25.4 (24.6)	33.1 (32.4)	37.1	0	18.5 (24.6)	25.5 (24.6)	33.0 (32.4)	39.3	2	17.7 (17.2)	24.9 (24.6)	32.4 (32.4)	39.5	0
June-August	17.3 (16.3)	24.5 (23.7)	32.1 (31.3)	41.2	3	17.4 (16.3)	24.7 (23.7)	32.3 (31.3)	42.2	5	18.6 (16.3)	26.1 (23.7)	33.6 (31.3)	43.0	15

persisted at low levels (0.7–2.3 adults per hour) until early July when the first locally produced adults increased the population 7–10 fold. The first locally produced generation was very small in 2013 with no detectable increase in numbers but the presence of fresh condition adults in late June–early July indicated newly eclosed individuals were present. A second generation of adults, as indicated by increase in population size and incidence of fresh individuals was produced in late July–mid-August in all years. The MRR data provided a maximum population estimate of 190 males on July 29 2015, which if a 1:1 sex ratio is assumed, corresponds to an estimated population of 380 individuals. Undoubtedly, some emigration and immigration occurred at the site but the relatively high recapture rate of tagged males (25–32%) and recaptures of males at the site up to 39 days after tagging, suggests good site fidelity. These populations of an estimated 300–400 adult *D. plexippus* at the 2.4 km² Lower Crab Creek site are remarkable and challenge our concepts of summer breeding of *D. plexippus* in the Pacific Northwest. Small, separated patches of milkweed which appear to be the 'normal' distribution of *Asclepias* spp. across the landscape in at least eastern and probably western North America (Hartzler 2010, Pleasants and Oberhauser 2013, Flockhart et al. 2012, Jepson and Hoffman Black 2015, Pyle 2015) will clearly not support large residential populations of *D. plexippus* like those documented in this study. Similar-sized largely resident populations of *D. plexippus* have been reported from large milkweed patches in Australia. James (1981) reported a winter breeding population of an estimated maximum of 348 adult *D. plexippus* during three months at a north Sydney site with milkweed in two 900 m² patches. Zalucki and Kitching (1984) in southeast Queensland estimated adult populations of 50–200 *D. plexippus* per milkweed patch site although the size of patches was not reported. However, the extent of milkweed in these Australian studies was not as great as the dominance of *A. speciosa* on the landscape at Lower Crab Creek. Isolated large areas of high density *Asclepias* spp. may be a characteristic of the arid zone of the western US and therefore an important part of the ecology of *D. plexippus* in the west. Clearly, there is an urgent need to determine the distribution and frequency of small and large, high density *Asclepias* spp. dominated sites in the western US.

In 2015 the second generation of locally produced *D. plexippus* adults at Lower Crab Creek was small and did not result in a population increase as in the previous two years. Instead the population declined by about 75%. The summer of 2015 was the hottest on record for many parts of central Washington (http://nws.weather.gov/blog/nwspendleton/wp-content/uploads/sites/13/2015/09/Season-In-Review_Summer_2015.pdf) and the climate data from the two weather stations near Lower Crab Creek reflected this. Whilst mean temperatures were 1.3–1.5 °C higher in 2015 than the

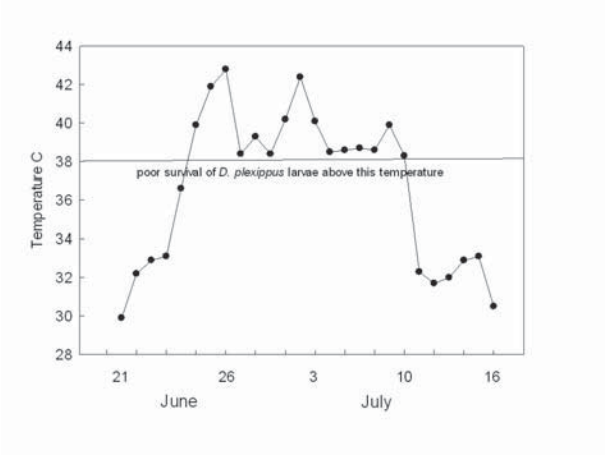

FIG. 9. Daily maximum temperatures (° C) at an Agweathernet station at Vantage 4 km west of Lower Crab Creek during June 21 to July 16 2015.

previous years and 2.3–2.4 °C higher than the eight year average, the more important statistic biologically was the number of consecutive days (15, June 26–July 10) with maxima above 38 °C. In 2013 and 2014, this number was 3 and 5, respectively. The immature stages of *D. plexippus* suffer increased mortality from temperatures of 36 °C and above depending on length of exposure and stage (Zalucki 1982). A constant temperature of 42 °C for 12 out of 24 hrs for two days resulted in 80–90% mortality of early and late instar larvae (Nail et al. 2015b). A constant temperature of 38 °C for 12 out of every 24 hrs for six days resulted in 30% mortality of third instar larvae (Nail et al. 2015b). While the 15 day period of daily maxima above 38 °C temperatures in June–July 2015 would not be as thermally severe as the examples above (night time temperatures ranged from 19.2–24.7 °C), it is possible that substantial heat-induced mortality occurred amongst immature stages of *D. plexippus* during this period. The peak of oviposition by migrant females arriving at the site likely occurred during the third week of June and larvae from these eggs would have been exposed to the 15 day heat wave. The first local generation of adults emerged in good numbers at the site from July 2–14, suggesting that pupae were not adversely affected by the heat. However, the anticipated second generation of adults did not occur until August 12 and numbers were relatively small. Egg to adult development of *D. plexippus* takes 25–28 days at temperatures of 25–35 °C (Zalucki 1982, James unpubl. obs), thus the second generation individuals would have developed after the heat wave. The expected time of second generation emergence based on 2013 and 2014 observations was late July–early August. The relatively low numbers of fresh newly eclosed individuals during this period in 2015 may have resulted from increased mortality in developing eggs and larvae during the 15 day heat wave in late June–early July. The effect of high temperatures on adult *D. plexippus* is uncertain although temperatures above 35 °C have been observed to cause individuals to rest in the shade (James, unpubl. obs., Masters et al. 1988). High temperatures may also cause dispersal. Observations (two to five monarchs per minute flying west along Crab Creek) made by Mark Genich (pers. comm.) on July 27 2015, 1.5 km southwest of the breeding site, support this.

Another factor in the population decline of *D. plexippus* during August 2015 may have been reduced nectar availability compared to the previous two years. The principal nectar sources used by *D. plexippus* at Lower Crab Creek are milkweed and Purple Loosestrife, the latter occurs only in parts of the site that retain at least some moisture during the summer. Milkweed finishes blooming by the end of July and blooming of loosestrife was very limited in 2015 due to dry conditions throughout the site with only 8 mm of rainfall during the monitoring period. Nectaring on Purple Loosestrife accounted for 19.7% of behavior observations in 2014 but only 2.4% of observations in 2015 because of the scarcity of blooming loosestrife. Pyle (1999) reported finding 'lots of monarchs' nectaring on Purple Loosestrife during southward migration near Hells Canyon in Idaho. Purple Loosestrife is a declared noxious weed in Washington (http://www.nwcb.wa.gov/detail.asp?weed=90) because of its propensity to disrupt wetland ecosystems by displacing native plants and animals. Its occurrence at Lower Crab Creek is limited however by moisture deficits minimizing adverse impacts on the ecosystem. In contrast, its high value as a nectar resource makes it an important factor in the suitability of Lower Crab Creek as a summer breeding area for *D. plexippus*. The issue of limited nectar resources in large areas of the arid west during mid-late summer may have a region-wide impact on monarch survival and deserves study. Aside from milkweed and loosestrife, the other plant which appears to be important to the persistence and survival of *D. plexippus* at Lower Crab Creek is *E. angustifolia* (Russian Olive). This large bush or small tree is also a declared noxious weed in Washington (http://www.nwcb.wa.gov/detail.asp?weed=187). Its importance to *D. plexippus* at Lower Crab Creek accrues from being the dominant shade tree. The observations reported here as 'resting on ground or vegetation' were largely made up of adults resting in the shade on Russian Olive. Additionally, female *D.*

plexippus were observed ovipositing on young milkweed plants growing in the shade underneath Russian Olives. Russian Olive is a nitrogen-fixing plant (Zitzer and Dawson 1989) raising the possibility that its presence may be a factor in the high abundance of milkweed at Lower Crab Creek. Pyle (1999) mentions Monarchs using Russian Olives as a night time roosting tree in various locations (including Lower Crab Creek) during his travels in the Pacific Northwest. Given that these two invasive plants have only been in eastern Washington since the early 1900s (see links above), it is possible that the existence of large summer breeding populations of *D. plexippus* at this site is a relatively recent phenomenon. Monarch occurrence at the site before the invasive plants arrived may have been limited to the period of milkweed flowering. Lower Crab Creek is described from 1879 to the 1950s by Bentley (2010) noting that the creek ran dry by September each year before the advent of irrigated agriculture in the 1950s. It is likely that Purple Loosestrife and Russian Olive did not establish at Lower Crab Creek until the 1950s.

The sex ratio of *D. plexippus* populations at the breeding site was consistently male-biased which accords with data from breeding populations in Australia (James 1981, Zalucki and Kitching 1984, Bull et al. 1985, Zalucki and Suzuki 1987). Interestingly, Davis and Rendon-Salinas (2009) reported increasingly male-biased sex ratios in the Mexican overwintering colonies over a 30 year period. However, the well-known aggressive male courtship and mating behavior of *D. plexippus* (Pliske 1974) is the most likely reason for the apparent low numbers of females at the Lower Crab Creek site during this study. Since the study area with almost contiguous milkweed was essentially one enormous patch of *A. speciosa*, it is likely that most oviposition occurred on the perimeters of the site away from frequent male interference. During the three years (108 hrs of observation) 20 oviposition events were recorded and all of them were either on the outer edges of the site or within the shade or thickets of Russian Olives located in central or perimeter areas. Oviposition within the shade and understory of Russian Olives provided females with protection from patrolling males as well as providing young, vigorous *A. speciosa* plants. This 'clandestine' oviposition behavior curiously appeared to reduce encounters with egg-laying females beyond what might be expected in a large population of *D. plexippus*. Zalucki (1993) suggested that female *D. plexippus* treat milkweed patches with resident males as resources and 'hang around' perimeter areas venturing in when amenable to courtship and mating. The single instances of courtship flight and mating seen over three seasons occurred within central areas of the site.

The Lower Crab Creek site appears to offer a valuable and dependable resource for migrant reproductive *D. plexippus* adults in early June. Peak bloom of *A. speciosa* occurs at this time and the noticeable fragrance emanating from the extensive area of plants could perhaps help attract *D. plexippus* to the site. This study demonstrates that the reproductive potential of small numbers of migrant *D. plexippus* colonizing a large, milkweed-rich site in early summer is substantial. Although the maximum population estimate derived from MRR for males and adjusted for both sexes was close to 400, it is likely there was some permanent emigration from the site, thus this site's contribution to the region's *D. plexippus* population likely exceeded 500 at least in 2014. It is interesting to speculate on the maximum population size that could be sustained by this site. Milkweed is unlikely to be a limiting resource for immature stages of *D. plexippus* at Lower Crab Creek so population regulation would likely be mediated by natural enemies, climate, nectar resources and/or adult behavior. Extreme heat and lack of nectar resources appeared to curtail maximum population size in 2015. Given sufficient nectar resources and average summer temperatures it is reasonable to expect a maximum potential population of 1000–1500 individuals at the site.

A key finding of a long term study on immature *D. plexippus* populations across the eastern United States was a positive association between survival and the number of plants in a milkweed patch (Nail et al. 2015a). The authors of this study suggested that 'conservation actions should encourage plantings with large numbers of milkweed plants, not only because more plants will support more Monarchs but also because survival is likely to be higher'. Thus, it would seem appropriate and beneficial for *D. plexippus* conservation strategies in arid areas of the western United States to focus on the protection, expansion and creation of isolated, *Asclepias*-rich sites. High production of *D. plexippus* in isolated, large, dense patches of milkweed like Lower Crab Creek may be more characteristic of the arid west than production on widely distributed small patches of milkweed. Large, isolated patches of dense milkweed in moister areas, is also characteristic of Arizona (Gail Morris, pers. comm.). The few currently known summer breeding sites of *D. plexippus* in eastern Washington all contain substantially less *Asclepias* in terms of number of plants and area occupied, than Lower Crab Creek. They also support substantially smaller populations of *D. plexippus* (James unpubl. obs.). It is possible that simply expanding the milkweed acreage at these sites may be the most effective and economic way of enhancing the

summer breeding potential of *D. plexippus* in Washington. However, more research is clearly needed on the relative production of Monarchs in large and small milkweed patches over broad areas of the inland Northwest to determine optimal strategies for increasing milkweed and Monarch populations.

ACKNOWLEDGMENTS

I thank Ann Potter and the Washington Department of Fish and Wildlife (WDFW) for facilitating the necessary permits for this research at Lower Crab Creek. I also thank WDFW for administering the Lower Crab Creek Wildlife Area and being sensitive to the needs of *Asclepias speciosa* and *Danaus plexippus*.

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Submitted for publication 10 November 2015; revised and accepted 12 February 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 194–200

THE LEPIDOPTERA OF WHITE SANDS NATIONAL MONUMENT, OTERO COUNTY, NEW MEXICO, USA 11. A NEW SPECIES OF *AROTRURA* WALSINGHAM 1888 (SCYTHRIDIDAE), ANOTHER ICONIC SPECIES FROM THE MONUMENT

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ABSTRACT. The U.S. National Park Service initiated a 10-year study of the Lepidoptera at White Sands National Monument, Otero County, New Mexico in late 2006. *Arotrura landryorum* **sp. n.**, described here, was discovered in 2007, during the first year of the study. The male and female adult moths and genitalia are illustrated.

Additional key words: Endemism, evolution, U.S. National Park Service, U.S. Army, White Sands Missile Range, Tularosa Basin, biological diversity, white gypsum dunes

In 2006, White Sands National Monument invited me to conduct a 10-year study of moths at the Monument. The study began in late 2006 and within the first few months, I found several species of moths new to science (unpublished data) including the one described here. Species of the family Scythrididae are very difficult to separate superficially. The North American genera of Scythrididae were revised by Landry (1991) in which he described six new species of *Arotrura*. He mentioned having seen more than 60 additional undescribed species in the genus. *Arotrura landryorum*, described here, is none of the undescribed species mentioned by Landry. Landry (1991) specifically explained that many species of Scythrididae are identical in outward appearance, thus making it very difficult for a positive identification without examining the genital organs. The description of this species is another in a series of publications describing new species of moths at White Sands National Monument (WSNM), Otero County, New Mexico, USA (see Metzler 2014, Metzler 2016, Metzler et al. 2009, Metzler & Forbes, 2011a, 2011b, 2011c, Metzler & Lightfoot 2014).

METHODS AND MATERIALS

Moths and other night flying insects were collected in U.S.D.A. type black-light traps, as described in Smith et al. (1974), in diverse habitats within the dunes in White Sands National Monument. A detailed description of the study methods is given by Metzler et al. (2009).

All, except easily identified (i.e. *Hyles lineata*) species of moths, were retained for further study. The specimens were sorted; selected specimens were spread and labeled. All nonlepidopterous insects from the traps were placed in 95% ethanol and deposited in the Museum of Southwestern Biology at the University of New Mexico, Albuquerque, New Mexico.

The moth genitalia were prepared and examined by following procedure outlined in Landry (1991). After cleaning, dissected parts were stained first with Orange G in lactic acid followed by Chlorazol Black in 70% ethanol. Dissected parts were dehydrated in 99.9% propanol and slide-mounted in Euparal. Small vinyl props were used to raise the cover slips of thick genitalia preparations. For males, different mounts were prepared for dorsal and lateral views.

Terms for elements of wing markings, regions of the wing, color, morphology, and genital structures follows Landry (1991). Forewing lengths were measured to the nearest 0.1 mm, from the base to the apex excluding fringe, using a Leica MZ 12 stereo-microscope with a Wild 15x ocular micrometer.

The photographs of the adults were taken with a Canon EOS 60D with a Canon 65mm MP-E lens, and a Nikon D7100 with an AF-S Micro Nikkor 105mm 1.28 GED lens. The images were processed with Zerene Systems software and Photoshop CS6 software. The photographs of the genitalia and slide-mounted

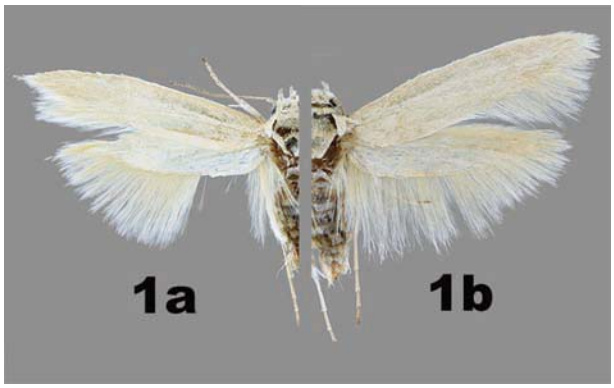

FIG. 1. *Arotrura landryorum* adults; **1a**, male holotype (photographed before dissection.) **1b**, female paratype (reversed left side shown, same scale as Fig. 1a);

structures were taken with Nikon DS-Fi1 digital camera mounted on a Nikon Eclipse 800 microscope at magnifications of 40× or 100×. Nikon's NIS 2.3 Elements was used to assemble multiple photos of different focal planes into single deep-focus images.

The coordinates for latitude and longitude on the labels of the specimens from the study are in degrees and decimal minutes. The coordinates were obtained with the aid of a Garman II Plus and confirmed by reference to Google™ Earth Pro 7.1.4.1529. Specimens of Lepidoptera cited in this paper are deposited in the following collections:

EHM	Eric H. Metzler, Alamogordo, NM, for subsequent transfer to MSUC
MSUC	Michigan State University's A.J. Cook Arthropod Research Collection
UNM	University of New Mexico's Museum of Southwestern Biology
USNM	United States Museum of Natural History (Smithsonian Institution), Washington, DC

RESULTS

Arotrura landryorum Metzler, **sp. n.**
Figs 1-6

Type material.

♂ Holotype, pinned, double-mounted, with labels as follows:

"New Mexico: Otero Co., W[hite]S[ands]N[ational] M[onument]; Dunes/no vegetation, 11-12 June 2007; 32°45.778'N, 106°11.391'W, 4,014 ft; Coll. E.H. Metzler & D. Adamski; Blacklight/WHSA 00131"; "Specimen # USNMENT01142721"; [blue label] "Barcode of Life Project Leg(s) removed, DNA extracted"; [upside down barcode] "USNMENT01142721"; [green label] "Genitalia slide by JFLandry, ♂ USNM 130,266"; [red label] "HOLOTYPE USNM; *Arotrura landryorum* Metzler". Deposited in USNM.

Paratypes: (♂7), (♀1): same locality/date as holotype:

CNCLEP00121863, ♂genitalia slide USNM 130,252 by JFLandry; CNCLEP00121864, ♂genitalia slide USNM 130,253 by JFLandry; USNMENT01142708, ♂genitalia slide USNM 130,255 by JFLandry; USNMENT01142713, ♂genitalia slide USNM 130,254 by JFLandry. Deposited in EHM, UNM, USNM. (♂4), specimens # USNMENT01142716, USNMENT01142719, USNMENT01142720: USA, NM, Otero Co., White Sands Nat [ional]. Mon [ument], interdunal vegetation, 32°45.685'N, 106°11.379'W, 4,000', 10 June 2013, WHSA8, Eric H. Metzler uv trp, Accss # WHSA 00131. Abdomens not dissected. Deposited in MSUC. (♀1) specimen # USNMENT01142732, ♀genitalia slide USNM 130,264 by JFLandry: USA, NM, Otero Co., White Sands Nat [ional]. Mon [ument], edge of dunes habitat, 32°45.724'N, 106°11.315'W, 4,000', 10 June 2013, WHSA8, Eric H. Metzler uv trp, Accss # WHSA 00131. Deposited in USNM.

Diagnosis. The diagnostic features are the yellow-white adults and the genital structures. The color, size, and shape of the moth help narrow the possibilities, yet three other undescribed species of Scythrididae, with identical habitus, but differing in genitalia, were collected microsympatrically and on the same date with *A. landryorum*. Dissections and/or DNA barcodes are required to distinguish the species.

Arotrura landryorum is a small (forewing length = 9.0–13.0 mm) pale white-yellow moth (Fig. 1). In the key to species groups of *Arotrura* based on genitalia (Landry, 1991), males of *A. landryorum* key out to the *A. divaricata* species group, which comprises two described species, *A. divaricata* (Braun, 1923) and *A. oxyplecta* (Meyrick, 1916). Both are dark gray species with a transverse zigzagged white fascia in the basal third of forewing, outwardly very different from the uniformly pale ivory-white *A. landryorum*.

The habitus of *A. landryorum* is superficially similar to some *Arotrura* in other species groups, notably *A. eburnea* and *A. formidabilis* in being pale white yellow. The differences between species can only be seen in specimens that are dissected, with the genitalia of both sexes of *A. landryorum* sharing the characteristic aspect of the *A. divaricata* group, namely the horned profile of the socii, the concavity with small pits in the fused medial region of the socii, and the slender aedeagus with a bulbous base set at the apex of a long, blade-like juxta.

Because of the paucity of specimens of *A. oxyplecta* coupled with, as yet, no clearly associated males and females (Clarke, 1965; Landry, 1991), I limit the genitalia comparison of *A. landryorum* to *A. divaricata*. The latter is also known from New Mexico, whereas the occurrence of *A. oxyplecta* outside of its type locality (Florida) remains unconfirmed.

In male genitalia, the distal 3/5 of the valvae of *A. landryorum* are markedly up curved with the apices at 90 degrees from the base and extended to the top of the socii, the horn-like socii are extended caudad more than the ventral lobes, the base of the socii is dorsally humped, adorned with long, recumbent setae, the anterior section connecting to the tegumen is narrow and constricted, the gnathos is long and extended below the valvae, and the sides of the tegumen are more slender with widely separated anterior margins

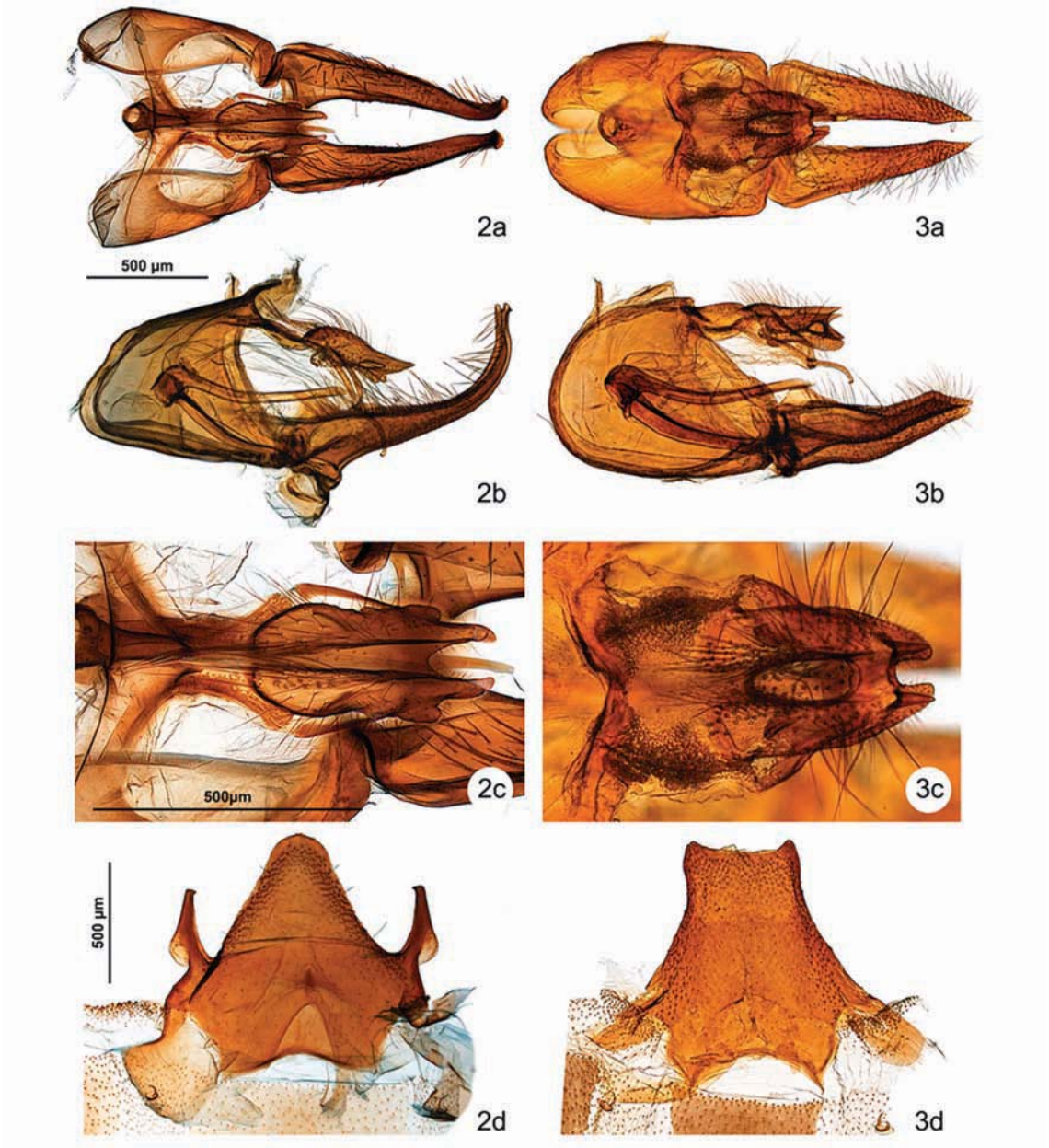

FIG. 2–3. *Arotrura* male genitalia and 8th abdominal tergite (T8); **2a**, *A. landryorum* male genitalia, dorsal view [slide USNM130252, paratype]; **2b**, *A. landryorum* male genitalia, lateral view [slide USNM130266, holotype]; **2c**, close up of uncus-socii in dorsal view [USNM130252, paratype]; **2d**, *A. landryorum* male abdominal T8 [slide USNM130252, paratype]; **3a**, *A. divaricata* male genitalia dorsal view [slide MIC6995]; **3b**, *A. divaricata* male genitalia, lateral view [slide MIC6995]; **3c,** close up of uncus-socii in dorsal view [slide MIC6995]; **3d**, *A. divaricata* male abdominal T8 [slide MIC6995].

(Fig. 2a–c). In *A. divaricata*, distal half of the valvae is only slightly up curved, with obliquely truncate apices below the socii-gnathos; the horn-like socii have the ventral lobe extended beyond the apex of the dorsal horns; base of the socii is nearly flat, with shorter, more erect setae, the anterior section broadly connected to the tegumen; the gnathos is short with the apex above the valvae; and sides of the tegumen are broadly rounded with nearly contiguous anterior margins (Fig. 3a–c).

The male T8 of *A. landryorum* is distally conical with a rounded apex, and has lateral angles that are markedly projected into elbow-like processes (Fig. 2d). In male *A. divaricata*, T8 is rectangular-conical with the apex subtruncate and dentiform corners, and the lateral angles are not projected (Fig. 3d).

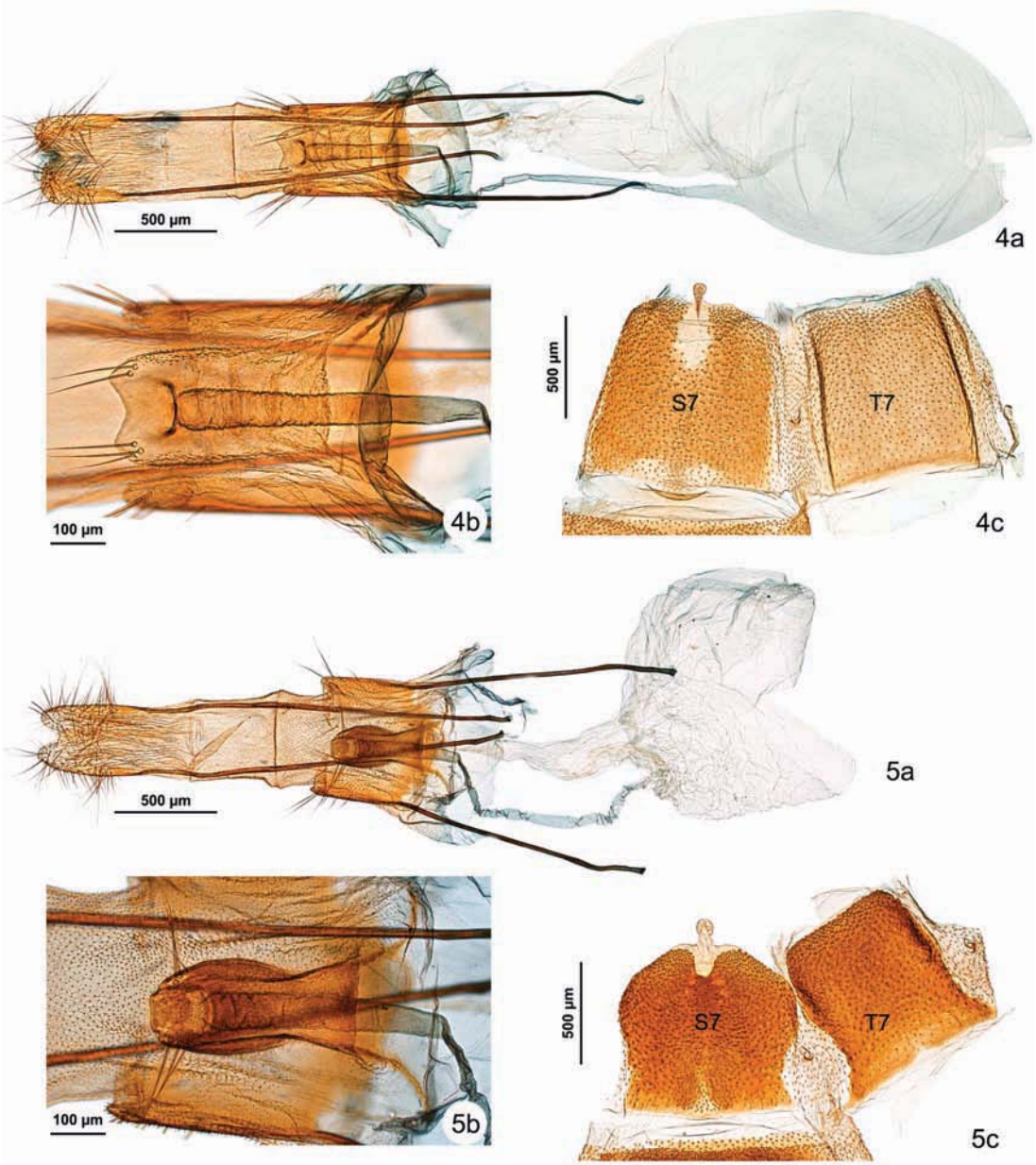

FIG. 4–5. *Arotrura* female genitalia and 7th abdominal segment; **4a,** *A. landryorum* [slide USNM130264, paratype]; **4b,** *A. landryorum*, close up of S8 and sterigma [slide USNM130264]; **4c,** *A. landryorum* 7th abdominal segment, **5a,** *A. divaricata* [slide MIC6996, bursa partly broken]; **5b,** *A. divaricata*, close up of S8 and sterigma [slide MIC6996]. **5c,** *A. divaricata* 7th abdominal segment

The female sterigma of *A. landryorum* is parallel-sided with an apical notch and the membrane of S8 is very finely spiculate (Fig. 4a–b), whereas in *A. divaricata* the sterigma is laterally dilated with the apex rounded and the S8 membrane is coarsely spiculate (Fig. 5a–b).

The female sternite 7 of *A. landryorum* is nearly rectangular with an anterior crescentic invagination of the intersegmental membrane and a postero-median unmelanized zone, and tergum 7 is parallel-sided (Fig. 4c); whereas in *A. divaricata* S7 is broadly scoop-shaped posteriorly, without anterior invagination and postero-median unmelanized zone, but with a notch instead, and the sides anteriorly sinuate (Fig. 5c).

Description. Adult male (Fig 1a): **Head**: Front and vertex smooth, scales directed forward and ventrally, pale yellow; palpi sickle-shaped, up curved to vertex, divergent apically, scales slightly shaggy ventrally, otherwise appressed, pale yellow. Base of haustellum densely scaled, scales appressed, pale yellow. Eye with long thin pale yellow lashes dorsally and ventrally. Antenna first

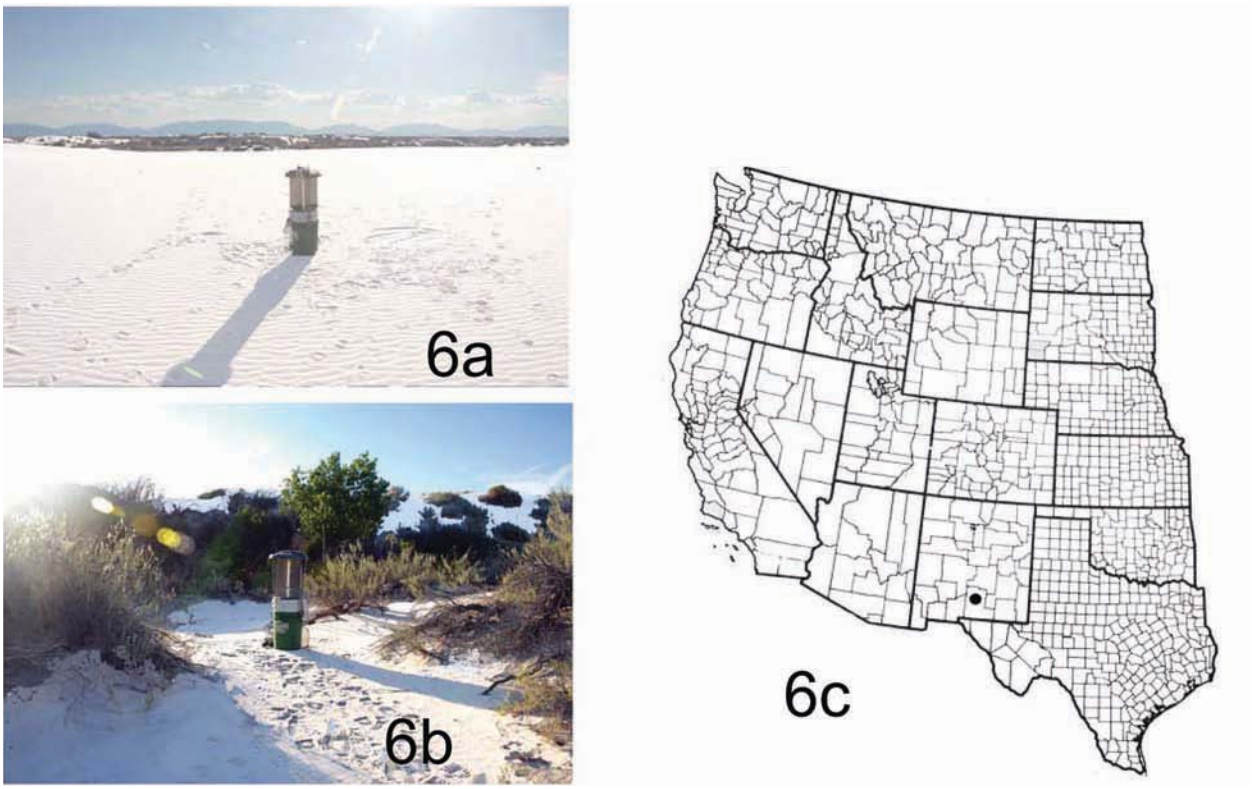

FIG. 6. *Arotrura landryorum* type locality, habitats, and distribution; **6a**, type locality of *A. landryorum*; **6b**, habitat of paratypes of *A. landryorum*; **6c**, distribution of *A. landryorum*.

segment robust, long hair-like scales directed anteriorly, densely scaled dorsally, shaggy, pale yellow; antennae to apex dorsum densely scaled, pale yellow; ventrally naked with dense short setae. **Thorax**: Thoracic scales appressed, pale yellow, tegulae with long hair-like scales directed posteriorly, pale yellow; underside, scales appressed, whitish; foreleg unarmed, scales appressed, whitish; midleg with terminal claw, scales appressed except ventrally shaggy, whitish; hind leg with two pair femur spurs, dorsally shaggy, ventral scales appressed, whitish; hind leg tarsomeres ventral surface with dense semi-erect scale bundles resembling short spurs, dorsal scales appressed, pale yellow. **Forewing** lanceolate, pale yellow to white, no markings, scattered gypsum colored scales; fringe long at tornus, concolorous; **Hindwing** lanceolate, reflective white scales appearing brown like mica, fringe long, pale yellow; underside fore wing disc and cell light brown, otherwise wing pale yellow; fringe long at tornus, pale yellow; underside hind wing costa light brown, otherwise whitish; fringe long, pale yellow/whitish. **Abdomen**: scales appressed, pale yellow. T8 (Fig. 2d) shield-shaped, distally narrowed to a rounded apex; lateral processes heavily sclerotized, directed lateral posteriorly, elbow-like. **Male genitalia** (5 preparations examined, Figs 2a–c): anterior part of tegumen broadly hood-shaped in lateral aspect, left and right sides connected dorso-anteriorly by narrow sclerotized region, anterior dorsal margin broadly V-shaped, tegumen posteriorly divided at articulation with valves; juxta slopes ventrally from anterior end to posterior end; aedeagus bulbous at anterior end; vinculum appears like human hip bones; posterior end of socii slightly upturned and divergent, appearing in lateral aspect "horn-like," convex dorsally, with broadly angled lobe directed ventrally at midpoint, setose, setae directed anteriorly; gnathos proximal arms directed posteriorly and broadly curved 90° ventrally; valvae elongate, bases widened dorsally and ventrally, distally convergent and narrowed, bent dorsally 90° beginning at 3/5 length, apex slightly toothed, setose on dorsal surface, and anterior surface when the apices are vertical.

Adult female (Fig 1b): Habitus nearly identical to male. **Forewing**: length 6.6 mm, n = 1. **Abdomen** (Fig 4c): S7 slightly elongate-rectangular with rounded posterior angles, anterior margin with distinct crescentic invagination and transverse median unmelanized zone, posteriorly with elongate spoon-shaped unmelanized zone and small, protruding, tongue-like membranous appendix. T7 slightly elongate-rectangular with squarish posterior angles, surface evenly melanized. **Female genitalia** (1 preparation examined, Fig. 4a–b): ovipositor about 0.2× length of abdominal segments 1–7; papillae anales densely setose, apically notched, with lateral portion around attachment of posterior apophysis slightly sclerotized; anterior and posterior apophyses each 0.3× length of T8; sterigma elongate, about as long as T8, parallel-sided, apex notched, with 2–3 setae, laterodistal portion finely spiculate, antero-lateral apodemes present but somewhat indistinct; ostium bursae subapical on sterigma; ductus bursae with sclerotized, transversely wrinkled antrum extended beyond anterior margin of S8, inception in middle of left side of corpus bursae; corpus bursae elongate-ovoid, tapered caudad of inception of ductus bursae;

Remarks. The new species is placed in the genus *Arotrura* based on the structure of the male and female genitalia, especially the enlarged male tergum 8 with anterolateral extensions fused alongside the tegumen and wishbone-like gnathos, and female sternum 7 with a small, tongue-like, protruding membranous lobe. The distally fused socii with an apically bihorned profile, slender aedeagus with bulbous base, valvae with "shouldered" bases in male genitalia, and proboscis-like sterigma with antero-lateral apodemes and apical setae in female genitalia place it in the *A. divaricata* species group as defined by Landry (1991). Individual specimens of *A. landryorum* have some widely scattered gypsum-colored scales on the fore wing.

DNA barcoding of the type series (BOLD:ACS7411) also showed the new species to be distinct from 37 other barcoded species of *Arotrura* (J.-F. Landry, unpublished data 2015; see http://www.boldsystems.org). Details of the barcoding protocol are as presented in Landry et al. (2013). A more extensive DNA barcode analysis of this and other *Arotrura* species occurring at WSNM will be presented in a subsequent paper.

Distribution and biology. *Arotrura landryorum* occurs in White Sands National Monument, Otero County, New Mexico (figs 6a, 6b, and 6c). The immature stages and host plant are unknown.

Etymology. The specific name of this species, *landryorum*, a noun in the genitive case, recognizes the contributions of Jean-François with support of his spouse, Marie Landry, to the study of Lepidoptera. Jean-François Landry and I share a personal and professional relationship going back to the early 1980s. Marie Landry, ever-supportive of Jean-François, and his constant companion in pursuit of lepidopterological studies, was a gracious hostess when I visited their home. I am pleased to recognize Jean-François and Marie Landry.

DISCUSSION

Most species of *Arotrura* in North America are undescribed. Landry (1991) listed 11 described species and mentioned 59 undescribed species of this genus, thus a comparison of *A. landryorum* to its congeners is limited. *Arotrura landryorum* is not one of the undescribed species previously seen by Landry.

The primary purposes of the 10-year study at White Sands National Monument were to compile an inventory of moths, and describe new species in habitats within and immediately adjacent to the white gypsum dunes in the Monument. White Sands National Monument preserves 284.9 km^2 (110 square miles), about 40%, of the world's largest snow-white gypsum dune field. The remainder of the 275 square miles dune field is under the jurisdiction of the U.S. Army via the White Sands Missile Range. The dune field is located in the northern Chihuahuan Desert in southern New Mexico's Tularosa Basin (Schneider-Hector, 1993). A complete description of the study site and some of its unique biological resources is in Metzler et al. (2009). Stroud (1950) reported twenty species of Lepidoptera from the Monument, all of them representing typical fauna of the region. In this survey during the period 9 February 2007 through 4 December 2015, more than 650 named species (unpublished data) of described Lepidoptera were recorded from the Monument. Additionally, twelve new species were so far described on the basis of moths discovered during this study at White Sands National Monument (Metzler 2014, Metzler 2016; Metzler et al. 2009, 2010; Metzler & Forbes 2011a, 2011b, 2011c, 2012; Metzler & Lightfoot 2014, Wright 2012, 2014, Wright & Gilligan 2015).

The lack of lepidopteran specimens seen until now can probably be attributed to the dearth of insect collecting in the gypsum dunes ecosystem in New Mexico because the dunes were private property, and are now under the control of the U.S. National Park Service and the U.S. Army.

ACKNOWLEDGMENTS

It is my recently formed opinion, that the spouses of lepidopterists, the ones who maintain the home-front and provide physical and moral support to the lepidopterist in the family, the ones who frequently prepare the meals, do the laundry, and perform the bulk of the childcare, do not receive enough recognition for their support. I am pleased to recognize Marie along with Jean-François in the name of this moth.

The Western National Parks Association, Tucson, Arizona contributed funding for travel and logistics for the study of Lepidoptera at White Sands National Monument. I am especially grateful for the financial support. . The El Paso Zoo Conservation Committee, El Paso, Texas, and the Association of Zoos and Aquariums' Terrestrial Invertebrate Taxon Advisory Group (TITAG), Seattle, Washington also contributed small grants. Their commitment to this research is rewarding. Jean-François Landry was very helpful with the identification and diagnosis of this and other new species I found in the Monument, and assisted with genitalia dissections, imaging, and DNA barcoding. Several executives, David Bustos, Marie Frias-Sauter, Hildy Reiser, Kevin R. Schneider, Cliff Spencer, Diane White, and Becky Burghart from the National Park Service were instrumental in arranging and promoting this study of the moths. I single out David Bustos, recipient of the U.S. National Park Service's 2014 Director's Trish Patterson Student Conservation Association Award for Natural Resource Management in a Small Park, for his enthusiastic support and for getting things done.

The National Park Service granted permits to take samples of moths and provided access to areas normally closed to the public. Michigan State University's A.J. Cook Arthropod Research Collection and the University of New Mexico's Museum of Southwestern Biology agreed to be repositories for the specimens collected during the study. Voucher specimens are also deposited in the U.S. National Museum of Natural History (Smithsonian).

Representatives from research collections and other institutions provided insect pins, alcohol, identification services, research consultation, and storage space for specimens collected leading to discovery and naming of this species. I thank the following persons for offering support from their respective institutions: Kelly B. Miller, Sandra L. Brantley, and David C. Lightfoot (University of New Mexico), Frederick W. Stehr, Anthony I. Cognato, and Gary L. Parsons (Michigan State University), J. Donald Lafontaine, Jean-François Landry, Vazrick Nazari, and B. Christian Schmidt (Canadian National Collection of Insects, Arachnids, and Nematodes), Larry Berger (Ohio Department of Agriculture), and David Adamski, John W. Brown, Mark E. Metz, David G. Furth, Patricia Gentili-Poole, and M. Alma Solis (United States National Museum of Natural History). David Adamski visited me in June 2007 to help collect the first few specimens in the type series. Patricia A. Metzler faithfully assisted me on many aspects of this study, and she provided funding. I thank Jean-François Landry, Vazrick Nazari and Kari Nupponen for reading the paper and offering valuable suggestions.

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Submitted for publication 5 January 2016; revised and accepted 4 April 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 201–204

SOOTY WING PHENOTYPE FOUND IN OFFSPRING OF A WILD-CAUGHT FEMALE OF *EUREMA MANDARINA* (LEPIDOPTERA: PIERIDAE)

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ABSTRACT. Here I describe a sooty wing phenotype in offspring produced by a wild-inseminated female of *Eurema mandarina* (Lepidoptera: Pieridae) collected in Tanegashima Island, Japan in May, 2014. Seven (3 females and 4 males) out of 36 offspring had a sooty phenotype on their wings, which is likely due to a simple genetic mutation (most likely a single recessive allele in an autosome). The expression of this phenotype, restricted to the ventral side of the wings, was not uniform—more conspicuous in hindwings than in forewings, and the affected areas were variable among individuals. Microscopic observation revealed that the sooty phenotype was attributed to the increased proportion of pigmented scales.

Additional key words: mutant, pigmentation, scale, ventral

In May, 2014, I collected female adults of the yellow butterfly *Eurema mandarina* (Lepidoptera: Pieridae) in Tanegashima Island (Kagoshima Prefecture, Japan). They were brought into the laboratory and allowed to lay eggs on fresh leaves of *Lespedeza juncea* var. *subsessilis* (Fabales: Fabaceae). Among 18 broods fed on an artificial diet containing *Albizia julibrissin* (Fabales: Fabaceae) leaves (Narita et al. 2007a) at 25°C with 16h : 8h (light : dark) photoperiod, one brood developed to adults consisting of individuals with a sooty phenotype in their wings (here referred to as sooty-wing or *sw*) as well as individuals with a wild phenotype (Table 1). The *sw* phenotype was expressed only on the ventral side of the wing (both forewing and hindwing) (Fig. 1; Fig. 2a), implying that this mutation may be governed by a gene responsible for dorsal/ventral separation of wing cells (such as apterous in *Drosophila*; Blair 1994, reviewed by Held 2002). The expression of this phenotype was more conspicuous in hindwings than in forewings, and the affected areas were variable among individuals—in the female hindwings for instance, the marginal area (code 013) and the interior area (codes 011 and 032) were conspicuously affected (Fig. 1a).

In *E. mandarina*, there are peculiar spots in the specific positions of the ventral side of the wings (both forewings and hindwings in males and females; Yata 1995). Each of these spots was also present in *sw* individuals (Fig. 3), indicating that the *sw* phenotype is not due to the mislocation of melanic scales which are to constitute these spots.

Because all the insects were reared at a constant temperature (25°C), the *sw* phenotype is not likely to be due to the thermal effects as has been observed in monarch butterflies, *Danaus plexippus* (Davis et al. 2005). The ratio of *sw* individuals (n = 7) to wild-type individuals (n = 29) was not significantly deviated from 1:3 (Table 1), a ratio predicted from the Mendelian segregation of an autosomal recessive allele *sw*, which was carried by both of their parents in the heterozygous condition (*sw*/+) ($P > 0.05$ by Fisher's exact probability test). On the other hand, the ratio of *sw* to wild-type individuals was significantly deviated from 1:1, a ratio predicted from the assumption that one of the parents was homozygous (*sw*/*sw*) ($P = 0.0125$ by Fisher's exact probability test). Although other possibilities such as polygenic effects remain, a single autosomal recessive allele is most likely to be responsible for the phenotype.

Close inspection under dissecting microscope revealed that the *sw* phenotype is due to the marked increase in number of blackish or brownish scales (Fig. 2c,d,e,f), which is probably due to the pigmentation by melanins. Thus, a disruption in the regulation of melanin biosynthesis may be responsible for the phenotype (True 2003). Darkening of color was first recognized at the late pupal stage, implying that the

TABLE 1. Phenotype of 36 offspring produced by a wild-caught *E. mandarina* female

	sw	Wild-type	Total
Female	3	11	14
Male	4	18	22
Total	7	29	36

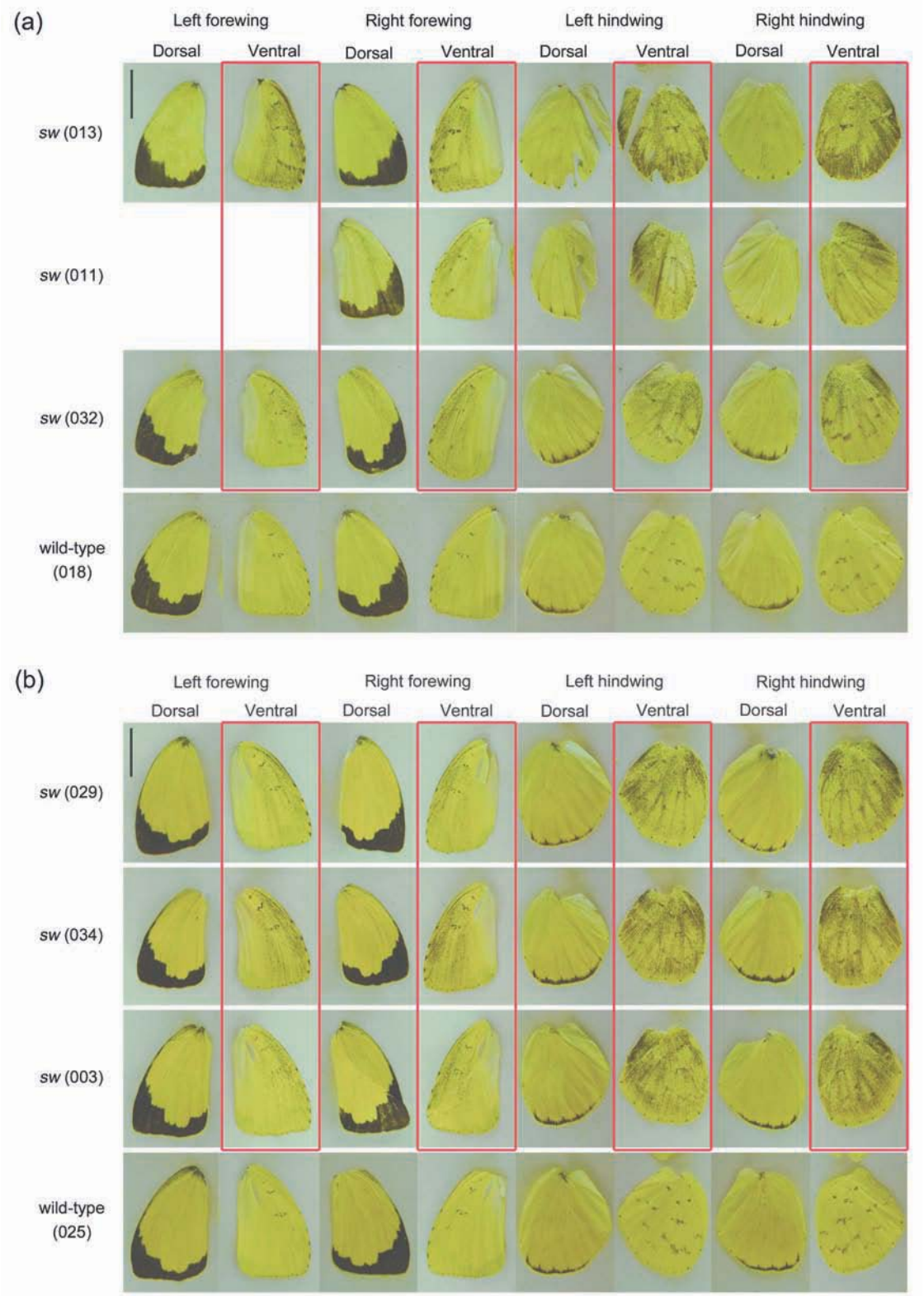

FIG. 1. Wings with sw phenotype. (**a**) Females. Left forewing of a sw individual (code 011) was crumpled and was not suitable for photograph (missing panels). (**b**) Males. Red rectangles indicate wings showing sw phenotype (restricted to the ventral side). Bars represent 10 mm.

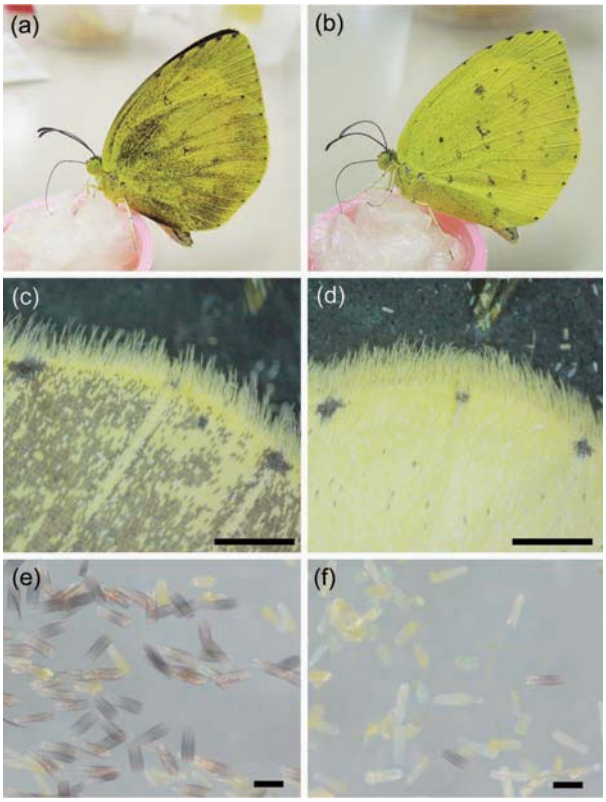

FIG 2. Individuals with sw phenotype (a,c,e) and wild-type individuals (b,d,f). (**a,b**) Butterflies drinking honey solutions. (**c,d**) Magnified image of peripheral areas of hindwings. Bars represent 1 mm. (**e,f**) Scales removed from the wing surface. Bars represent 200 m m.

appearance of this phenotype is associated with the extension of invaginated wing discs in *E. mandarina* (Švácha 1992, Heming 2003).

When an *E. mandarina* butterfly is at rest, the ventral side of the wings is more conspicuous than the dorsal side (Fig. 2a). Hence, *sw* phenotype would be prone to be subjected to natural selection, be it positive or negative. In some butterflies, adaptive significance of wing melanism as a thermoregulatory mechanism is established (Watt 1968, Roland 1982, Guppy 1986).

E. mandarina is a model species for the study of *Wolbachia*, a host manipulating intra-cellular bacteria (Werren et al. 2008, Kageyama et al. 2012). Two strains of *Wolbachia* were described in *E. mandarina*—a cytoplasmic-incompatibility-inducing *w*CI, being almost fixed except for the northernmost part of Japan (Hiroki et al. 2005; personal observation by DK); a feminizing wFem (always coexisting with *w*CI), being found only from Tanegashima Island and Okinawa-jima Island (Hiroki et al. 2004, Narita et al. 2007a, b, Kern et al. 2011). Here, I diagnosed all the 36 individuals of the brood (both *sw* and wild-type individuals) by PCR that was designed to specifically detect each of the strains (Hiroki et al. 2004), and found that they were all singly infected with *w*CI.

ACKNOWLEDGEMENTS

I am grateful to Masatsugu Hatakeyama for helpful discussion on wing development, and Markus Riegler, an anonymous reviewer, and the editor Keith S. Summerville for constructive comments to the early version of the manuscript.

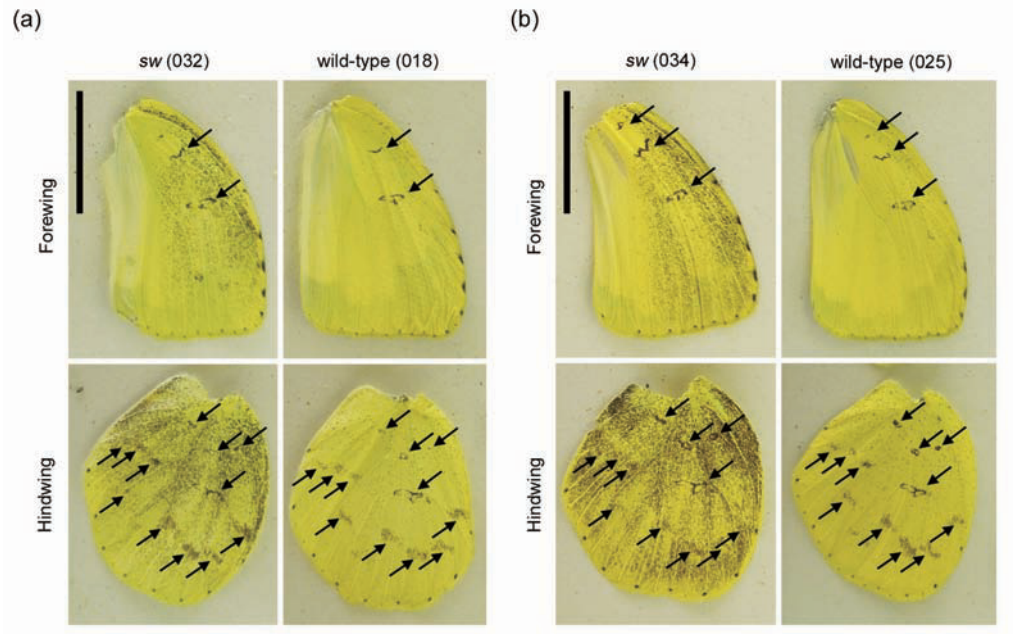

FIG 3. Magnified images of some panels of Fig. 1. (**a**) Female left wings. (**b**) Male left wings. Arrows represent markings present in both wild-type and sw individuals. Bars represent 10 mm.

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Submitted for publication 25 December 2015; revised and accepted 20 February 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 205–210

IMMATURE STAGES OF *TERIOCOLIAS ZELIA ANDINA* FORBES (PIERIDAE: COLIADINAE)

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ABSTRACT. The egg, larva and pupa of *Teriocolias zelia andina* Forbes, 1928 (Lepidoptera, Pieridae, Coliadinae) are described and illustrated for the first time based on specimens collected on *Senna birostris* var. *arequipensis* (Fabaceae) on the western slopes of the Andes of northern Chile. The morphology of the egg and first instar enables separating *T. z. andina* from species of its sister genus *Leucidia* Doubleday, 1847. The larva of *T. z. andina* passes through five instars, which can be accurately identified by head width.

Additional key words: Andes, Folivory, *Leucidia*, Monophagy, *Senna birostris*

Teriocolias Röber, 1909 (Lepidoptera: Pieridae: Coliadinae) is a monotypic genus of the Neotropical Coliadinae whose only species, *T. zelia* (Lucas, 1852), currently includes four subspecies which are mostly associated with the Andes of Argentina, Bolivia, Chile and Peru (Lamas 2004). Molecular phylogenetic studies have confirmed a close evolutionary relationship of the genus with the also Neotropical *Leucidia* Doubleday, 1847 (Braby et al. 2006, Wahlberg et al. 2014), as suggested earlier by Klots (1928) based on morphological characters of the adult stage.

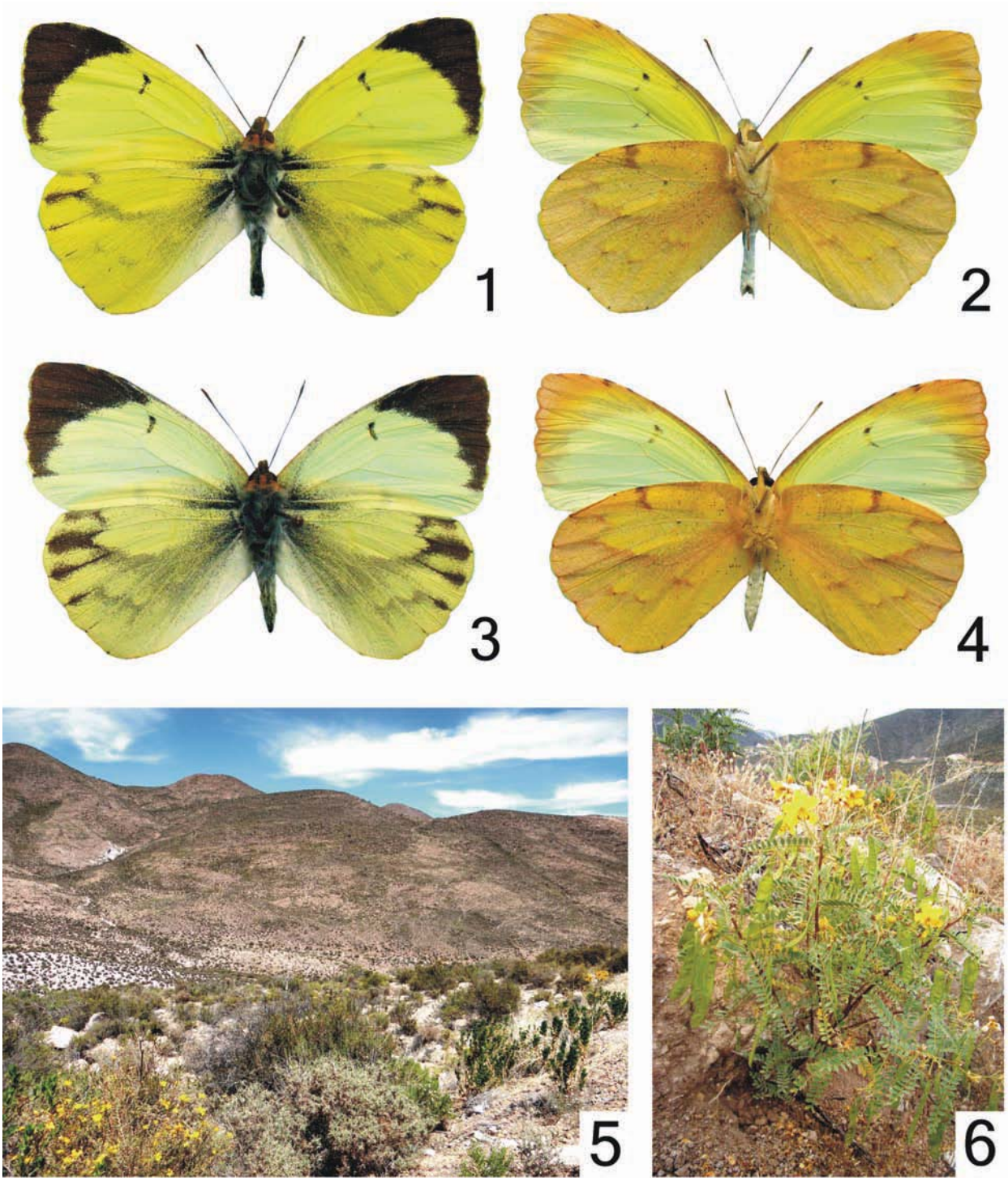
Teriocolias zelia andina Forbes, 1928 (Figs. 1–6) was originally described from Peru (Forbes 1928). It is the only subspecies of *T. zelia* recorded in the Andes of northern Chile (Peña & Ugarte 1996). Although it is one of the most characteristic butterflies of the prepuna belt of the Parinacota Province (Benyamini 1995), its biology remains insufficiently known. Based on the few biological data available, *T. z. andina* appears to be a highly host-specialized (monophagous) butterfly, at least at the local level, because its eggs have been found only on the native shrub *Senna birostris* var. *arequipensis* (Vogel) H. S. Irwin et Barneby (Fabaceae) despite intensive field surveys, while the recently eclosed first instar is unable to feed on leaves of other native or exotic Fabaceae plants growing in this arid landscape (Vargas 2012). Furthermore, although sometimes eggs are laid on mature leaves of the host plant by the females, egg-laying is performed preferentially on new leaves (Vargas & Benítez 2013). It has been shown that the egg phenology of *T. z. andina* is mostly associated with the availability of plant substrate adequate for egg-laying and subsequent larval feeding (Vargas & Benítez 2013).

The importance of morphological studies of immature stages for the understanding of the systematics and evolution of Lepidoptera has been widely recognized (Scoble 1995). Furthermore, the close relationship between *T. z. andina* and *S. b. arequipensis* suggests that this butterfly-plant system could be used as a model for insect phenological studies in the arid environments of the Andes. However, the immature stages of the butterfly remain undescribed, hindering their identification in the field. Accordingly, the morphology of the egg, larva and pupa of *T. z. andina* are described and illustrated for the first time.

MATERIAL AND METHODS

Eggs of *T. z. andina* were searched for on plants of S. b. *arequipensis* near Socoroma village (18°16'S, 69°35'W), at about 3,300 m elevation on the western slopes of the Andes of the Parinacota Province of northern Chile between April 2011 and September 2015. The site is characterized by a tropical xeric bioclimate with about 9° C annual mean temperature and about 160 mm annual precipitation mostly concentrated between December and February (Luebert & Pliscoff 2006). The vegetation cover is seasonal, reaching higher levels after rains, during April–May (Muñoz & Bonacic 2006).

Leaflets with eggs were collected and brought to the laboratory in individual plastic vials, where they were maintained until larvae emerged. Fresh leaves of the host plant were added daily ad libitum until the last instar stopped feeding to start to prepare for pupation. Ten immature specimens of each instar and stage were stored in ethanol 70% and were used to perform metric measurements with the aid of a graduated ocular mounted on a stereomicroscope. Vouchers were deposited in the "Colección Entomológica de la Universidad de Tarapacá" (IDEA), Arica, Chile. The remaining individuals were reared through the life cycle to obtain adults to confirm the taxonomic identification. The adults obtained were mounted following standard procedures and were deposited in IDEA.



FIGS. 1–6 Adult, habitat and host plant of *Teriocolias zelia andina* Forbes, 1928 in the Andes of northern Chile. **1**) Male in dorsal view. **2**) Male in ventral view. **3**) Female in dorsal view. **4**) Female in ventral view. **5**) Habitat close to Socoroma village at about 3,300 m elevation. **6**) The only host plant species in the study site, *Senna birostris* var. *arequipensis*.

RESULTS

Life history. The larva passes through five instars, which are all folivorous. The first instar makes a hole sub-apically to exit the chorion and consumes it variably, after which it begins to consume the leaflet. The leaflet consumption begins thereafter. The first and second instars scrape the leaflet, leaving the cuticle of the opposite side intact, similar to leaf skeletonizer larvae. In contrast, leaflets are completely consumed by the third, fourth and fifth instars. The fifth instar stops eating 1–2 days before pupation, moving toward shoots or raquis to pupate. The pupa is secured to the substrate by silk threads spun on the cremaster, and by a silk girdle surrounding the anterior abdominal segments and the wings.

Egg (Fig. 7, 8). Fusiform, upright, white immediately after deposition, subsequently orange-yellow. Chorion weakly striated longitudinally by about 40 ridges, most of which extend the length of the chorion, some are interrupted close the apex; an undetermined number of poorly differentiated transverse ridges; translucent, the larva may be seeing before eclosion. Height: 1.34 mm (1.30–1.40 mm); width: 0.42 mm. (0.38–0.46 mm); n = 10. Duration: 5–6 days (n = 10).

First instar (Fig. 9). Head black, thorax and abdomen yellow immediately after eclosion, greenish yellow after meal; legs, prothoracic shield, anal shield, pinnacles and setae black. Maximum length: 2.5–3.0 mm (n = 10). Duration: 6–7 days (n = 10).

Second instar (Fig. 10). Head yellowish brown; thorax and abdomen pale green, with many translucent secondary setae. Maximum length: 4.5–4.8 mm (n = 10). Duration: 4–5 days (n = 10).

Third instar. Color similar to second instar. Maximum length: 6.0–6.7 mm (n = 10). Duration: 4–5 days (n = 10).

Fourth instar. Color similar to fifth instar. Maximum length: 9.5–11.0 mm (n = 10). Duration: 4–7 days (n = 10).

Fifth instar (Fig. 11). Head, thorax and abdomen light green; small light gray dots on thorax and abdomen which are sometimes absent; a white longitudinal stripe from prothorax to A10. Maximum length: 18.0–19.0 mm (n = 10). Duration: 7–9 days (n = 10).

Instar identification. As described above, the only instar clearly different from any other is the first, based on body coloration and shape. Using the same attributes the second instar may be easily confused with the third, while the fourth instar may be confused with the fifth. However, the cephalic widths of successive instars are clearly different with no overlaps among them. Thus, this measurement may be used successfully for instar identification in *T. z. andina* (Table 1).

TABLE 1. Mean and standard deviation (SD), interval of variation (IV) and growth rates (GR) of the head capsule width in larval instars of *Teriocolias zelia andina* Forbes reared on *Senna birostris* var. *arequipensis* (Fabaceae).

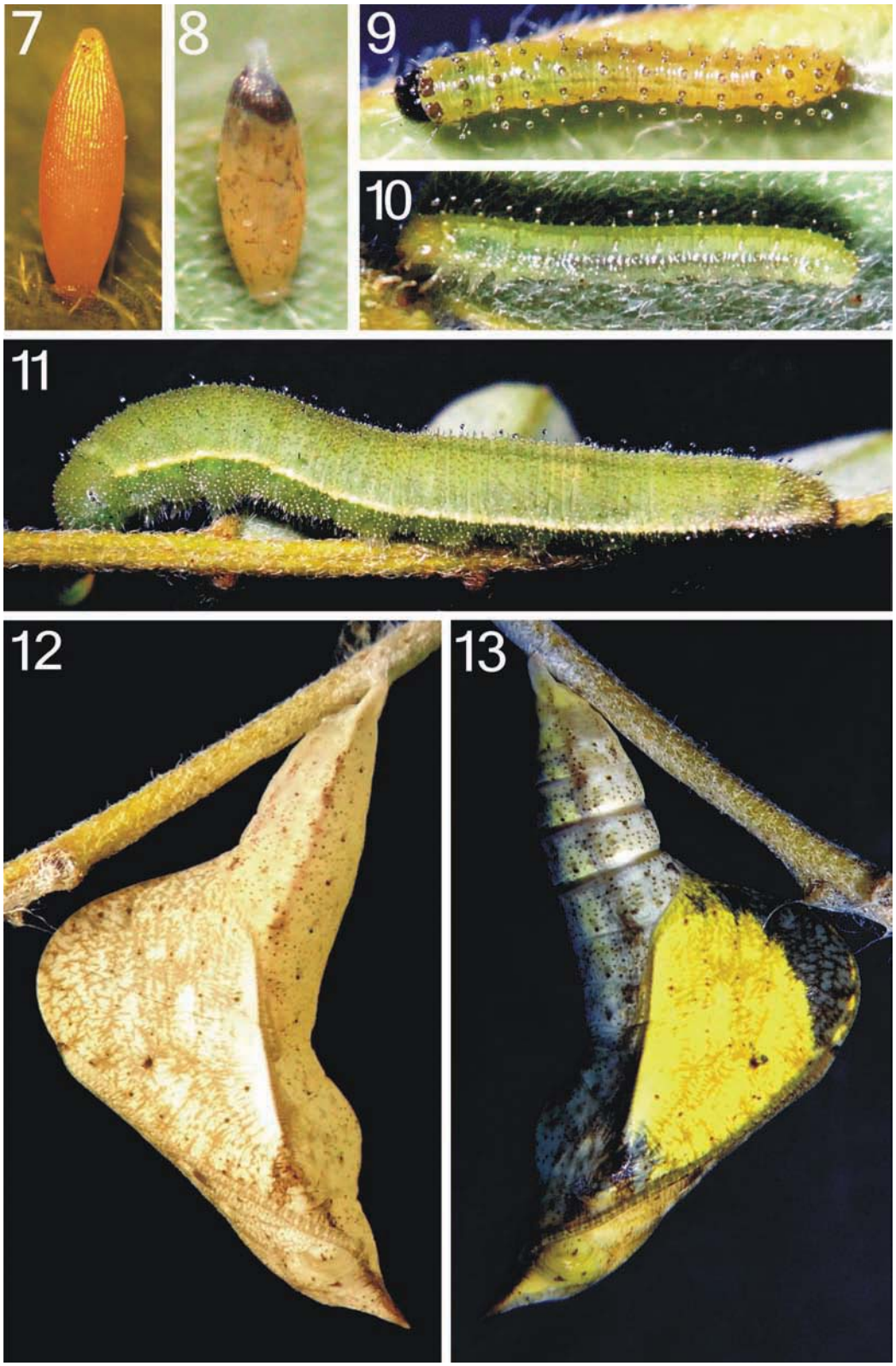
		Head capsule width (mm)		
Instar	N	Mean ± SD	IV	GR
I	10	0.33 ± 0.0083	0.32 – 0.34	
II	10	0.51 ± 0.0208	0.48 – 0.56	1.55
III	10	0.80 ± 0.0272	0.74 – 0.84	1.57
IV	10	1.20 ± 0.0479	1.10 – 1.22.	1.50
V	10	1.94 ±0.0562	1.86 – 2.12	1.62

Pupa (Fig. 12, 13). Translucent integument; cream white initially; wing pattern of the adult may be easily observed through the integument before eclosion. Head with a conspicuous anterior projection; wings broadly projected ventrally and flattened laterally. Length: 14.0–15.0 mm (n = 10). Duration: 10–15 days (n = 10).

DISCUSSION

Knowledge of morphology and life history of immature stages of Lepidoptera is useful in studies of systematics and evolution (Freitas 2006, Kaminski & Freitas 2008, Dias et al. 2015, Salik et al. 2015, Neves & Paluch 2016) being also a valuable tool in ecological studies requiring field identification of immature stages (Pessoa-Queiroz & Diniz 2007, Nelson 2015; Silva et al. 2016). However, although the immature stages of several Neotropical Pieridae have been described (Shapiro 1979, 1989, 1991; Aiello 1980; Braby & Nishida 2007, 2011; Kaminski et al. 2012, Hernández-Mejía et al. 2014, 2015) these remain unknown for many genera and species.

This is the first description of the immature stages of *T. z. andina*. The morphological pattern generally fits that described for the sister genus *Leucidia* (Freitas 2008); however, some differences were detected. Freitas (2008) described the egg of *Leucidia* as four times longer than wide, with 16–18 weakly marked longitudinal ridges and 30–35 transverse ridges. Contrastingly, the egg of *T. z. andina* is 3.19 times longer than wide, with the chorion finely sculptured by a large number of longitudinal ridges and an undetermined number of poorly differentiated transverse ridges. Interestingly, the morphology of the chorion of *T. z. andina* appears to be different to that of other Coliadinae genera already described in detail by Hernández-Mejía et al. (2014).



FIGS. 7–13 Immature stages of *Teriocolias zelia andina* Forbes, 1928 collected on *Senna birostris* var. *arequipensis* in the Andes of northern Chile. **7**) Egg two days after oviposition. **8**) Egg with the first instar ready to eclose. **9**) First instar in dorsal view. **10**) Second instar in lateral view. **11**) Fifth instar in lateral view. **12–13**) Pupa recently molted and with adult ready to emerge, respectively, in lateral view.

The first instar of *Leucidia* and *T. z. andina* also can be differentiated: this is completely green or pale green in *Leucidia* (Freitas 2008), contrasting with the black coloration of the head, pinnacles and setae in *Teriocolias*. Although no obvious morphological differences were detected for the subsequent instars, an important difference was found in development, since five larval instars were observed in *T. z. andina*, while the larvae of *Leucidia* pass through only four instars (Freitas 2008). As mentioned above, the instars of *T. z. andina* can be accurately separated by the measurement of the cephalic width. Accordingly, further ecological studies with this species can be complemented with detailed age characterizations of samples.

The results obtained in this study for the egg and first instar suggest that the morphological characters of immature stages may be helpful in further comparative studies of Coliadinae. However, it is evident that more detailed analyses, including scanning electron microscopy (SEM), are needed to know better the external morphology of *T. z. andina*, because SEM is useful either to find subtle diagnostic characters among species with highly conserved morphology and to find characters defining close genera of butterflies (Duarte & Robbins 2009; Vargas et al. 2014). Studies should be expanded to the other subspecies of *T. zelia* and also to additional genera of Coliadinae close to *Terocolias*. Furthermore, the incorporation of additional genera of Coliadinae in morphological studies of immature stages will be highly valuable to assess the character evolution in this butterfly group in the light of current phylogentic hypotheses (Braby et al. 2006, Wahlberg et al. 2014).

ACKNOWLEDGMENTS

I thank two anonymous reviewers for valuable comments and suggestions on a preliminary version, Marcelo Vargas-Ortiz for editing the figures, Lafayette Eaton for checking the English, and Darli Massardo and André Victor Lucci Freitas for providing literature. Financial support was obtained from Project DIEXA-UTA 9710-10 from the Universidad de Tarapacá.

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Submitted for publication 28 February 2016; revised and accepted 11 March 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 211–217

PHYLOGENETIC ANALYSIS SUPPORTS THE RECOGNITION OF *ALBUNA BEUTENMULLERI* SKINNER AS A SPECIES DISTINCT FROM *A. PYRAMIDALIS* WALKER (LEPIDOPTERA: SESIIDAE)

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ABSTRACT. *Albuna beutenmulleri* Skinner (Lepidoptera: Sesiidae) is endemic to eastern and southern Utah, feeds on primrose (*Oenothera pallida* Lindl.), and is distinctly colored with opaque, orange-red wings. However, *A. beutenmulleri* was synonymized as a color variant of the widely distributed *A. pyramidalis* (Walker). Thus, given the criteria of monophyly, we tested the species status of *A. beutenmulleri* in the context of *Albuna* species. Phylogenetic analysis of mitochondrial DNA revealed that specimens of *A. beutenmulleri* and *A. pyramidalis* were reciprocally monophyletic. The nucleotide difference between species was ~8% which was similar to the distance among other *Albuna* species and exceeded the intraspecific difference by ten times. Given phylogenetic and life history evidence, we recognize *A. beutenmulleri* as a distinct species from *A. pyramidalis* and describe a yellow variant of *A. beutenmulleri*.

Additional key words: Sesiidae, *Albuna beutenmulleri*, *Albuna pyramidalis*, DNA taxonomy

Walker (1856) described *Albuna pyramidalis* from a specimen collected along the Albany River near Hudson's Bay, Canada. Since then, specimens of the species have been collected within the endemic range of fireweed and willow-herb, (*Chamerion angustifolium* (L.) and *C. latifolium* (L.), (Onagraceae), respectively). Its current distribution includes Alaska, Canada, the Rocky Mountains, the northern tier of the United States and the Cascades and Sierra Nevada to the Pacific coast (Eichlin & Duckworth 1978, 1988). Color variants exist throughout its extensive range (Beutenmüller 1901)(Fig.1). Of these, beutenmulleri was the most distinct and was described as a new species from an individual collected in Stockton, Utah (Skinner 1903). However, it was synonymized with *A. pyramidalis* (Fig. 2 and 3) given that the color patterns showed degrees of intergradation throughout *A. pyramidalis* populations (Engelhardt 1946). Engelhardt (1946) recognized various color forms in addition to the typical form, which range from the nearly entirely black "coloradensis" Hy. Edwards to "rubescens" Hulst with orange-red wings, to "beutenmulleri" Skinner with opaque, orange-red wings. Eichlin and Duckworth (1988) investigated the genitalia among the various color forms of *A. pyramidalis* but they did not find differences that would support the recognition of different species and maintained these phenotypes as variants of *A. pyramidalis*. They maintained that *A. pyramidalis* was readily recognized by the broad oblique discal mark on the forewings despite distinct color variation throughout its range.

FIG. 1. Current geographic distribution of *Albuna pyramidalis* (shaded area) and the Utah collection sites of *Albuna beutenmulleri* (black dots). Adapted from Eichlin and Duckworth, 1978.

However, along with the color differences, beutenmulleri exhibits differences in its geographic locality, phenology and host use. Specimens of the beutenmulleri color morph have only been collected or observed from localities in Utah between elevations of 1723–1905 meters. Adults emerge as early as April and no later then June 3 which is 2 weeks before the emergence of *A. pyramidalis* (Fig. 4, Table 1 and 2). The larvae of beutenmulleri are associated with various primrose species including (*Oenothera pallida* Lindl.) as compared to various fireweeds, which are used by the typical color morph (Engelhardt 1946). These observations suggest potential reproductive barriers and ecological differences between beutenmulleri and the typical color morph of *A. pyramidalis*.

FIG. 2. *Albuna beutenmulleri*, Type female from ANSP. J.D. Weintraub (ANSP) photo.

FIG. 3. *Albuna pyramidalis* from Alberta. G. Anweiler photo.

Phylogenetic analysis using DNA sequence data offers a solution for delimiting species (Rubinoff & Powell 2004, Cognato & Sun 2007). Monophyly of suspect species with association of biological traits often provides evidence for the recognition of new species under the phylogenetic species concept (sensu Wheeler & Platnick 2000). Many cryptic Lepidoptera species, including sesiids, have been confirmed or discovered with the use of molecular phylogenies and morphology (e.g., Kallies 2002, Lumley & Sperling 2010, Dumas et al. 2015). Therefore, we reconstruct a phylogeny using mitochondrial cytochrome oxidase I DNA data for specimens of *Albuna pyramidalis* including the beutenmulleri and the typical color morph, *A. fraxini*, and species of *Carmenta, Paranthrene, Synanthedon* and *Zenodoxus* for outgroup comparison in order to test the monophyly of the beutenmulleri color morph.

MATERIALS AND METHODS

Collection of specimens. In May of 2014, David Wikle collected two males of the beutenmulleri color morph along Lick Wash just off the Skutumpah Road (37.36428, -112.18711), elev. 1905 m, in Grand Staircase-Escalante National Monument in Kane County, Utah (Fig. 5). These adults were attracted to the lesser peachtree borer pheromone lure taped to his net. The habitat was at the interface between Ponderosa Pine above and Great Basin scrub below (*Artemisia*

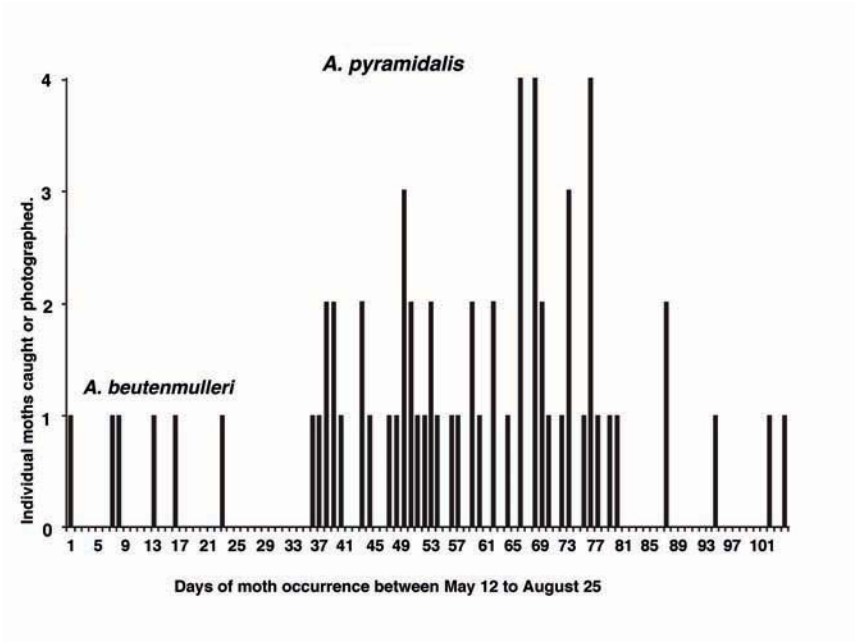

FIG. 4. Flight records for *A. beutenmulleri* (1902–2014) and *A. pyramidalis* (1902–2014).

TABLE 1. *Albuna pyramidalis* - North American records

Country	Location	Flight date	Reference
Canada	New Brunswick, Westmorland Co.	6/23, 2013	Bugguide.net
Canada	Newfoundland, Terra Nova Nat.Pk.	7/10, 2010	BOLD data
Canada	Newfoundland, Terra Nova Nat.Pk.	7/10, 2010	BOLD data
Canada	Newfoundland, Terra Nova Nat.Pk.	7/11, 2009	BOLD data
Canada	Quebec, Laurentides Park	8/15, 1956	MSU-A.J.CookM.
Canada	Ontario, Fulton Twp.	7/20, 1939	MSU-A.J.CookM.
Canada	Ontario, Hymers	6/24, 1930	Bugguide.net
Canada	Saskatchewan, Creighton	6/23, 2012	Bugguide.net
Canada	Saskatchewan, Prince Al. Nat. Pk.	6/16, 2010	BOLD data
Canada	Alberta, Banff National Park	7/26, 2010	Bugguide.net
Canada	Alberta, Banff National Park	7/27, 2014	Bugguide.net
Canada	Yukon, Teslin	7/30, 2014	Bugguide.net
Canada	Yukon, Fox lake	6/28, 2013	Bugguide.net
USA	Alaska, Delta Junction	7/27, 2013	Bugguide.net
USA	Alaska, Wrangell	7/20, 2007	Bugguide.net
Canada	British Columbia	7/07, 1988	MSU-A.J.CookM.
Canada	British Columbia	7/17, 1988	MSU-A.J.CookM.
Canada	British Columbia, Cariboo	6/27, 2007	Bugguide.net
Canada	British Columbia, Fernie	7/26, 2009	Bugguide.net
Canada	British Columbia, Vancouver Island	6/29, 2010	Bugguide.net
Canada	British Columbia, Vancouver Island	7/04, 2007	Bugguide.net
Canada	British Columbia, Vancouver Island	6/29, 2010	BOLD data
Canada	British Columbia, Vancouver Island	8/01, 2011	BOLD data
Canada	British Columbia, Whistler	8/06, 2011	BOLD data
Canada	British Columbia, Whistler	8/06, 2011	BOLD data
Canada	British Columbia, Yoho Nat. Pk.	7/24, 2010	BOLD data
Canada	British Columbia, Yoho Nat. Pk.	7/21, 2010	BOLD data
Canada	British Columbia, Yoho Nat. Pk.	7/24, 2010	BOLD data
Canada	British Columbia, Muncho Lake	7/13, 2004	Bugguide.net
USA	Washington State, Leavenworth	7/04, 2013	Bugguide.net
USA	Washington State, Bandera Mt.	7/05, 2013	Bugguide.net
USA	Oregon, Marion Forks	6/30, 1962	MSU-A.J.CookM.
USA	Oregon, Union County	6/17, 2013	Bugguide.net
USA	Oregon, Mt. Emily	8/23, 2011	Bugguide.net
USA	Oregon, Silverton	7/24, 2009	Bugguide.net
USA	Oregon, Clackamas Co.	7/27, 1994	MSU-A.J.CookM.
USA	California, Alpine Co.	6/29, 1989	MSU-A.J.CookM.
USA	California, Oman Valley	7/17, 1938	Bugguide.net
USA	New Mexico, San Miguel Co.	7/27, 2012	Bugguide.net
USA	New Mexico, Taos Canyon	6/14, 1956	MSU-A.J.CookM.
USA	Colorado, Grand County	7/19, 2014	Bugguide.net
USA	Colorado, Jackson Co.	7/02, 1962	MSU-A.J.CookM.
USA	Colorado, Colorado Springs	10/17,1937	Bugguide.net
USA	Wyoming, Johnson Co.	7/15/1993	MSU-A.J.CookM.
USA	Montana, Missoula	8/25, 2011	Bugguide.net
USA	Montana, Glacier County	7/23, 2006	Bugguide.net
USA	Montana, Glacier County	7/08, 2007	Bugguide.net
USA	Utah, Lake Co.	7/19, 1962	MSU-A.J.CookM.
USA	Minnesota, St. Louis Co.	6/30, 2015	Bugguide.net
USA	Michigan, Cheboygan Co.	7/03, 1937	MSU-A.J.CookM.
USA	Michigan, Dickinson Co.	6/19, 1984	MSU-A.J.CookM.
USA	Michigan, Dickinson Co.	6/20, 1984	MSU-A.J.CookM.
USA	Michigan, Dickinson Co.	7/13, 1983	MSU-A.J.CookM.
USA	Michigan, Dickinson Co.	7/19, 1984	MSU-A.J.CookM.

TABLE 2. *Albuna beutenmulleri* records

Location	Flight date	Reference or Depository
Utah, Tooele Co.	5/24, 1902	ANSP
Utah, Tooele/Utah Co.	4-5/1901-1913	Engelhardt, 1946
Utah, Beaver Co.	6/--, 1904	Engelhardt, 1946
Utah, Carbon Co.	5/12, 2013	Opler/CSUC
Utah, Kane Co.	5/18, 2013	Wikle collection
Utah, Kane Co.	6/03, 1981	MONA 5.1
Utah, Kane Co.	5/19, 2014	Opler/CSUC
Utah, Kane Co.	5/27, 2014	Wikle collection

ANSP= Academy Natural Sciences, Philadelphia
CSUC= Gillette Museum of Arthropod Diversity, Fort Collins

tridentata with abundant subshrubs and forbs). A year earlier in May 2013, Paul Opler caught a male and female of the beutenmulleri color morph at Cat Canyon (39.55083,-110.64098), elev. 1723 m, near Wellington, Carbon County, Utah. The female was net collected in steady flight and the male came to a pheromone strip taped to Opler's net rim. Here the habitat was open pinyon-juniper woodland at the base of a steep escarpment. The male was found amongst a patch of *Oenothera pallida* (Fig. 6) along a dirt track. The plants were all in full bloom in May 2013 but only a single plant was found in flower a year later apparently as a result of an ongoing drought (U.S. drought monitor, April 2014).

DNA sequence data and phylogenetic analyses. At Michigan State University, DNA was extracted from three hand-collected specimens initially killed with ethyl acetate and subsequently pinned and stored at 15–22° within C.P. Gillette Museum of Arthropod Diversity at Fort Collins (CUSU). Specimens were dissected by removing the meta-leg from the thorax. The leg was ground with a pestle in a 1.5 ml microfuge vial and DNA extractions were performed using a Qiagen DNeasy blood and tissue kit (Hilden, Germany) following the manufacturer's protocol. Specimens were vouchered in the A. J. Cook Arthropod Research Collection at Michigan State University. The purified DNA was used to amplify ~650 base pairs of the 5' end of mitochondrial cytochrome oxidase I gene using PCR primers LCO1490 and HCO2198 and following the PCR protocol of Herbert et al. (2003). PCR products were purified with EXO-SAP-IT (USB Corp., Cleveland, OH, USA) and following the manufacturer protocols. Purified PCR products were sequenced in the Michigan State University Research Technology Support Facility using a Big-Dye Terminator v 1.1 (Applied Biosystems, Foster City, CA, USA) and visualized using an ABI 3730 Genetic Analyzer (Applied Biosystems). Sense and antisense strands were compiled using Sequencher (Ann Arbor, MI) to trim sequences of

FIG. 5. Habitat of Lick Wash, Grand Staircase—National Monument, Utah. David Wikle photo.

FIG. 6. *Oenothera pallida* Lindl. Chicago Botanical Garden/CLM internship program photo.

TABLE 3. Specimens used for phylogenetic analysis and corresponding Genbank and BOLD identification numbers.

Albuna beutenmulleri 1	KU926960
Albuna beutenmulleri 2	KU926961
Albuna beutenmulleri 3	KU926962
Albuna pyramidalis 1	LALPA976-11
Albuna pyramidalis 2	LCHP135-07
Albuna fraxini 1	GSCMA460-10
Albuna fraxini 2	GSCMA991-11
Carmenta anthracipennis	GSCMA959-11
Carmenta mimuli	GSCMA143-10
Synanthedon bibionipennis	GSCMW712-12
Synanthedon pini	GSCMB701-12
Synanthedon refulgens	GSCMB711-12
Paranthrene tabaniformis	GBGL0315-06
Paranthrene tabaniformis	GBGL7235-10
Paranthrene insolita	PHLAW067-13
Paranthrene simulans	LGSMC752-05
Zenodoxus mexicanus	GSCMA967-11
Zenodoxus rubens	GSCMA123-10

primer sequences, to examine for ambiguities and to create consensus sequences. Final sequences of 654 bp were deposited in Genbank (Table 3).

DNA sequences of the same locus were obtained from the BOLD data base (Ratnasingham & Hebert 2007) for 15 sesiid specimens representing *Albuna pyramidalis* (typical color morph), *A. fraxini*, *Carmenta, Paranthrene, Synanthedon* and *Zenodoxus* (Table 3). A phylogeny for these species and the specimens of the beutenmulleri color morph was reconstructed using PAUP* (Swofford 2003). Heuristic searches for the most parsimonious and most likely trees were conducted. Each search consisted of 500 stepwise random additions with tree bisection-reconnection. The model for the likelihood searches corresponded to HKY85+I+G. Bootstrap values for the parsimony tree were calculated by heuristic searches with simple sequence additions for 1,000 pseudoreplicates. Intraspecific and interspecific pairwise uncorrected "p" distances were computed in PAUP*.

RESULTS

Inspection of the COI sequences produced for the three specimens of the beutenmulleri color morph revealed no evidence of nucleotide ambiguities or pseudogenes. As anticipated, the sesiid sequences aligned without the need of gaps. Subsequent phylogenetic analyses recovered mostly congruent trees. Four most parsimonious trees were reconstructed which were most resolved in a strict consensus (Fig. 7). The one most likely tree shared the topology of one of the four most parsimonious trees. All genera, *Albuna* species, and individuals of the beutenmulleri color morph were monophyletic. The likelihood tree suggests a sister relationship between the clades of the beutenmulleri color morphs and the typical color morphs however this relationship was unresolved among the four most parsimonious trees.

On average the intraspecific distance was 0.0097 for the beutenmulleri color morphs, which falls in the range (0–0.02446) of other *Albuna* species. The average interspecific distance among the beutenmulleri color morphs and the other *Albuna* species was 0.0868, which was greater than the interspecific distance observed between *A. pyramidalis* and *A. fraxini* (0.06575). The distance among species of the outgroup genera ranged between 0.07–0.17. Taken together, these values suggest that the amount of genetic divergence of the beutenmulleri color morphs is similar to the divergences observed for other *Albuna* species and sesiid genera.

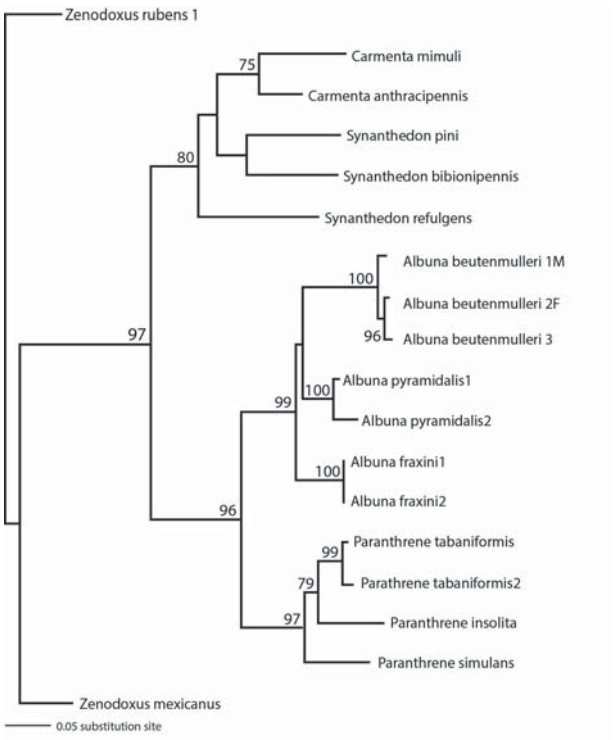

FIG. 7. Likelihood tree (-Ln likelihood = 3395.865) of *Albuna* species and outgroup genera which represents 1 of 4 parsimonious trees. Numbers are bootstrap values determined by parsimony analysis. Clades without bootstraps were unresolved in the strict consensus of the four most parsimonious trees.

FIG. 8. Black form male of *A. beutenmulleri* from Carbon Co. UT.

FIG. 9. Yellow form male of *A. beutenmulleri* from Kane Co., UT.

FIG. 10. Yellow form female of *A. beutenmulleri* from Carbon Co., UT.

The correspondence of monophyly, genetic divergence, and distinct biological characteristics strongly suggests that the beutenmulleri color morphs represent a lineage with a unique evolutionary trajectory. Given the phylogenetic species concept (Wheeler & Platnick 2000), there is much evidence to support the resurrection of *A. beutenmulleri*. For completion, we reprint the original description of *A. beutenmulleri* and describe a yellow morph of this species discovered during this investigation.

Albuna beutenmulleri Skinner, species resurrected

Black form female (Original type description, Skinner 1903) (Fig. 2): "Expanse 20 mm. Antennae, palpi, head, thorax, abdomen and legs, black. Segments of abdomen slightly differentiated by being somewhat bluish and shining. Under side of abdomen and thorax with blue-black shining metallic scales. Hind legs clothed with long black hairs. Wings bright red, edged narrowly with black. Fringes black. Fore wings with a translucent spot at outer third, divided by the red veins into four parts. There is also a similar spot at inner third in the centre of the wing, which is linear. These spots are covered with beautiful, very light greenish or bluish opalescent scales. The base of the wing is black and the black margin breaks and runs slightly into the wing from the inner margin. The hind wing has two spots of the same character " one beyond the middle resting on the costa and divided into two parts, and a larger one near the base, divided into three parts by the veins. This spot extends the width of the wing. Base of wing black.
This species somewhat resembles *Euhagena nebraskae* H. Edw. but the red color is brighter and shining. *E. nebraskae* lacks the beautiful opalescent spots."
Black Form Male (Fig. 8) Similar to the female description above except unlike the female the black form male has a black hind wing instead of red.
Specimens: Utah, Carbon Co. 5/12/2013. P.A. Opler. (CSUC-1 specimen)
Yellow Form, Male (Fig. 9): Head with vertex yellow; front pale yellow with white scales dorsally; occipital fringe pale yellow; labial palpus roughened, pale yellow with brown/black laterally; antennae bipectinate, dark orange dorsally and ventrally with upper half black; thorax black with yellow laterally near anterior margin and beneath wings, yellow band on collar, yellow scales on tegula; abdomen blue black dorsally with wide yellow banding on segments 4–7; anal tuft light orange, legs with coxa of fore leg mostly yellow mixed with a few black scales anteriorly; femora and upper third of tibia black; remainder of tibia covered with long yellow hair-

like scales, tarsal spurs and tarsi yellow; Wings bright red with narrow black margins and black fringes. Forewing with a narrow elongated, translucent area before the distal mark, divided by red veins into 4 parts; behind the distal mark another translucent area, narrowly triangular, terminating before the wing base. Hindwing bright red on the outer two-thirds with two irregular translucent patches. Specimens: Utah, Kane Co. 5/19/2014. D. Wikle. (CSUC-1 specimen)

Female (Fig. 10): Similar to male except, Head with vertex yellow; front and occipital fringe pale yellow; labial palpus roughened, pale yellow; antennae orange; abdomen blue black dorsally with wide yellow banding on segments 2,4,6, and 7; anal tuft yellow.
Specimens: Utah, Carbon Co. 5/12/2013. P.A. Opler. (CSUC-1 specimen)

Discussion

Intraspecific color variability is a common phenomenon among sesiid species (e.g. *Paranthrene fenestrata, Euhagena emphytiformis, Vitacea polistiformis, Synanthedon polygoni, Carmenta giliae*) (Beutenmüller 1901, Engelhardt 1946, Eichlin & Duckworth 1988). These color variants often occur at the same location and respond to specific pheromones suggesting their unity as a species (Eichlin & Duckworth 1988). In the case of *A. beutenmulleri*, the apparent isolation by geography, flight period, and/or host plant has allowed for genetic divergence equivalent to divergence observed for other species. Although the red and black scaling and translucent areas of the fore and hind wing based diagnose *A. beutenmulleri*, color variation (black versus yellow) exists among individuals. The extent of this variation within the species is unknown because *A. beutenmulleri* is only known from a limited number of individuals from five locations in Utah. Further investigation, utilizing DNA data and detailed biological observations may yield additional cryptic sesiid species as observed with other Lepidoptera (e.g., Wilson et al. 2010).

Acknowledgments

The authors would like to thank J. D. Weintraub, Collection Manager, Department of Entomology Academy of Natural Sciences (ANSP), Philadelphia, PA. for providing the type photograph. For additional digital images and/or specimens, we thank David Wikle, Gary Anweiler, Todd Losee and the Chicago Botanic Garden/CLM internship program. For DNA COI data, we thank Franz Puhringer, project manager for Global Sesiidae initiative on the BOLD system.

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Submitted for publication 10 January 2016; revised and accepted 17 March 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 218–228

DEMOGRAPHIC PARAMETERS OF *AUTOGRAPHA GAMMA* (NOCTUIDAE) AFFECTED BY SUGAR BEET CULTIVARS

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ABSTRACT. *Autographa gamma* (Linnaeus) (Lepidoptera: Noctuidae) is one of the important polyphagous pests of various crops in Iran and many countries of the world. The effect of eight commercial sugar beet cultivars (Peritra, Karolina, Paolita, Lenzier, Tiller, Ardabili, Persia and Rozier) on demographic parameters of *A. gamma* was determined under laboratory conditions (25 ± 1 ºC, 65 ± 5% RH, a 16:8 h light-dark photoperiod) using age-stage, two-sex life table method. A delay in the developmental time of pre-adult was observed when larvae were fed on cultivar Rozier. The lowest net reproductive rate (R_0) was observed on cultivars Paolita and Persia. Also, the intrinsic rate of increase (r_m) was the highest when larvae were reared on cultivar Karolina and the lowest when they were fed on cultivars Peritra, Paolita and Persia. The results of this study revealed that the Peritra, Paolita and Persia were the most unsuitable cultivars for population growth of *A. gamma*. The information obtained from this study could be used, along with other management tactics, to control of this pest by sowing resistant sugar beet cultivars.

Additional key words: *Autographa gamma*, sugar beet, intrinsic rate of increase, population growth

Sugar beet (*Beta vulgaris* L.) and sugar cane (*Saccharum officinarum* L.) are the two sources of sucrose derived exclusively from cultivated crops (Draycott 2006). Owing to the importance of sugar beet as a rotational crop, and the absence of reliable and safe chemical control measures against insect pests of this crop, alternative control options are required to prevent the economic damages. It seems that an integrated pest management (IPM) program (i.e. application of selective pesticides and sowing of resistant sugar beet cultivars) could supply the best management option for control of *A. gamma* in Iran.

Sugar beet can be attacked by a large number of pests from different orders and families (Hein et al. 2009). The silver Y moth, *Autographa gamma* (Linnaeus) (Lepidoptera: Noctuidae), is a polyphagous pest, with more than 200 host plant species (Nash and Hill 2003), distributed in Europe, Asia, and Northern Africa (CABI 2007). Sugar beet has been reported as one of the main host plants of *A. gamma* in Africa, the former Soviet Union (Dochkova 1972, CAB 2003), and Iran (Kheyri 1989). The larvae of *A. gamma* cause economic losses to sugar beet crops by feeding on leaves, and subsequently reducing yields (CABI 2007).

Climatic conditions, host plant quality, migration, and natural enemies can influence population dynamics of *A. gamma* (Maceljski & Balarin 1974, Honek et al. 2002). *A. gamma* is capable of long distance migration, which allows it to remain an important pest in regions where populations can not survive throughout the year (Palmqvist 2001, Chapman et al. 2012, 2015). For the most herbivorous insects that have non-feeding adults, host plant quality is an important factor for determinant of their fitness, because reproductive potential of adults is limited by food accumulated during their larval stage (Slansky & Rodriguez 1987, Awmack & Leather 2002).

Life table studies are useful tools to understand the most detailed and correct scheme of an insect population dynamics (Wittmeyer & Coudron 2001). Studies related to the traditional life tables, however, are only based on the survival and fecundity of female populations. Moreover, male individuals, the stage differentiation and variable developmental time among individuals have been neglected (Chi & Su 2006). Therefore, to incorporate variable developmental rates for both sexes (male and female), Chi & Liu (1985) and Chi (1988) developed an age-stage, two-sex life table model.

Among the life table parameters, the intrinsic rate of increase (r_m), as an index of herbivores performance (Buitenhuis et al. 2004), is the most important parameter that can be used to estimate the population growth of insect species under specified conditions (Andrewartha & Birch 1954). Previously, several authors evaluated the effect of different host plants on the life table parameters of lepidopteran insects (Jha et al. 2012, Karimi-Malati et al. 2012, Naseri et al. 2014, Alami et al. 2014). However, despite *A. gamma* causes large economic damages on different host plants, there is no published papers about the life table parameters of this pest on different sugar beet or other host plant cultivars. The aim of this study was to compare the demographic parameters of *A. gamma* on eight commercial sugar beet cultivars and to quantify

resistance or susceptibility of these cultivars to the pest. Therefore, determining the effect of different sugar beet cultivars on the demography and other biological parameters of *A. gamma* is an initial step toward designing comprehensive strategies in an IPM program of this pest.

MATERIALS AND METHODS

Sugar beet cultivars. The untreated seeds of eight sugar beet cultivars including Peritra, Karolina, Paolita, Lenzier, Tiller, Ardabili, Persia and Rozier were obtained from the Plant and Seed Modification Research Institute of Sugar Beet (Ardabil, Iran). They were cultivated in the research farm of the University of Mohaghegh Ardabili (Ardabil, Iran) in May 2014. Selection of these cultivars was based on their importance as the most cultivated sugar beets in different regions of Iran and some other countries of the world. The experiments were started when sugar beet cultivars reached the eight-leaf stage. The young leaves, with equal size, of each sugar beet cultivar were transferred to a growth chamber (25 ± 1 ºC, 65 ± 5% RH, and a 16:8 h light-dark photoperiod) and were used in the experiments.

Rearing of *A. gamma*. The eggs of *A. gamma* were collected from sugar beet fields from Northern Khorasan, Iran. The insect populations were reared for two generations on the leaves of each sugar beet cultivar before performing the experiments. All experimental insects were kept inside a growth chamber at above-mentioned conditions.

After emergence of adult moths and to obtain the same aged eggs of *A. gamma*, 10–15 pairs of both sexes (male and female) were kept inside transparent egg-laying containers (11.5 cm in diameter, 9.5 cm in height), which were closed at the top with a fine mesh net for ventilation. The internal walls of each container were covered with the same mesh net as an egg-laying substrate. Fifty eggs laid within 12 hours were collected from the egg-laying container, and were used for the experiment. Neonate larvae were individually transferred into plastic Petri dishes (8 cm in diameter, 2 cm in height) with a hole (1 cm in diameter) covered with a fine mesh net for aeration. These Petri dishes were contained fresh leaves of each tested sugar beet cultivar. To keep freshness, the petioles of detached leaves were inserted in cotton soaked in water. The leaves of each cultivar were replaced daily. Mortality or molting were recorded daily up to pupation and emergence of adults. For pre-pupation and pupation, fifth instar larvae were kept in cylindrical plastic containers (3 cm in diameter, 5 cm in height). Duration of larval, pre-pupal and pupal stages and developmental time of total pre-adult were recorded on different sugar beet cultivars.

After emergence of adult moths, a pair of female and male moths was transferred to the same egg-laying containers as described above. Number of eggs laid was counted and the experiments were continued up to the death of the last adult moth. To supply a source of carbohydrate for adult feeding, a small cotton wick soaked in 10% honey solution was inserted into the egg-laying containers. In this study, adult pre-oviposition period (APOP: the time between the emergence of an adult female and the initiation of its oviposition), total pre-oviposition period (TPOP: the duration from egg to first oviposition), oviposition period, fecundity (eggs laid during the reproductive period) and adult longevity were recorded.

Life table analysis. All individuals' data were analyzed according to the age-stage, two-sex life table model (Chi & Liu 1985; Chi 1988). The survival rate (s_{xj}) (i.e. x is the age and j is the stage) was calculated; the first stage is the egg, the second stage is the larva–prepupa, the third stage is the pupa, the fourth and fifth stages are female and male, respectively. The fecundity (f_{xj}), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters were measured accordingly (Huang & Chi 2012). The intrinsic rate of increase (r_m) was calculated using bisection method from the Euler-Lotka formula:

$$\sum_{x=0}^{\infty} e^{-r_m(x+1)} l_x m_x = 1$$

with the age indexed from 0 (Goodman 1982). The mean generation time (T) is defined as the length of time that a population can increase to R_0-fold of its population size at the stable stage distribution ($\lambda^T = R_0$), which it was then calculated as $T = (\ln R_0)/r_m$. The gross reproductive rate (GRR) was calculated as $GRR = \sum m_x$. To estimate the means, variances, and standard errors of the population parameters, the bootstrap technique (Efron & Tibshirani 1993), which included TWOSEX-MSChart (Chi 2013), was used. The obtained data were then analyzed by one-way ANOVA followed by comparison of the means with LSD test at $\alpha = 0.05$ using statistical software Minitab 16.0.

RESULTS

Statistics of analysis of variance for the effect of different sugar beet cultivars on the demographic parameters of *A. gamma* are listed in Table 1.

Developmental time. The results of the effect of different sugar beet cultivars on development time of total pre-adult of *A. gamma* are given in Table 2. Larval period, pupal period and developmental time of pre-

TABLE 1. Statistics of analysis of variance for the effect of different sugar beet cultivars on demographic parameters of *Autographa gamma*

Parameters	*df*	*F*	*P-value*
Incubation period (day)	-	-	-
Larval period (day)	7, 171	3.77	<0.05
Pupal period (day)	7, 173	16.04	<0.05
Pre-adult (day)	7, 171	16.29	<0.05
Male longevity (day)	7, 53	5.62	<0.05
Female longevity (day)	7, 67	1.43	0.21
Male life span (day)	7, 53	8.77	<0.05
Femle life span (day)	7, 67	0.62	0.73
APOP [a] (day)	7, 31	3.34	<0.05
TPOP [b] (day)	7, 31	2.32	<0.05
Oviposition period (day)	7, 83	1.64	0.13
Fecundity (eggs per female)	7, 52	2.46	<0.05
R_0 [c] (offspring)	7, 625	91.20	<0.05
GRR [d] (offspring)	7, 674	165.22	<0.05
r_m [e] (day^{-1})	7, 488	106.11	<0.05
λ [f] (day^{-1})	7, 706	59.42	<0.05
T [g] (day)	7, 638	68.62	<0.05

[a] Adult pre-ovipostion period, [b] Total pre-oviposition period, [c] Net reproductive rate, [d] Gross reproductive rate, [e] Intrinsic rate of increase, [f] Finite rate of increase, [g] Mean generation time

TABLE 2. Mean (±SE) pre-adult duration (days) of *Autographa gamma* fed on different sugar beet cultivars under laboratory conditions.

Cultivar	Incubation	*n*	Larval period	*n*	Pre-pupal period	*n*	Pupal period	*n*	Total pre-adult	*n*
Peritra	3.00	30	14.43 ± 0.28 abc	28	2.00	23	9.39 ± 0.24 b	23	26.78 ± 0.35 b	23
Karolina	3.00	30	14.34 ± 0.29 abcd	25	2.00	23	9.04 ± 0.13b c	23	26.39 ± 0.31 b	23
Paolita	3.00	30	13.65 ± 0.18 d	29	2.00	29	7.65 ± 0.21 e	29	24.41 ± 0.21 cd	29
Lenzier	3.00	30	15.00 ± 0.35 a	15	2.00	15	8.53 ± 0.37 cd	15	26.53 ± 0.50 b	15
Tiller	3.00	30	14.78 ± 0.39 a	19	2.00	19	9.00 ± 0.30 bc	19	26.78 ± 0.41 b	19
Ardabili	3.00	30	13.76 ± 0.21 cd	27	2.00	25	6.85 ± 0.21 f	25	23.68 ± 0.33 d	25
Persia	3.00	30	13.92 ± 0.09 bcd	30	2.00	25	8.00 ± 0.20 de	25	24.92 ± 0.16 c	25
Rozier	3.00	30	14.60 ± 0.18 ab	20	2.00	20	10.30 ± 0.52 a	20	27.80 ± 0.59 a	20

The means followed by different letters in the same column are significantly different (LSD, P<0.05).

The *n* value shows the number of insect tested on each cultivar.

TABLE 3. Mean (±SE) adult longevity (days) and life span (days) of *Autographa gamma* fed on different sugar beet cultivars under laboratory conditions.

Cultivar	Male longevity	*n*	Female longevity	*n*	Male life span	*n*	Female life span	*n*
Peritra	7.33 ± 1.56 c	14	13.88 ± 1.96 a	9	34.00 ± 1.57 c	14	40.88 ± 1.56 a	9
Karolina	8.67 ± 2.28 c	12	14.71 ± 2.20 a	11	34.67 ± 2.26 c	12	41.00 ± 1.99 a	11
Paolita	10.22 ± 1.05 bc	15	14.09 ± 1.95 a	14	34.44 ± 1.13 c	15	40.00 ± 1.56 a	14
Lenzier	18.57 ± 1.51 a	8	15.57 ± 1.89 a	7	45.43 ± 1.51 ab	8	41.57 ± 2.30 a	7
Tiller	19.00 ± 1.98 a	10	18.22 ± 2.30 a	9	45.50 ± 1.96 ab	10	41.10 ± 1.84 a	9
Ardabili	11.75 ± 1.58 bc	14	16.89 ± 1.98 a	11	34.75 ± 1.54 c	14	41.22 ± 2.17 a	11
Persia	15.42 ± 2.20 ab	12	19.46 ± 1.50 a	13	40.50 ± 2.14 b	12	44.23 ± 1.44 a	13
Rozier	18.20 ± 1.16 a	8	14.00 ± 1.61 a	12	48.00 ± 1.55 a	8	41.00 ± 1.58 a	12

The means followed by different letters in the same column are significantly different (LSD, $P<0.05$).

The *n* value shows the number of insect tested on each cultivar.

TABLE 4. Mean (±SE) oviposition period (days), and fecundity (eggs laid during reproductive period) of *Autographa gamma* fed on different sugar beet cultivars under laboratory conditions.

Cultivar	APOP[a]	TPOP[b]	Oviposition period	Fecundity	*n*
Peritra	10.33 ± 3.18 a	36.00 ± 3.00 ab	2.67 ± 0.33 a	38.70 ± 14.30 b	9
Karolina	3.60 ± 1.12 b	29.40 ± 1.17 c	4.40 ± 0.67 a	212.90 ± 80.60 a	11
Paolita	12.33 ± 0.66 a	36.33 ± 0.66 a	2.30 ± 0.57 a	26.30 ± 12.70 b	14
Lenzier	6.00 ± 1.15 b	32.33 ± 2.03 abc	3.33 ± 1.20 a	79.80 ± 35.70 b	7
Tiller	5.40 ± 1.12 b	32.00 ± 1.45 abc	2.40 ± 0.74 a	91.20 ± 41.00 b	9
Ardabili	5.38 ± 1.03 b	29.75 ± 1.30 c	2.88 ± 0.58 a	72.40 ± 29.70 b	11
Persia	5.75 ± 0.91 b	30.62 ± 0.88 c	2.50 ± 0.46 a	31.38 ± 8.46 b	13
Rozier	6.25 ± 1.97 b	31.75 ± 2.14b c	2.25 ± 0.62 a	58.40 ± 21.70 b	12

The means followed by different letters in the same column are significantly different ($P<0.05$, LSD).

[a] Adult pre-oviposition period [b] Total pre-oviposition period

The *n* value shows the number of female moths tested on each cultivar.

TABLE 5. Mean (± SE) two-sex life table parameters of *Autographa gamma* fed on different sugar beet cultivars under laboratory conditions.

Cultivar	R_0[a] (offspring)	GRR[b] (offspring)	r_m[c] (day^{-1})	λ[d] (day^{-1})	T[e] (days)
Peritra	11.74±0.72 d	43.25±2.73 c	0.059±0.001 f	1.05±0.00 d	38.23±0.20 a
Karolina	36.05±1.62 a	138.12±5.59 a	0.104±0.001 a	1.10±0.00 a	34.89±0.08 c
Paolita	6.54±0.24 e	22.97±0.89 d	0.050±0.000 f	1.05±0.00 d	37.91±0.11 a
Lenzier	24.57±1.28 b	59.31±3.52 b	0.094±0.003 b	1.08±0.00 b	34.35±0.26 cd
Tiller	19.25±1.35 c	45.91±2.80 c	0.063±0.005 e	1.06±0.00 c	34.04±0.21 de
Ardabili	19.55±0.93 c	59.01±3.32 b	0.083±0.001 c	1.08±0.00 b	33.66±0.39 ef
Persia	8.48±0.36 e	13.18±0.57 e	0.059±0.000 f	1.05±0.00 d	35.82±0.14 b
Rozier	19.07±1.35 c	45.07±2.54 c	0.079±0.002 d	1.08±0.00 b	33.10±0.24 f

The means followed by different letters in the same column are significantly different (P<0.05, LSD).

[a] Net reproductive rate, [b] Gross reproductive rate, [c] Intrinsic rate of increase, [d] Finite rate of increase,

[e] Mean generation time

adult were significantly different on sugar beet cultivars. The longest larval period was observed on cultivars Lenzier (15.00 ± 0.35 days) and Tiller (14.78 ± 0.39 days), and the shortest larval period was seen on cultivar Paolita (13.65 ± 0.18 days). The pupal period and developmental time of total pre-adult were the longest on cultivar Rozier (10.30 ± 0.52 and 27.80 ± 0.59 days, respectively), and the shortest on cultivar Ardabili (6.85 ± 0.21 and 23.68 ± 0.33 days, respectively).

Adult longevity and life span. Table 3 shows the adult longevity and life span (from egg stage to adults' death) of *A. gamma* fed on eight sugar beet cultivars. Different sugar beet cultivars as larval food had no significant effects on the longevity and life span of female *A. gamma*. However, significant differences were observed for the longevity and life span of male moths fed on eight sugar beet cultivars. The shortest male longevity was observed on cultivars Karolina (8.67 ± 2.28 days) and Peritra (7.33 ± 1.56 days). Moreover, the longest male life span was recorded on cultivar Rozier (48.00 ± 1.55 days) compared with the other cultivars.

Oviposition period and fecundity. The APOP, TPOP, oviposition period and fecundity of adults of *A. gamma* which came from larvae reared on different sugar beet cultivars are given in Table 4. Oviposition period of *A. gamma* was not significantly different on different sugar beet cultivars. However, tested sugar beet cultivars showed significant effects on the APOP, TPOP and fecundity of this pest. The APOP was the longest when larvae were fed on cultivars Paolita (12.33 ± 0.66 days) and Peritra (10.33 ± 3.18 days), and TPOP was the longest when they were fed on cultivar Paolita (36.33 ± 0.66 days). Furthermore, fecundity of *A. gamma* on cultivar Karolina (212.90 ± 80.60 eggs) was higher than the other tested cultivars.

Life table analysis. The age-stage specific survival rates (s_{xj}) of *A. gamma* on different sugar beet cultivars are shown in Figure 1. Noticeable stage overlapping was observed because of variation in the development rate among individuals on sugar beet cultivars. The highest age-stage specific survival rate of larva-prepupa, pupa and adult male of *A. gamma* was on cultivar Persia. Also, the highest age-stage specific survival rate of adult female was on cultivars Persia and Rozier, and the lowest was on cultivar Peritra (Fig. 1).

The age-stage life expectancy (e_{xj}) gives the expected life span that an individual of age x and stage j can live after age x. The e_{xj} of *A. gamma* on different sugar beet cultivars are presented in Figure 2. The highest value of the life expectancy for egg stage was 39.00 days on

cultivar Persia. Life expectancy of larval and pupal stages was the highest on cultivars Persia (36 days) and Tiller (30.52 days), respectively. Moreover, the life expectancy of female moths was the highest (21.00 days) on cultivars Persia and Tiller. The life expectancy of the male moth of *A. gamma* was the highest (21.33 days) on cultivar Rozier.

Age-specific survival rate (l_x) is the probability that an egg will survive to age x; therefore, the curve l_x (Fig. 3) is the simplified version of s_{xj}. Our results showed that the death of the last female occurred at the ages of 47, 51, 44, 52, 52, 50, 54 and 52 days on cultivars Peritra, Karolina, Paolita, Lenzier, Tiller, Ardabili, Persia and Rozier, respectively (Fig. 3). The age-stage specific fecundity (f_{x4}) gives daily number of offspring produced by *A. gamma* individual at age x and stage j. Since only females produce offspring, there is only a single curve f_{x4} (i.e. the adult female is the fourth life stage). The maximum age-stage specific fecundity on these cultivars (the same order mentioned above) was 32, 48, 6, 24, 28, 30, 5 and 23 eggs female^{-1} day^{-1}, respectively that occurred at the ages of 39, 36, 41, 30, 31, 30, 32 and 32 days, respectively. The oviposition of the first female on the tested sugar beet cultivars (the same order mentioned above) started at the ages of 28, 25, 30, 29, 28, 25, 26 and 28 days, respectively. Also, the highest age-specific fecundity (m_x) of *A. gamma* adult emerging from the larvae reared on above-mentioned cultivars was 32, 31, 5, 12, 14, 25, 2 and 18 females female^{-1} day^{-1}, respectively that occurred at the ages of 39, 33, 41, 30, 31, 40, 32 and 32 days, respectively (Fig. 3).

The demographic parameters calculated by using the age-stage, two-sex life table for *A. gamma* on different sugar beet cultivars are listed in Table 5. The net reproductive rate (R_0) was found to be significantly different depending on the sugar beet cultivars on which individual insects were reared. The R_0 value of *A. gamma* was the lowest on cultivars Paolita (6.54 ± 0.24 offspring) and Persia (8.48 ± 0.36 offspring), and the highest on cultivar Karolina (36.05 ± 1.62 offspring). The gross reproductive rate (GRR) of this pest was the highest on cultivar Karolina (138.12 ± 5.59 offspring) and the lowest on cultivar Persia (13.18 ± 0.57 offspring). The highest intrinsic rate of increase (r_m) was on cultivar Karolina (0.104 ± 0.001 day^{-1}) and the lowest values were on cultivars Peritra (0.059± 0.001 day^{-1}), Persia (0.059± 0.000 day^{-1}) and Paolita (0.050 ± 0.000 day^{-1}). Moreover, the finite rate of increase (λ) showed significant differences, which was the highest on cultivar Karolina (1.10 ± 0.00 day^{-1}). The longest mean generation time (T) of *A. gamma* was observed on cultivars Peritra (38.23 ± 0.20 days) and Paolita (37.91 ± 0.11 days), and the shortest value was seen on cultivar Rozier (33.10 ± 0.24 days).

Discussion

The results of this study demonstrated that the life table parameters of *A. gamma* were significantly differed on eight sugar beet cultivars. Variation in host plant quality can affect the life cycle characteristics of herbivores and play an important role in regulating insect populations (van Lenteren & Noldus 1990, Awmack & Leather 2002, Umbanhowar & Hastings 2002).

A delay in the development of *A. gamma* larvae on cultivars Lenzier and Tiller could be attributed to unsuitablity of these cultivars for larval feeding and development. The mean larval period of *A. gamma* on eight sugar beet cultivars, in this study, is shorter than that reported by Taha et al. (2012) for *A. gamma* fed on artichoke (19.14 days). Differences in the host plant species could explain such discrepancy. *A. gamma* required a longer time to complete its immature stages when reared on cultivar Rozier than when reared on the other tested cultivars. The extension of the developmental time of pre-adult of *A. gamma* on cultivar Rozier could increase the risk of encountering immatures by their natural enemies or being exposed to unfavorable environmental conditions (Al- Zubaidi & Capinera 1984).

The results of this study showed that the longevity and life span of female moths of *A. gamma* were constant on different sugar beet cultivars; however, the longevity and life span of male moths were affected by tested sugar beet cultivars. Since nutrient regulation in lepidopteran larvae is sex-specific (Lee 2010), nutritional requirements of male and female *A. gamma* larvae are likely different with each other. Such difference could affect the life history traits of female and male moths of *A. gamma* such as the adult longevity.

It is noticeable that, in phytophagous insects, larval feeding on nutritionally-rich plants can increase fecundity of adults (Verkerk & Wright 1996). Therefore, high fecundity of *A. gamma* on cultivar Karolina suggests that this cultivar is more suitable than the others for feeding of this pest. In our study, the range of fecundity of *A. gamma* (Table 4) on different sugar beet cultivars is lower than that reported by other authors (Dochkova 1972, Harakly 1975, Spitzer et al. 1984), suggesting that these cultivars are more unsuitable than other host cultivars for oviposition of the pest . However, Taha et al. (2012) reported that the female moths of *A. gamma* which came from larvae reared on artichoke could lay 57.67 eggs at 25 ºC. Some

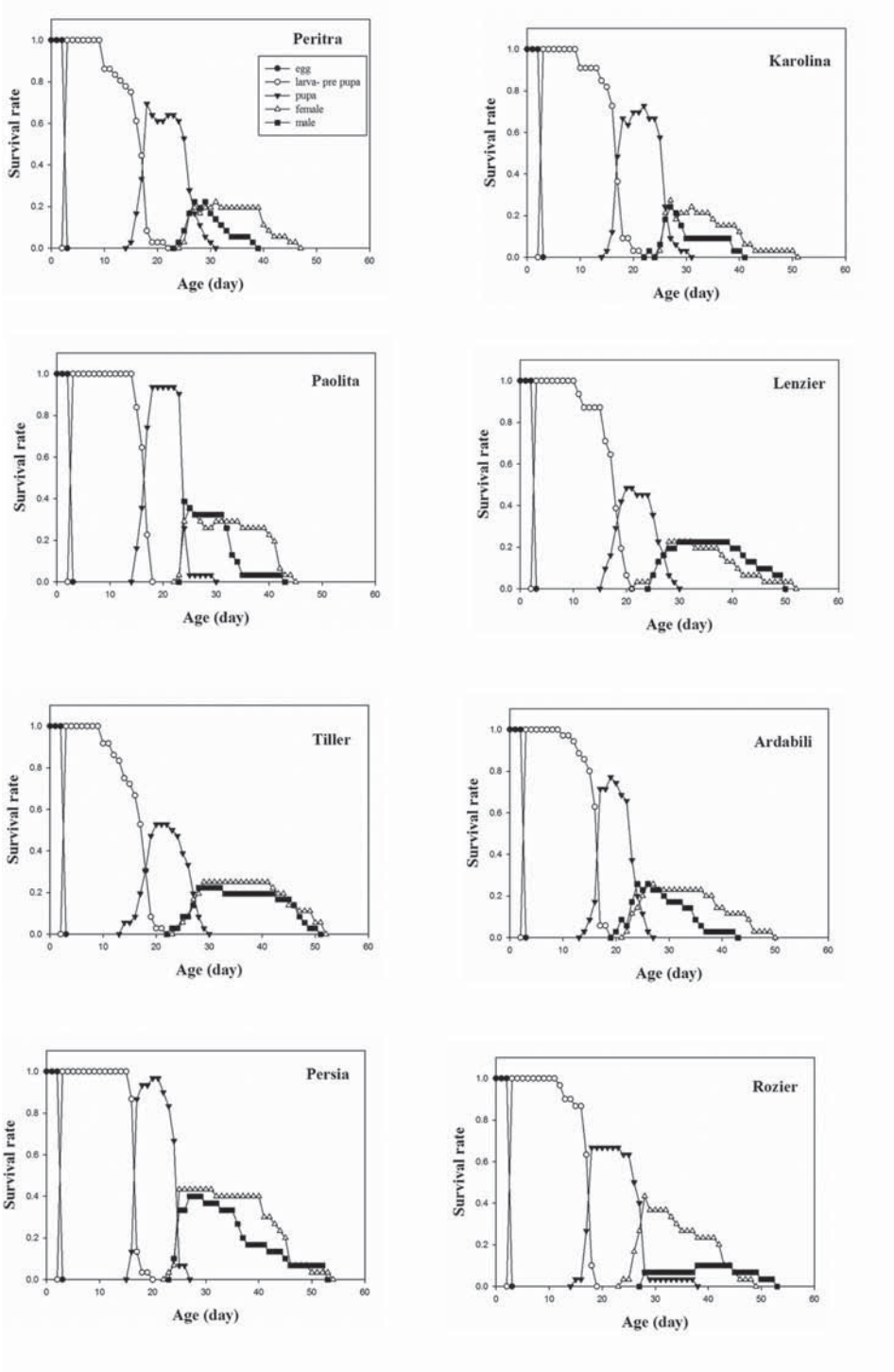

FIG. 1. Age-stage specific survival rate (s_{xj}) of *Autographa gamma* fed on different sugar beet cultivars under laboratory conditions.

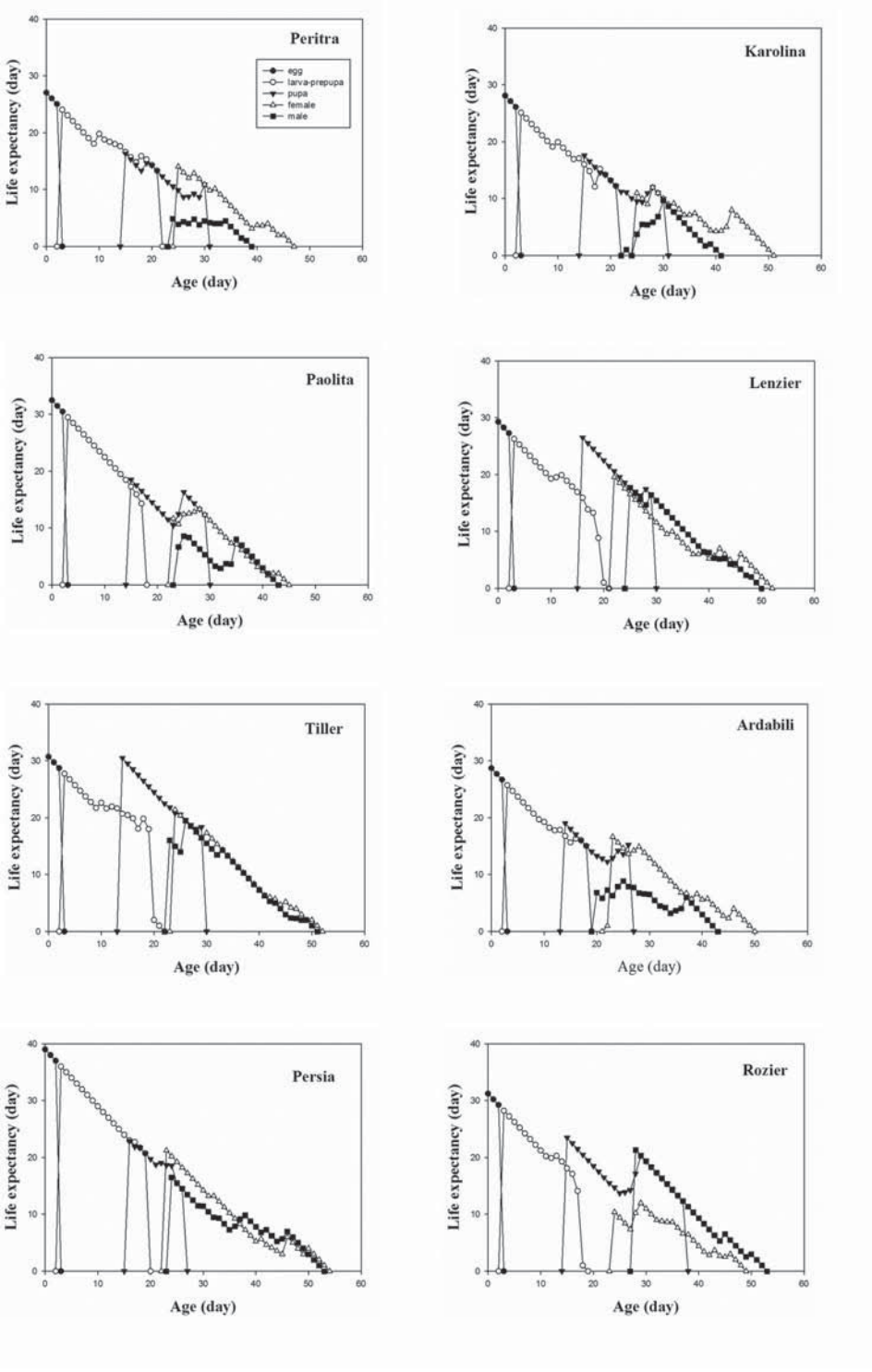

FIG. 2. Age-stage specific life expectancy (e_{xj}) of *Autographa gamma* fed on different sugar beet cultivars under laboratory conditions.

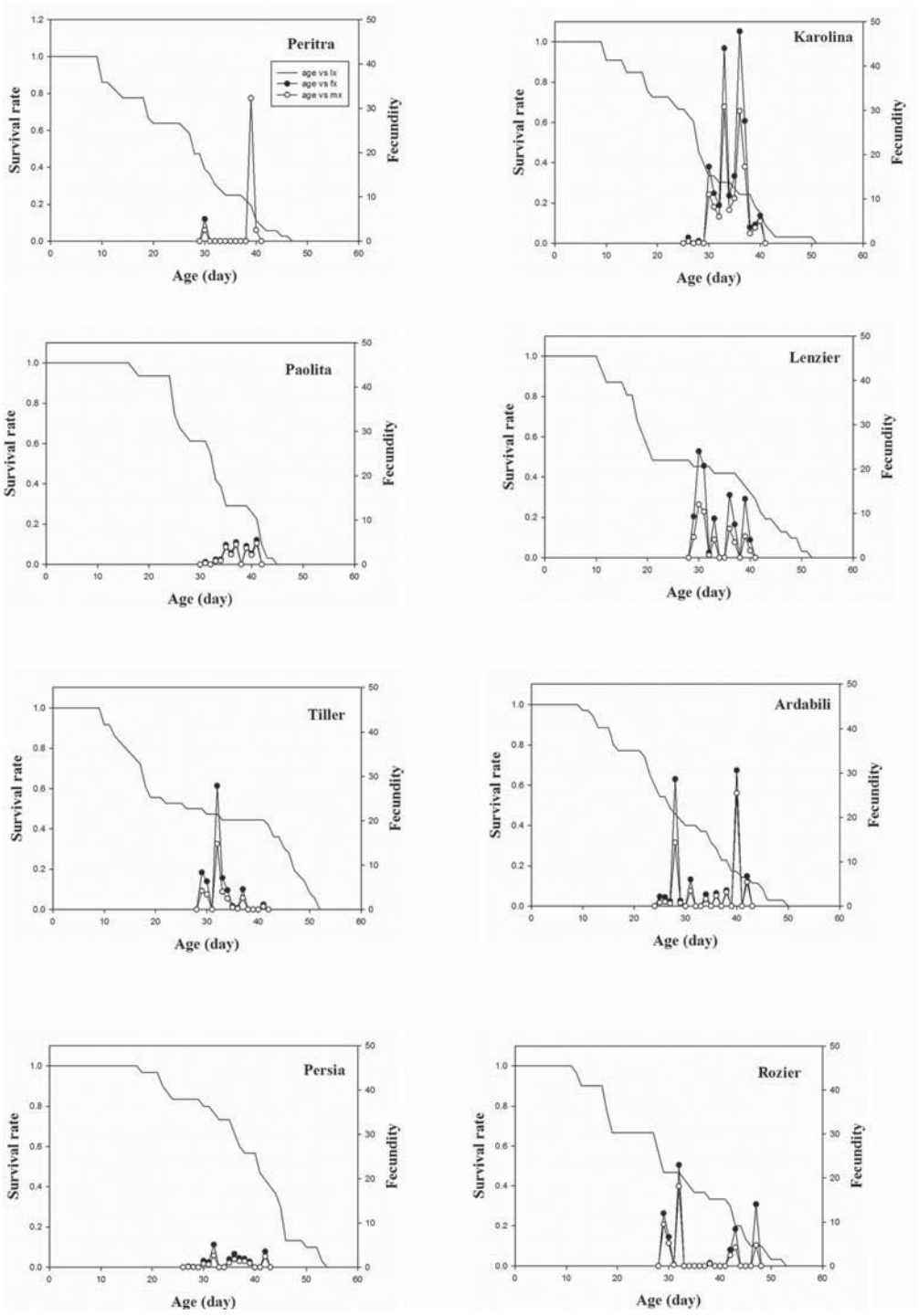

FIG. 3. Age-specific survival rate (l_x), age-stage specific fecundity (f_{x4}), and age-specific fecundity (m_x) of *Autographa gamma* fed on different sugar beet cultivars under laboratory conditions.

probable reasons for this inconsistency could be attributed to the variations in geographic populations of *A. gamma* or differences in the tested host plants.

The bootstrapping was used to estimate the population parameters in this study. It is known that this technique is more valid in estimating means and variances of population parameters than jackknife procedure (Huang & Chi 2012). It was accepted that the age of the first reproduction of insects can affect the intrinsic rate of increase (r_m). Moreover, when fecundity not change, the shorter pre-oviposition period will result in a higher intrinsic rate of increase (Lewontin 1965, Huang & Chi 2012, Jha et al. 2012). The higher r_m value of *A. gamma* on cultivar Karolina was mainly owing to the greater fecundity and the shorter APOP and TPOP of the pest reared on this cultivar. Thus, lower r_m value on cultivars Peritra, Paolita and Persia was mainly owing to the lower fecundity and longer APOP and TPOP of *A. gamma* on these cultivars. A high value of r_m shows that the host plant species/cultivars are relatively suitable to insect feeding and vice versa. Thus, cultivar Karolina, among sugar beet cultivars, was the susceptible host, and population growth of *A. gamma* was the highest on this cultivar. However, the lowest value of r_m was on cultivars Peritra, Paolita and Persia, suggesting that they are partially resistant to *A. gamma* compared with the other cultivars.

According to the results obtained from this research, cultivars Peritra, Paolita and Persia are relatively unsuitable (resistant) and cultivar Karolina is relatively suitable (susceptible) hosts for population growth of *A. gamma*. Owing to the polyphagous nature of *A. gamma*, future studies should be focused on testing the wide range of host plants for development of *A. gamma*. Moreover, assessing the chemical components of host plants is required for better understanding of host plant suitability. As the selected cultivars are commercially grown sugar beets in Iran, the control programs of *A. gamma* would be successful if farmers switch to growing the most resistant cultivars. Moreover, the wide host-range of this pest and the long-range migration capacity of adults (Chapman et al. 2015), contribute to its high potential for establishment in different countries of the world. Therefore, identification of cultivars resistant to *A. gamma* by studying demographic analysis of the pest is an important step for area-wide pest management.

Knowledge of how the quality of tested sugar beet cultivars affects the demographic parameters of *A. gamma* will be helpful to understand the population dynamics of this pest. Furthermore, we emphasize the significant effects of the tested sugar beet cultivars (as representatives of the gene pool of sugar beet) on the demographic parameters of *A. gamma* and discover the crucial importance of the cultivar selection and breeding in management programs of the pest.

ACKNOWLEDGEMENTS

We gratefully appreciate M. Ghollarata and S. Alami for their valuable assistance in this research. This work was financially supported by the University of Mohaghegh Ardabili (Ardabil, Iran), which is appreciated.

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Submitted for publication 9 January 2016; revised and accepted 14 March 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 229–237

THE OZARK BALTIMORE CHECKERSPOT, *EUPHYDRYAS PHAETON OZARKAE* (NYMPHALIDAE): LIFE HISTORY IN NORTHERN ARKANSAS

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ABSTRACT. Ecological and geographical differences between eastern and western populations of the Baltimore Checkerspot have led to a division into two subspecies, *E. p. phaeton* and *E. p. ozarkae*. Research concerning *E. p. ozarkae* is sparse, and prior to our work, many aspects of the life history of this subspecies were not known. An accurate assessment of its status has been hindered and confused through the use of data obtained from the eastern subspecies (e.g., mesic habitats and larval food plant) to characterize Ozark populations (e.g., glade habitats and a different larval food plant). From 2011 to 2014 we studied this butterfly in the Ozark Mountains of northern Arkansas. We identified the availability of the primary larval host plant (*Aureolaria flava*) as a potential limiting factor and investigated limitations to the distribution of this plant. We found many similarities concerning the timing of development between the two subspecies but ample evidence to demonstrate the uniqueness of Ozark populations. Our findings provide valuable information for future research, management, and conservation of the Ozark Baltimore Checkerspot.

Additional key words: Specialist, univoltine, metapopulation, species identity, Arkansas

Life histories provide critical insight into the biology and ecology of organisms. Detailed information concerning life stages allows investigators to focus research efforts on prominent issues. Relationships involving food resources, predators, and competitors promote greater understanding of the organism in question, which subsequently leads to better conservation and management efforts to promote sustainable populations.

The Baltimore Checkerspot, *Euphydryas phaeton*, is a nymphalid butterfly that occurs in much of the eastern United States. Ranging from Maine southward along the Appalachians to Georgia and westward to Oklahoma, Kansas, and Texas, the species is divided into two subspecies, *E. p. phaeton* Drury and *E. p. ozarkae* Masters. Both are univoltine and exist in local, isolated populations that are interconnected through adult dispersal. With the majority of their lifetime spent as aggregations of relatively immobile larvae and a flight season of only three weeks, these subpopulations are subject to conditions of the local habitat, sensitive to habitat fluctuations (such as extreme or atypical weather and acute destructive events), and prone to establishment and extirpation on a generational scale, each characteristic of metapopulations (Hanksi & Gilpin 1991, Hanski & Singer 2001).

The subspecies are most readily differentiated by their geographic range and larval host plant. Members of the genus *Euphydryas* specialize on plants containing iridoid glycosides (Bowers 1983), which larvae sequester to render them unpalatable to some vertebrates (Bowers 1980, Belofsky et al. 1989). These compounds are found in relatively large amounts in all chosen host plants (Bowers et al. 1992), but contrasting environmental conditions are present depending upon host plant selection. *E. p. phaeton* are normally associated with White Turtlehead, *Chelone glabra* L. (Plantaginaceae), a wetland species occurring in flooded meadows and stream banks. *C. glabra* has been reported to be in decline due to disappearing wetlands brought about by urbanization (Durkin 2009; wetland urbanization effects highlighted in Johnson et al. 2013). Exotic Ribwort Plantain (*Plantago lanceolata* L., Plantaginaceae) has provided a viable alternative to White Turtlehead (Stamp 1979, Bowers et al. 1992) and occurs in a broader range of habitats, such as meadows, upland grasslands, river banks, and cliffs (Preston et al. 2002), expanding the potential range for *E. p. phaeton*. In contrast, *E. p. ozarkae* uses False Foxglove, *Aureloaria flava* L. and *Aureolaria grandiflora* Bentham (Orobanchaceae) as its primary hosts (Masters 1968, Bauer 1975, Scholtens 1991). These *Aureolaria* spp. occur in dry, upland oak and pine-oak woodlands and are believed to be hemi-parasitic of oak species (Musselman 1996).

Considering different hosts (and habitats), metapopulation dynamics, short flight seasons, geographic distance between, and ecological time

separating *E. phaeton* subspecies (>85 generations; Brower 1930), speciation is a possibility. These circumstances have been the basis for subspecies separation and have driven the question of whether *E. phaeton* is a single wide-ranging species or two species. This query arises frequently in studies and references involving *E. p. ozarkae* (e.g., Bauer 1975, Vawter & Wright 1986, Opler & Malikul 1998) and is a topic of interest in our study.

Here we provide life history data for *E. p. ozarkae*, the largely under-studied subspecies found primarily in the Ozarks of Arkansas and Missouri. Research on this subspecies has been overshadowed by studies concerning *E. p. phaeton* and therefore lacks focused efforts to understand its status and behavior, which may differ from the nominate subspecies. As such, we compare and contrast the two subspecies when possible.

METHODS

Four persistent (Buckridge, Cozahome, Longbottom, and Tilly) and two ephemeral (Spring Creek and Maumee) populations of Baltimore Checkerspot were located along the Buffalo River and surrounding areas in Arkansas. Sites occupied three counties (Newton, Pope, and Searcy) and were named after prominent nearby landmarks (e.g. land features, roads, and cities). All study areas are dominated by elevated hardwood, oak-hardwood forests of the Ozark subdivisions of the Boston Mountain, Salem, and Springfield Plateaus. Sandstone and shale provide well-drained north-south facing slopes that have been incised into plateaus of elevations upwards of 790 m. Soils are predominantly alluvial, consisting of sandy and/or silt loam. Precipitation averages 48–50 inches and temperatures average 57–59°F (Gentry et al. 2013). Baltimore Checkerspots in these areas use *Aureolaria flava* as their primary host.

Surveys of browsing upon host plants were conducted at all sites in August 2011, excluding Tilly, and July 2013, including Tilly, to estimate the effect of herbivory. Browsing was identified by the removal of the growing tip of any shoot (primary or auxiliary) and exposure to the hollow center of the plants. Because young larvae feed on leaf material, we concluded browsing was due to large herbivores. As such, no corrections or groupings were made to discriminate effects of browsing by herbivores or larvae. Plants housing communal tents, aggregations of *E. p. ozarkae* larvae within structured webbing (also called nests), were categorized as having been browsed or unbrowsed. Data were used to construct a 2×2 contingency table to examine the relationship between browsing and tent presence for both years (SAS 2011; proc freq).

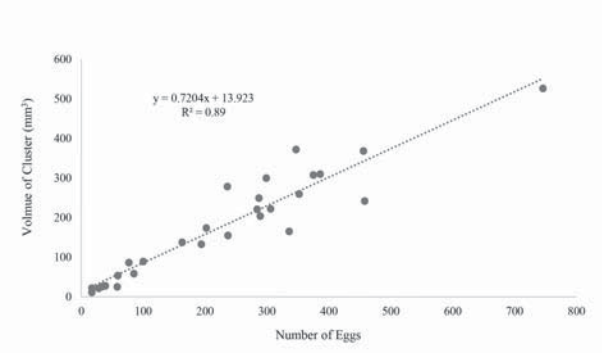

FIG. 1. Regression model generated from volume measures and absolute counts of 29 egg clusters collected in 2013 and 2014.

Observations of *Euphydryas phaeton ozarkae* in our study span the majority of three generations, beginning August 2011 and ending June 2014. Study sites were visited as frequently as every two weeks but no less than once every two months, at which times data concerning behavioral patterns, life cycle stages, morphological characteristics, and biotic and abiotic stressors were collected.

Egg clusters were located in 2012 and their coloration in relation to development recorded. Individual egg counts were obtained by dissecting egg clusters in 2013 and 2014 (n = 29). Using electronic calipers dimensional data (length, width, and height) were obtained from as many clusters as could be located in these years. Egg counts from the collection effort were correlated to

$$x = \frac{(y - b)}{a}$$

volumetric data to generate a predictive model where x is the estimated egg count using the measured volume y, in determining egg numbers (SAS 2011; proc reg; Fig. 1). The model was then used (r^2 = 0.89) to determine the approximate number of eggs in each cluster for which measures were taken. Descriptive statistics were calculated for all egg counts (SAS 2011; proc univariate).

Length measurements of larvae were made in 2012 to estimate growth of instars 2–6 at three of the four persistent locations (Buckridge, Coazhome, and Longbottom; throughout the study larval development was consistently two weeks behind at Tilly), with two measurements taken during the fourth instar (late summer and early winter). All sites were visited on three consecutive days during which larvae were assumed to be at specified instars based on coloration, size, date, and presence of recent molted exoskeletons. Measurements,

FIG. 2. Photographs from adult tagging effort in 2013: (left) tagging apparatus with male no. 1 carefully restrained and (right) male no. 2 with tag placed on the venter of the left hindwing.

made from head to anus without respect to time within instars (i.e., early, mid, or late) due to variation in development between tents and individuals, were taken using electronic calipers. Data were compared among sites using ANOVA (SAS 2011; proc GLM) before combining each site into one homogeneous data set. Each data set amassed for instars was analyzed and summarized through descriptive statistics (SAS 2011; proc univariate). Fourth instars were measured twice; during pre-hibernation (late summer) and hibernation (early winter). Fourth-instar data sets were compared by first reducing the pre-hibernation data set (n = 239) using 25 iterations of bootstrap resampling to generate a random sampling equal to the hibernation sample size (n = 32). The resampled data set was then compared to hibernating fourth instars using Student's t-test (SAS 2011; proc ttest).

Anecdotal observations of adults in 2012 were made at Buckridge, Cozahome, and Longbottom. Adults at Tilly, chosen for logistical ease, in 2013 were individually tagged and monitored for life-history characteristics and concurrently studied with ovipositing as the primary focus. Adults were captured, GPS location recorded, and activity described. Tagging was performed using a strip of paper folded into a triangle with circular windows punched out to facilitate marking (Fig. 2). Adults were placed in the paper triangle and secured with a clothespin. Scales were removed from the venter of the left hindwing at the location of the most appropriate window using tape and a gentle scraping motion. Tags were generated using individual identification codes on lightweight printer paper and were fixed to the left hindwing using super glue (Loctite®). Prior to being released unharmed at the site of capture, adults were sexed and forewing lengths measured from the thorax to the wing tip using electronic calipers. Subsequent to release, marked adults were identified through a slow approach and use of binoculars; recaptures were avoided to prevent disrupting ongoing behavior. Forewing lengths were compared between sexes using Student's t-test (SAS 2011; proc ttest).

Communal tents housing larvae were observed during the summer and fall of 2012 (June–October) and 2013 (July–October) to establish larval persistence. Larval dispersal in the spring made it difficult to quantify group size, as counts became highly variable and unreliable. In 2012, tents were tagged for identification using 5 mL Eppendorf tubes containing numbered strips. Larvae were counted during each visit and numbers estimated to categories of 10 (i.e., 1–10, 11–20, 21–30, etc.). Following data collection, categories were reduced to a single average for each group (i.e., 5.5, 15.5, 25.5, etc.). In 2013, tent surveys were made of areas outlined by GPS data. Using a sweep-survey method, all active tents within areas were counted.

During diapause (4th instar) in 2011 a small sample of tents outside the primary area of study were monitored for nest repair and post-damage survival. Nests of pre-hibernating larvae were located and the number of larvae estimated; a 3–6 cm incision was then cut into tents to expose larvae to the external environment. Ten of these incisions were subsequently covered using leaves from the area surrounding the nests to imitate leaf fall and to determine if debris affected repair. Nests were revisited the day following incision and weekly

thereafter for a total of three returns across three weeks. Data were recorded as the relative percent of enclosed area (0–4; 0 = no repair, 1 = 25% repaired, etc.) of the incised area with newly formed webbing.

RESULTS

In 2011 and 2013, 79 and 61%, respectively, of all sampled plants without larvae had been browsed. In contrast, only 44 and 43%, respectively, of plants with nests had suffered browsing by the same seasonal period (Table 1). Overall, browsing was more frequently observed on plants without than plants with tents (X^2 = 11.92; $p < 0.0001$).

Egg deposition occurred in late spring to early summer with differences as great as four weeks between years and two weeks among some sites (information concerning each stage/instar summarized in Table 2). Variation between years seemed to be correlated with temperature; at a higher elevation, Tilly maintained cooler temperatures throughout larval development. Clusters averaged 271.4 eggs (n = 186, s.d. = 156.4). Eggs took approximately three weeks to develop, following the same color progression each year (bright yellow - red - purple - black - dark grey; in some cases all colors were visible within one cluster, producing a "corn maize" pattern).

Larvae hatched en masse (early June to early July) approximately three weeks after eggs were laid and then proceeded to the growing tip of the stalk on which they hatched. First instars immediately began to construct the first pre-diapause tents, characterized by loosely organized silk, encompassing only the growing tip of the *Aureolaria flava*. Feeding was confined to surficial portions of leaves, creating window-pane injuries.

TABLE 1. Among site browse comparisons for 2011 and 2013.

		Plants without nests		Plants with nests	
Year	Site	Browsed	Unbrowsed	Browsed	Unbrowsed
2011	Buckridge	1244	249	12	9
	Cozahome	331	109	4	21
	Longbottom	48	12	13	10
	Maumee	32	31	4	4
	Spring Creek	174	104	2	1
	Combined	1829	506	34	45
2013	Buckridge	1019	149	64	39
	Cozahome	146	152	20	57
	Longbottom	153	76	30	34
	Tilly	356	691	22	50
	Combined	1674	1068	136	180

Second instar nests grew to encompass the majority of the upper portion of the plant, including most flowering portions of the stalk (typically occurring on secondary stalks near the top of the plant). Individuals continued to feed on leaves but also fed on under- and fully-developed flowers; the latter prevented several plants from reproducing (evidenced by the lack of seed pods).

Third instars constructed larger tents, which often covered the entire plant or lower portions where green leaves remained. Tops of plants were often "browning" and appeared to be dead or in a state of senescence. During this instar it was not uncommon for larvae to

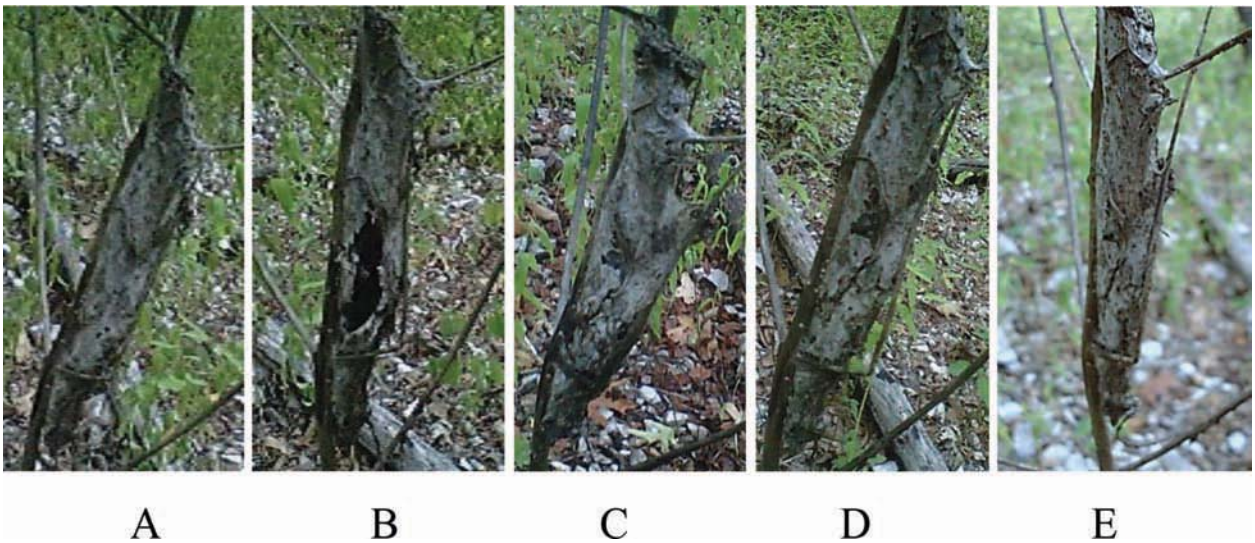

FIG. 3. Sequential photographs of tent repair for nest 20: (**a**) prior to incision, (**b**) incision, (**c**) first revisit, (**d**) second revisit, and (**e**) third revisit.

TABLE 2. Summarization of results for all butterfly stages; dashes indicate that no data were taken.

Stage/Instar	Approximate start date	Estimated duration	Size	Appearance	Organization	Typical relative location
Egg	mid-May - mid-June	3 weeks	-	yellow - dark grey	clustered	mid-plant
First instar	early June - early July	1.5 weeks	-	colorless; spineless	aggregated	growing plant tip
Second instar	mid-June - mid-July	2 weeks	4.89 mm ± 1.46 (n = 370)	pale; undeveloped spines	aggregated	upper 2/3 of plant
Third instar	early July - early August	3 weeks	7.66 mm ± 1.20 (n = 316)	orange and metallic-blue; developed spines	aggregated	entire plant; original or adjacent host
Fourth instar1	early - late August	3 months	7.18 mm ± 1.06 (n = 239)	orange and metallic-blue; developed spines	aggregated	mid-plant
Fourth instar2	mid-October - early December	4 months	3.96 mm ± 0.80 (n = 32)	orange and metallic-blue; developed spines	aggregated	leaf litter
Fifth instar	late February - early March	3 weeks	25.24 mm ± 5.85 (n = 180)	orange and metallic-blue; developed spines	loosely aggregated	plant rosettes; wandering
Sixth instar	late March - early April	4 weeks	40.02 mm ± 4.90 (n = 68)	orange and metallic-blue; developed spines	loosely aggregated	growing plants; wandering
Pupa	late April - mid-May	2 weeks	-	mostly white with orange and black markings	dispersed	(see text)
Adult	mid-May - early June	3 weeks	females larger (see text)	mostly orange, black, and white adult butterfly	dispersed	(see text)

[1] Pre-hibernating fourth instar.

[2] Hibernating fourth instar.

leave their original hosts to find new plants, a behavior seemingly dependent on the availability of leaf material on the original host. By some unknown mechanism, new tents on adjacent plants seemed to receive most, if not all, of the individuals from the previous nest and perhaps individuals from other adjacent nests. Larvae at this instar continued to feed on leaf material and were observed feeding on coarser stem material (i.e., not confined to new leaves/shoots).

The fourth instar lasted the longest and extended from late summer to early spring when larvae broke diapause. Once molting took place, larvae constructed pre-hibernation webs that had more compact webbing and were considerably smaller. Pre-hibernation webs seemed to vary in size depending on the number of larvae present. In this state, larvae seldom fed and activity was much reduced, only occasionally exiting the web on excessively warm days or rainy weather. Beginning in October to early December, larvae abandoned pre-hibernation nests and hibernated in leaf material at the base of the host plant. Fourth instars in hibernation were significantly smaller when compared to early fourth instar ($t = 14.55$; $p = < 0.0001$). During hibernation larval activity was seemingly non-existent. In early spring larvae resumed activity, feeding on emerging *A. flava* rosettes.

Larvae gained considerable length during the short period between emergence and the fifth instar. Feeding increased greatly and it was common for emerging larval groups to reduce plant rosettes of the original plant, and those plants in the immediate vicinity, to stubs. Plants subjected to such feeding did not recover until the following year (a phenomenon resulting in the displacement of central location of the following butterfly generation). During this period, larvae continued to specialize on *A. flava*, though two confirmed instances of larvae feeding on Coral Honeysuckle (*Lonicera sempervirens* L., Acanthaceae) were documented.

By the onset of the sixth instar plants had grown enough that foliage was readily available and larvae had noticeably increased in length. As in the fifth instar, sixth instars would move considerable distances to obtain food (one distance measured more than 20 m, but observations suggested even greater distances). Movement by spring-instar larvae often seemed to include full groups (unclear whether they remained in groups or if groups converged). However, it was not uncommon to find larvae alone on plants where no others had previously been. Dispersal during the spring period was widespread and apparently random, seemingly owing to the availability of food plants.

FIG. 4. Anchor Stink Bug (*Stiretrus anchorago* Fabricius) with third instar Baltimore Checkerspot larva.

Pupae were exceptionally difficult to find (n = 9), but were most frequently located less than 25 cm (n = 6) from the ground, attached to any number of items. Substrates included a fallen log (n =1), a branch on a sapling (n = 2), a rock (n = 1), a fallen branch (n = 1), a greenhouse (n = 1), a non-host plant (n = 1), and an axillary shoot of *A. flava* from a previous year (n = 2).

Adult males eclosed approximately 5–7 days prior to females. Females were significantly larger than males, with male wings averaging 30.4 mm and female wings averaging 36.4 mm (males: n = 57, s.d. = 1.4; females: n = 45, s.d. = 1.8; t = 18.91, p < 0.0001). Adults spent much of early morning basking on leaves in the understory. By 1000 (until late afternoon), males most frequently perched at the top of plants, with occasional patrols of surrounding areas. Females could be found searching for oviposition plants, ovipositing, or simply flying during this time. When two *E. p. ozarkae* came into contact, they often flew straight upward, circling one another. Females were less likely to be seen following tagging, with few secondary observations and only one tagged female observed laying twice within the study area. Both sexes were heavy nectar feeders, with multiple cases of adults spending greater than 45 minutes on a single flowering plant. Nectaring plants varied, but included Purple Milkweed (*Asclepias purpurascens* L.), Butterfly Milkweed (*Asclepias tuberosa* L.), Pale Purple Coneflower (*Echinacea pallida* Nutt.), and Wild Quinine (*Parthenium integrifolium* L.). Puddling was seen infrequently. Adult females mated shortly after emerging and copulation was maintained for as long as three hours; polyandry was not observed. Two tagged females were observed laying their first mass of eggs within 24 hours of mating. Egg deposition averaged 88.2 min (n = 28, s.d. = 38.2 min), but on three occasions was interrupted due to harassment by a paper wasp (Vespidae sp.; n = 1) and high-wind events (n = 2). With secondary observations of males declining dramatically after approximately two weeks of first captures, and females similarly disappearing one week later, adult lifespan was estimated to be about two weeks with a flight season of about three weeks; females persisted later than males.

The number of larvae in 2012 dropped by 76% prior to hibernation. Buckridge experienced the most dramatic reduction, with 88% of individuals perishing, missing, or otherwise absent from original tents. Cozahome and Longbottom persistence was reduced by 81 and 64%, respectively. The majority of disappearances during 2012 occurred prior to July, with 35, 56, and 34% of individuals being unaccountable for Buckridge, Cozahome, and Longbottom, respectively. Nests remained active but individual disappearance was high during this period. Nests experienced the greatest decline in August, with losses of 47, 57, and 50% for Buckridge, Cozahome, and Longbottom, respectively. Similarly, the number of nests in 2013 was dramatically reduced in August. Buckridge, Cozahome, Longbottom,

FIG. 5. Photographs showing interactions between chalcid wasp (superfamily Chalcidoidea) and *E. p. ozarkae*: wasp attacking a sixth-instar (top), a healthy chrysalis (lower left), and a parasitized chrysalis (lower right).

and Tilly experienced reductions of 46, 40, 64, and 49% (respectively) during this month. The overall decline of nests within these sites during the study period was 62, 48, 78, and 58%, respectively; total loss among all sites was 61%.

Repair was observed in 18 of the 20 pre-hibernation tents damaged artificially (Fig. 3). All larvae died in unmended tents by the end of the experimental period, as did those in three additional partially mended tents. Furthermore, three of the five nests where all larvae died contained only 1–10 individuals at the beginning of the experiment.

Predation and parasitism were common throughout all life stages, though the constituents changed over the lifespan of larvae. Few marked egg clusters were removed, but if they were, a slight imprint on the leaf was the only evidence that remained. These disappearances were attributed to an unidentified neuropteran larva and an ant species (actual feeding not observed), each found in close proximity to multiple clusters. Early instars were eaten by Araneae, specifically jumping spiders (Salticidae) and Hentz's Orbweaver (*Neoscona crucifera* Lucas). One instance of attack by a paper wasp (Vespidae) was observed during an early instar (2–3). Early instars (1–3) suffered massive losses to the Anchor Stink Bug (*Stiretrus anchorago* Fabricius, Fig. 4). The stink bug, which often remained close to tents until all individuals had been consumed, patrolled the outer surface of pre-diapause tents inserting its proboscis through the webbing to attack larvae. Larvae ceased feeding and aggregated behind layers of loose webbing at the center of nests when this predator was present. Late instars (5–6) were attacked by a parasitoid wasp (superfamily Chalcidoidea). A single attack on a sixth instar by this parasitoid was observed (Fig. 5). The wasp maintained a distance of about 15–20 mm from its prey. The larva was noticeably agitated in the presence of this parasitoid, thrashing defensively with either the front or rear portions of its body when perturbed. This larva had seemingly punctured itself with its spines, but eventually killed the attacker with this defense. Other larvae were observed that lacked many of their rear spines and it was presumed these spines were lost in similar interactions. Chrysalides formed by larvae infected by this wasp were formed prior to healthy counterparts and developed a darker exoskeleton. The parasitoid is believed to be polyembryonic, with one collected chrysalis containing 137 wasp pupae. Adult wasps emerged soon after healthy adult butterflies eclosed, as shown by the presence of hollow, darkened chrysalides with obvious exit holes.

Discussion

The use of an alternate host plant (irreversible?) between subspecies, and to which Ozark populations have become dependent, is fundamental to understanding the basic biology and continued evolution of the Baltimore Checkerspot. *E. p. phaeton* is reported to persist in areas where *Chelone glabra*, *Aureolaria flava*, and *Plantago lanceolata* are its primary hosts (Shapiro 1974, Stamp 1979, Scholtens 1991, Bowers et al. 1992). Masters (1968) and Scholtens (1991) noted populations of *Chelone glabra* in Ozark sites occupied by *E. p. ozarkae*, but neither found the butterfly associated with this plant. Bauer (1975) found *E. p. ozarkae* to be intolerant to changes in original larval food plant when switched from either *Lonicera* (a reported alternate primary host to *Aureolaria* for *E. p. ozarkae*; unconfirmed in this study) or *Chelone* to the other. Vawter and Wright (1986) report that *E. p. ozarkae* performs significantly better on *Aureolaria*, while *E. p. phaeton* performs equally well on both *Aureolaria* and *Chelone*. Scott (1986) notes that after hibernation, larvae of *Euphydryas* spp. are more tolerant and occasionally may forage on plants that are botanically very different. A recent plant atlas for Arkansas (Gentry et al. 2013) shows *Plantago lanceolata* to be found in all counties within which our study occurred, but *E. p. ozarkae* was never found with this plant. Further, no records of *E. p. ozarkae* exist from north-eastern areas of Arkansas, where *C. glabra* is reported to grow.

Herbivore browsing may represent an important threat to populations of *E. p. ozarkae*. *A. flava* in all areas was heavily browsed. The majority of browsing was attributed to populations of White-tailed Deer (*Odocoileus virginianus* Zimmermann). Study sites are largely secluded from human populations and deer were frequently seen within study areas, except Tilly (private homestead with free-ranging dogs). Heavy herbivory of host plants by deer is a common threat to butterfly species (Schweitzer et al. 2011). Large populations are known to alter plant communities in forest understories (Côté et al. 2004), reducing available hosts for many specialized invertebrate herbivores. Schweitzer et al. (2011) suggest herbivory by large populations of deer is a primary cause of decline and imperilment for as many as 15 rare butterfly species. *Chelone glabra* in Maryland, where the Baltimore Checkerspot is state-listed (S-3), is also reported to suffer heavy losses to deer herbivory (Durkin 2009). The importance of herbivory during key periods of the present study is supported by the fact that more larval nests/tents were on unbrowsed plants, which may suggest a preference for unbrowsed plants that are generally not in great abundance.

Larval persistence on hosts in late-summer and early-fall seemed dependent on food availability and predation. Individuals in 2012 experienced the greatest loss within one month of hatching, the same period that the presence of *Stiretrus anchorago* was noticed. Many nests remained active with much fewer individuals into August, the period when nests most frequently deteriorate and when *A. flava* begins to senesce and larvae enter diapause. Food consumed by this point must provide enough of a reserve to carry larvae through winter. In 2012 an increase in larval numbers was recorded during this period. It is possible that larvae at this time aggregate in larger groups, perhaps because there may be energetic or defensive benefits to this behavior.

Comparisons between the two subspecies reveal a number of similarities but there are major differences. Behavior and life-stage characteristics seem similar between the two subspecies (Bowers 1978). Larval group size within communal tents appears to be smaller in *E. p. ozarkae* (present study) than reports for *E. p. phaeton* (Stamp 1982b), though precise data were not taken. An interesting difference in behavior regarding conditions for exiting pre-hibernation tents was noted. More animals were observed outside of nest webbing immediately following rain, perhaps owing to the dryer conditions of the habitat, and on atypically warm days, likely due to the increase of temperatures in aggregate groups. In winter, all larvae aggregated at the base of the host plant, in contrast to observations by Scholtens (1991) concerning *E. p. phaeton* in the Great Lakes region. Feeding behavior in spring larvae was similar between subspecies. Both exhibited broader diets and both aggressively feed on available resources, even to the point of detriment to later life stages (Bowers & Schmitt 2013). The adult flight season is believed to be shorter in *E. p. ozarkae* (Masters 1968) than that of *E. p. phaeton*, which persist up to four weeks. Our results are similar to Masters (1968) in that adult *E. p. ozarkae* persisted for three weeks, with an estimated lifespan of only two weeks for individuals. Wing lengths reported for males and females by Masters (1968) were of similar magnitude in our study, with females being significantly larger than males. It has been reported that *E. p. ozarkae* is larger and darker than the nominate subspecies (Heitzman & Heitzman 1996), but we have not yet made these comparisons.

E. p. ozarkae exhibited a much greater variance in the number of eggs per cluster by comparison to *E. p. phaeton*. Eggs were laid in similar quantities per cluster between subspecies (*E. p. phaeton*: 273.8, Stamp 1982a; *E. p. ozarkae*: 271.4). However, the standard deviation of 156.4 for *E. p. ozarkae* as compared to 23.1 for *E. p. phaeton* represents a nearly seven-fold difference. The magnitude of this difference is largely the result of differences in host relationships among our study sites. Tilly, which maintains much larger plants (more abundant, shorter stalks with greater numbers of leaves) and experiences less browsing (free-ranging dogs) by large herbivores, had the highest within site average for eggs per cluster (Robertson 2015).

Both subspecies are able to break diapause for nest repair (Bowers 1978, present study). Our findings concerning the number of individuals and persistence may point to a potential benefit of larger group size. Tent damage was observed frequently, mostly as circular holes. These holes appeared to have been caused by organisms residing within the protective webbing, particularly katydids (Tettigoniidae).

E. p. ozarkae had a different suite of predators and parasitoids than reported for the nominate subspecies and exhibits behavior that suggest adaptation over an extended period of association. Eggs were likely preyed upon by an unidentified ant species and a chrysopid larvae, both reported by Stamp (1982b) to occupy early instar communal tents. While vespids have not been reported as predators for the eastern subspecies, these insects are generalists and likely do impact *E. p. phaeton*. Predation by *Stiretrus anchorago* (unreported as a predator of *E. p. phaeton*) was common and observed at all sites. Larvae behaved differently when pre-diapause webs were being patrolled by this predator, which may suggest recognition and predator-specific behavioral responses. The impact of this predator was noticeable, with multiple nests lost to its appetite. Parasitism by a chalcidoid wasp is also unrecorded for *E. p. phaeton* and changes in behavior were noted during this interaction; the timing of the attacks (April) seemed specific to the 6th instar of *E. p. ozarkae*.

CONCLUSION

We have shown through the study of life-history traits that there are important differences between Ozark populations and those of the nominate subspecies of the Baltimore Checkerspot. Given the nature of this butterfly (univoltine, short-adult flight period, etc.) and the fact that Ozark populations have received very little study, our findings are noteworthy. The importance of shifts in host plant and differences in ecological environments among populations of phytophagous insects has been shown to lead to speciation (Matsubayashi et al. 2010). Ozark populations of this checkerspot have thrived on different host plants than its more eastern counterpart for at least 85 generations [first report of *E. p. ozarkae* by Bower (1930)]. Throughout this period, which has to have been much

greater in duration, the Ozark subspecies has experienced distinctly different habitat conditions and been affected by different predators than the nominate subspecies. The extent to which these differences are sufficient to raise each of the two subspecies to the level of full species has been of interest for many years (e.g., Bauer 1975, Vawter & Wright 1986, Opler & Malikul 1998). Vawter and Wright (1986) found little genetic evidence among 25 allozyme loci to suggest species separation. In comparing mtDNA (COI) between subspecies (4 Arkansas specimens vs 1 Maryland specimen), we too found little genetic difference (0.25 to 0.38%, unpublished data). However, differences in habitat and behavior between the two may supersede lack of genetic evidence (Vawter & Wright 1986, present study). Whether full species or subspecies, there should be no denying that *E. p. ozarkae* populations constitute butterflies that, from a conservation and management perspective, should receive greater consideration. Continuing to treat *E. p. ozarkae* populations of the Baltimore Checkerspot as extensions of the nominate subspecies is not justified and management activities should take this into account.

ACKNOWLEDGMENTS

We thank the National Park Service (Buffalo National River) and the Arkansas Game and Fish Commission for permits. Additional appreciation is extended to the National Park Service for providing lodging during our study, in particular we thank Mark DePoy, Faron Usrey, and Chuck Bitting. We will always be grateful to Ruth and John Andre for allowing us to use their property for the duration of our study. For assistance in the field we thank Dana Frederick, Mary-Kate Williams, Patrick Gibson, and Amanda Anthony. Financial assistance was provided directly to SMR through the Arkansas Audubon Trust, with additional support to WHB from the National Park Service, the Arkansas Game and Fish Commission, and the University of Arkansas at Little Rock. Constructive suggestions by the Editor and two anonymous reviewers helped to clarify and improve the manuscript.

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Submitted for publication 23 December 2015; revised and accepted 15 February 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 238–248

A NEW CRYPTIC *EUPSILIA* FROM NORTHEASTERN NORTH AMERICA (NOCTUIDAE)

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ABSTRACT. A new species of *Eupsilia* Hübner phenotypically allied to *E. cirriplaea* (Franclemont, 1952) and *E. sidus* (Guenée, 1852) is described from northeastern North America. Identification of *E. schweitzeri*, **n. sp.**, is most reliably made on the basis of larval morphology or genetic data, although most adults can be determined using subtle forewing features, including their derived forewing scale type. The adult, genitalia, forewing scales, and larvae of the new species are illustrated. Keys are provided to adults and larvae of the seven northeastern *Eupsilia*. *Eupsilia walkeri* (Grote, 1864) is synonymized under *E. vinulenta*, **n. syn.** *Scopelsoma colorado* (Smith, 1903) is removed from synonymy with *Eupsilia sidus* and given valid species status as *Eupsilia colorado*, **rev. stat.**, and a neotype is designated for *E. sidus*.

Additional key words: cryptic species, larval key, polyphagous, ground feeder, shelter former, CO1 barcodes

Eupsilia (Hübner, 1821) species are among the most abundant fall- and spring-active moths in the Northeast (Wagner et al. 2011). On warm winter and early spring nights, they account for many of the orange-brown moths seen in car headlights when driving through wooded habitats, and often outnumber all other moths at bait from January to early April, with densities sometimes reaching 50–200 or more moths at a single bait patch. There are seven species of *Eupsilia* in the northeastern United States (Forbes 1954, this work)—one of the more common of which has gone undescribed until now. The new taxon is named after Dale F. Schweitzer, who first recognized the new species based on larval characters. This new species is commonly confused with *Eupsilia cirripalea* Franclemont 1952, *E. sidus* (Guenée, 1852), and *E. vinulenta* (Grote, 1864). All seven *Eupsilia* fly sympatrically and synchronically at some locations (e.g., East Rock State Park in New Haven, Connecticut). Their abundance, species diversity, variability, and overlapping co-occurrence, collectively made it possible for this common eastern moth to go unnamed.

In this paper we describe the new species, illustrate its adult and larval stages, and provide an adult key and the first larval key for eastern *Eupsilia*. We identify and illustrate three types of forewing scales possessed by *Eupsilia*. We briefly describe the life history, as far as known (there is little knowledge of late instar feeding habits because larvae leave their natal hosts and are likely nocturnal). We also compare the new species to the western taxon, *E. colorado* (Smith, 1903), which appears to be closely allied to *E. sidus* and our new species.

MATERIALS AND METHODS

The adult description of *E. schweitzeri* is based on reared specimens from Connecticut and New Jersey. The larval description of *E. schweitzeri* is based on living larvae, preserved larvae, and larval images. Larvae were compared to reared specimens and larval images of *E. cirripalea*, *E. devia* (Grote, 1875), *E. morrisoni* (Grote, 1874), *E. sidus*, *E. tristigmata* (Grote, 1877), and *E. vinulenta*. High-resolution photographs of forewing scales were taken with a MacroSolutions® stacking system. Genitalia of the male holotype and female paratype were prepared and mounted according to Lafontaine (2004). Eighty-two slide mounted genitalic preparations made by John G. Franclemont were examined from the CUIC; as were 3 additional slides made by Tim McCabe in the NYSM. COI sequences were generated by the Barcodes of Life Project (BOLD); sequences for two *E. schweitzeri* specimens have been submitted to BOLD. Additional sequences of the new species have also been submitted by J. Bolling Sullivan. Adult paratypes of *E. schweitzeri* (n=87) have been deposited in the following collections:

CNC—Canadian National Collection, Ottawa, Ontario, Canada.
CUIC—Cornell University Insect Collection, Ithaca, NY, USA
ISIC—Eric Quinter Collection, Windham, CT, USA.
NYSM—New York State Museum, Albany, NY, USA.

PMNH—Peabody Museum of Natural History, Yale University, New Haven, CT, USA.
UCMS—University of Connecticut, Storrs, Connecticut, USA.
USNM—National Museum of Natural History, formerly United States National Museum, Washington DC, USA

Eupsilia schweitzeri Lavitt & Wagner, **n. sp.**
(Figs 1–3, 14–16, 19, 23–26, 28, 31)

ZooBank: urn:lsid:zoobank.org:pub:82B54624-AF80-4C34-A7CC-BF8C3B378AE5

Material Examined. HOLOTYPE male (Figs 1, 14, 15). CT: Windham Co., 0.7 mi. ESE Windham 240ft. junction Follett Rd. and Potters Brook // ♀#1 coll. in bait trap bred ex ova on *Prunus serotina*, emerged: ix.21-xi.8.2013, Eric L. Quinter, collector // Barcodes of Life Project DLW-000168, Specimen ID CNCLEP 00116373 // Barcode of Life leg removed DNA extracted // Genitalia CNC slide # ♂ 16800 (CNC). **Paratypes (adults)** (51 males, 36 females). **Connecticut:** New Haven County. Ex Ovis, pair A87, from CONN.: New Haven, West Rock, coll. March 1987 ♀ white spot, ♂ yellow Ecl. August 18-September 15 1987 reared Dale Schweitzer // Sleeved late April to mid June, started on apple, 4th instar to *Carya glabra*, last instar on *Juglans nigra* at Dedham, Mass. (18 ♂, 6 ♀); same data as above except: Ecl. August 22-August 31 1987 Reared indoors on cuttings: oaks, hickory, apple, wild cherry etc. April-June 1987 (1♂, 2 ♀); same data as above except: Reared Dale Schweitzer Sleeved late April to mid June 1987 on apple at Dedham, Mass. (6♂, 1 ♀) (PMNH, UCMS); New Haven east base West Rock Jct. Baldwin Dr & Wintergreen Ave 11 March 1982 D. F. Schweitzer // McCabe gen. slide 1729 (1♀) (NYSM); New Haven east base West Rock Jct. Baldwin Dr & Wintergreen Ave 17 August 1981 D. F. Schweitzer // [tlm 81-12 Reared ex ovo on *Quercus ilicifolia*] // McCabe gen. slide 1683 (1♂) (NYSM). Windham County. CT: Windham Co., 0.7 mi. ESE Windham 240ft. junction Follett Rd. and Potters Brook // ♀#1 coll. in bait trap bred ex ova on *Prunus serotina* (1 ♀), emerged: ix.21-xi.8.2013, Eric L. Quinter, collector // Specimen ID CNCLEP 00116374 // Barcode of Life leg removed DNA extracted (CNC). Ct: Windham Co., 0.7 mi. ESE Windham 240ft. junction Follett Rd. and Potters Brook // [ex ova] Eric Quinter, [adults emerged] ix.21-xi.2013, host: *Prunus serotina* (9 ♀, 12 ♂) (ISIC, UCMS, USNM); Ct: Windham Co., 0.7 mi. ESE Windham 240ft. junction Follett Rd. and Potters Brook // [ex ova] Eric Quinter, [adults emerged] Sept. 22–Oct. 4, 2007, host: *Prunus serotina* (10 ♂, 12 ♀) (ISIC). **Massachusetts:** USA: Miles Standish SF Mass.: Plymouth Co 26 Sept. 1987 D. F. Schweitzer // [tlm 87-4 Reared ex ovo on: *Quercus ilicifolia*] (2 ♂) (NYSM, UCIC); USA: Miles Standish SF Mass.: Plymouth Co 26 Sept. 1987 D. F. Schweitzer // [tlm 87-4 Reared ex ovo on: *Quercus ilicifolia*] // McCabe gen slide 5076 (1♀) (NYSM). **New York:** Albany County. USA: Albany Pine Bush N.Y.: Albany County 42.33.43 – 73.51.54 24 November 1979 T. L. McCabe 100 m // McCabe gen. slide 506 (1♂) (NYSM. Orange County. USA: Bear Mtn. N.Y.: Orange Co. 41.18.14 – 74.00.24 26 Sept. 1989 T. McCabe 400 m // [tlm 89-13 L6 found feeding on *Cirsium*] // McCabe gen. slide 5306 (1♂) (NYSM). Tompkins County. Six Mile Creek Tompkins Co., N.Y. August 1958 JG Franclemont [Reared ex ovo on *Prunus serotina*] (2 ♂) (NYSM).

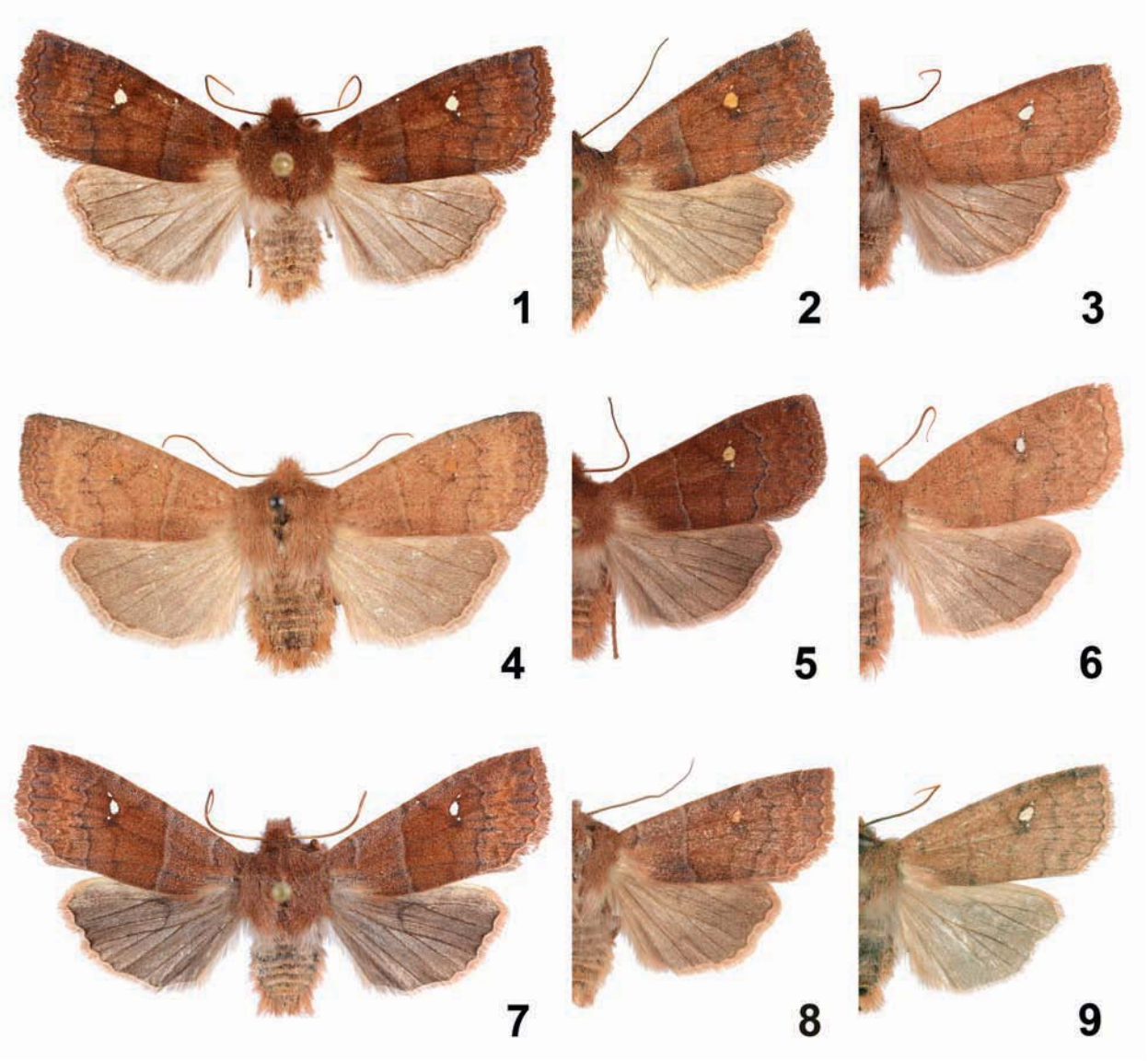
Etymology. We name this new species *Eupsilia schweitzeri*, after Dale F. Schweitzer, whom we credit for first recognizing this new species in the late 1980s.

Diagnosis. Adult.

Eupsilia schweitzeri (Figs 1–3) is closely similar to *E. sidus* (Figs 5, 6) and *E. cirripalea*, especially when weather worn or having overwintered. Fresh adults usually have three bands of violaceous scales (inside the antemedial, outside the postmedial, and inside of the dark crenulated adterminal lines). *E. sidus* tends to have a stronger sienna aspect to the FW color; *E. cirripalea* tends to have a more uniform brown aspect to the FW color; and *E. colorado* has a light-brown to tan FW with a faint cast of umber (bearing in mind that specimens we had for study are 120 years in age). *E. schweitzeri* and *E. sidus* tend to have a more conspicuous silvery-white, pale to fiery-orange, or stramineous reniform spot on the FW than is typical for either *E. cirripalea* or *E. colorado* (Fig. 4). The outer FW margin is distinctly crenulated in *E. cirripalea*; modestly crenulated in *E. schweitzeri*, but decidedly less crenulated in *E. colorado* and *E. sidus*. The FW scales beyond the postmedial line of *E. cirripalea*, *E. sidus*, and *E. schweitzeri* have strongly recurved outer teeth (excelsior scales of Franclemont); *E. cirripalea* and *E. sidus* often have greater numbers of modified scales distal to the postmedial relative to *E. schweitzeri*. (see also Figs 17–22). Distal to the postmedial line the FW scales of *E. colorado* are less recurved than those of *E. cirripalea*, *E. schweitzeri*, and *E. sidus*, and are essentially unmodified (flattened and shingled). (Contrary to keys and claims by earlier workers, *E. morrisoni* also possess modified wing scales (Fig. 18), and these tend to be in the basal half on the wing, sparser in number, and less overlapping.) In the male genitalia, the new species lacks the diagnostic medial blister on the juxta of *E. cirripalea* (Franclemont 1952, Forbes 1954). It can be separated from *E. sidus* and *E. colorado* by its more robust (broader) valve (in *E. colorado* and *E. sidus* the valve is more elongate and narrowed through much of its length) (Figs 10, 12, 14); the cucullus is relatively broader than that of that of *E. cirripalea*, *E. colorado*, and *E. sidus*. The digitus is longer and more attenuated in *E. cirripalea* and *E. schweitzeri* than that expressed in *E. colorado* and *E. sidus*.

Description of Adult

Head. Setae orange to reddish brown often darkened or hoary apically. Some scales forked apically on head but not on antennae. Lamellar, tan to grey scales on dorsal surface of antennae. **Thorax.** Thorax densely covered with hairlike setae. Scales forming middorsal ridge. Thorax concolorous with wings, ranging from orange brown to purple or reddish brown. Apices of setae often deeply forked and hoary. **Forewing** (Figs 1–3). Orange to reddish brown with hoary, gray violet color, often with purple luster especially basad of antemedial line and over outer third of wing. Sinuous postmedial line bulging outward beyond cell with dark dentate projections sometimes present on R_5, M_2, M_3–Cu_2. Reniform spot small, orange, yellow, or white, often flanked by upper and lower satellite spots; orbicular almost always absent, but sometimes faintly discernible; claviform obsolete. Reared and freshly flown individuals have three bands of violaceous scales: inside antemedial, outside postmedial, and inside of dark crenulated adterminal line. Outer margin of wing convex, rounded, modestly crenulated. Fringe concolorous or slightly paler than FW ground color. Indistinct medial line of darker scales, jogging outward



FIGS. 1–9. *Eupsilia* adults. **1**, *E. schweitzeri* Holotype reared male. **2**, *E. schweitzeri* reared female. **3**, *E. schweitzeri* flown male. **4**, *E. colorado* flown male. **5**, *E. sidus* Neotype, reared male. **6**, *E. sidus* flown male. **7**, *E. vinulenta* reared male. **8**, *E. vinulenta* flown male. **9**, *E. vinulenta* flown male, pale form.

to reniform spot through cell. **Hindwing** (Figs 1–3). Uniformly fuscous with only slightly pinker fringe, often concave between M_1 and M_3. Crescent-shaped reniform spot weakly expressed. Ventral surface of hindwing with poorly differentiated discal spot and postmedial bands (relative to many *Eupsilia*). **Abdomen.** Fuscous, more or less concolorous with hindwings. **Male genitalia** (Figs 14, 15). Very similar to those of *E. cirripalea* and *E. sidus*. Uncas strongly hooked, drawn into spine; gnathos subquadrangular, juxta pentagonal with truncated dorsal margin and lacking medial conical projection (of *E. cirripalea*); valve elongate, roughly four times as long as wide. Prominent corona, with outer margin slightly curved, running nearly in same axis as lower valve margin, bearing 20–31 bristles. Digitus very long, projecting past lower margin of valve; cavity between cucullus and corona shallow. Inflated vesica with six basal pouches (in two groups of three); near midlength, rosette of ca. 25–35 thickened setae, with finely attenuated, twisted apices. **Female** (Fig. 16). Bursa copulatrix with four signa, two of which are rudimentary, i.e., only slightly differentiated, from the walls of the bursa; all four extend nearly full length; mesal surface of signa serrulate. Numerous concentric creases of bursa microserrulate; caudal end of bursa copulatrix with two pouches. Ductus bursae more strongly sclerotized about antrum opening to pouch at midlength.

Diagnosis of Living Final Instar (Figs 26, 28). Thoracic venter from T1–A1 bright cherry red, contrasting with following abdominal segments, which are pale and largely unpigmented. Spiracular stripe thin, broken or absent between T1 and A1; thickened on A7 and A8. Middorsal and subdorsal stripes, white, thin, sometimes interrupted; supraspiracular stripe mostly obliterated. Ground color of dorsum red brown over thorax but giving way to frosty grey or brown over abdomen (this same area becoming brown in the mature larvae and prepupa); laterally, brown below subdorsal and above supraspiracular stripe; contrasting with reddish-brown, maroon, or wine

supraspiracular region. **Middle Instars** (Figs 23–25) with more gray over abdomen; red pigments in supraspiracular more evident, stripes white, and abdominal subventer and venter mostly unpigmented.

Distribution. Because *E. schweitzeri* represents a cryptic species, its range is poorly circumscribed. We have examined specimens from southeastern Ontario, Massachusetts, and New York, southward to Pennsylvania and New Jersey, though the moth becomes scarce in the southern parts of New Jersey. Its range extends farther south in mountainous areas; confirmed records extend through mountainous areas of North Carolina (Bo Sullivan unpubl. data) into northern Georgia (Adams 2015). What appears to be the same taxon occurs as far west as Missouri according to Dale Schweitzer (personal communication).

Barcoding. Of 154 barcoded *Eupsilia* from North America, with reads >500 base pairs, *E. schweitzeri* clusters as a single entity (n=10) outside of *E. cirripalea-morrisoni-sidus-tristigmata-vinulenta* assemblage (Don Lafontaine unpubl. data). Thus, there is little suggestion from CO1 that *E. schweitzeri* is especially close to any of the three species with which it has been confused in collections, i.e., *E. cirripalea*, *E. sidus*, or even *E. vinulenta*. (Known specimens of *E. colorado* are too old for barcoding—see below.)

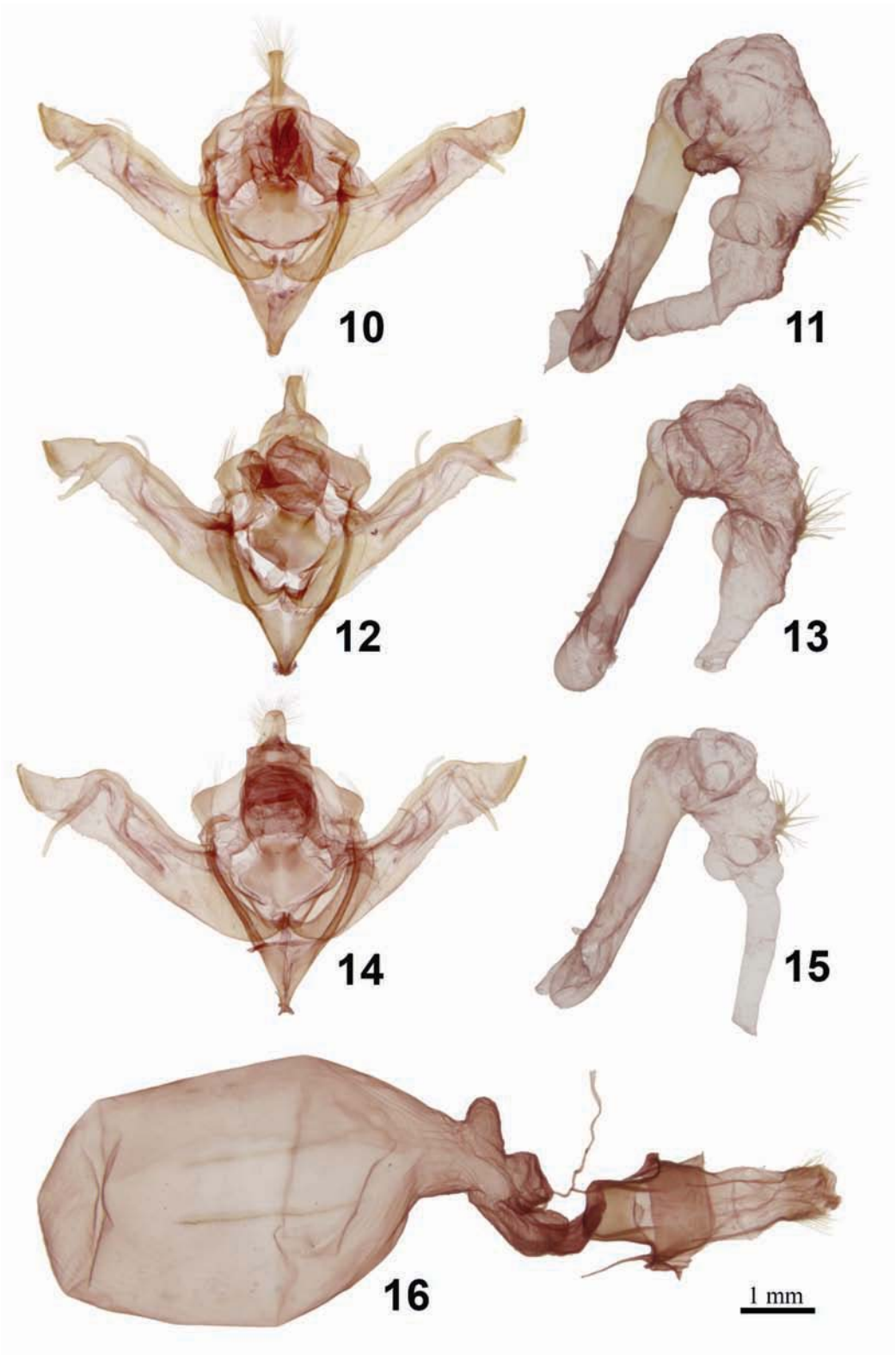
Key to Adults of Eastern North American *Eupsilia*. Our key extends the effort of Forbes (1954). All characters refer to forewing characters unless otherwise noted. Species identifications in *Eupsilia* can be challenging. In some species and forms, no single character may work to assure certain identification, especially in flown or overwintered individuals. Likewise both male and female genitalia are frustratingly uniform across the genus.

1. FW postmedial line not crenulate; basal, antemedial, and postmedial lines of pale scales; outer side of reniform often marked by crenulate tan line 2
   FW postmedial line crenulate; basal, antemedial, and postmedial lines mostly of dark scales; outer side of reniform not marked by crenulate tan line 3
2. Ground color gray without reddish tints; basal third of FW gray; excelsior scales absent ..... *devia*
   Ground color with orange or reddish tints; basal third of FW concolorous with rest of wing; modified scales sometimes present basad to postmedial line ..... *morrisoni*
3. Reniform with dark spot at its base; orbicular often distinct; basal line usually including a dark spot; often with dark subapical spot along costa ..... *tristigmata*
   Reniform without dark spot on its lower edge; orbicular obsolete; basal line without dark spot; without dark subapical spot along costa 4
4. FW scales distal to postmedial line lamellar (scales below the reniform are shown in Fig. 22)..... *vinulenta*
   Many FW scales distal to postmedial line of excelsior type, i.e. deeply cleft with outer spines curled and overlapping, and scale edges often rolled upwards (as in Figs 17, 19, 20) 5
5. Male juxta with central conical projection; female bursa with two signa; outer margin of forewing crenulated; ground color of FW more uniformly brown (and less reddened) than those that follow)..... *cirripalea*
   Male juxta without central conical projection; female bursa with four signa (two of which are weakly expressed); outer margin of forewing crenulated or smooth; ground color of FW more reddish brown and sometimes with violet luster 6
6. Three bands of violaceous scales (inside antemedial, outside postmedial, and inside of dark crenulated adterminal line) (in fresh individuals); FW color more brown than red; outer margin modestly crenulated; FW scales distal to postmedial often with numerous lamellar scales; valve robust, 4x longer then broad; digitus longer and more attenuated than that of *sidus* (cf. Figs 10, 14)..... *schweitzeri*
   Lacking three bands of violaceous scales; FW color more reddish than brown; outer margin crenulate; FW scales distal to postmedial dominated by excelsior scales; valve slender, 5x longer than broad; digitus shorter and less attenuated than that of *schweitzeri* (cf. Figs 10, 14) ..... *sidus*

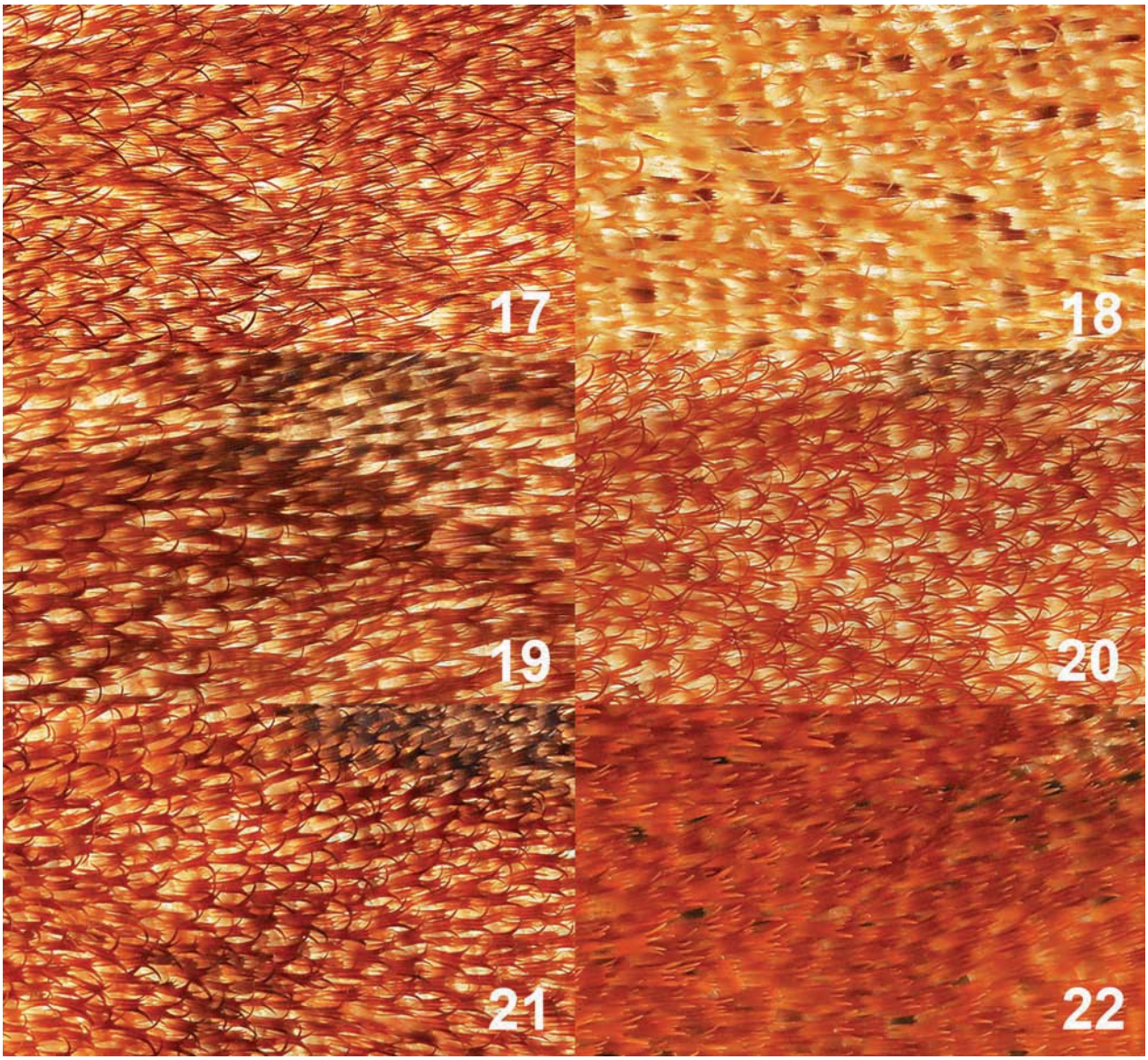
Key to Last Instar Northeastern *Eupsilia* Larvae

Note: Our key works best for fifth and submature sixth instars. Fully fed last instars and prepupae lose diagnostic coloration and become muddied in color to the extent that coloration characters become unreliable. Likewise, early instars of northeastern *Eupsilia* can be so similar that identifications based on this key, or our images, should be considered provisional. For example, the diagnostic red thoracic venter of *E. schweitzeri* (in late instars) is seen in early instars of other *Eupsilia*. We also caution that some larval characters given in previous works, i.e., the absence of subdorsal white stripes on T1 in *E. tristigmata*, are not deemed reliable. All seven eastern *Eupsilia* are figured in Wagner et al. (2011).

1. Venter green (including thorax), mostly unpigmented (sometimes pinkish anteriorly) 2
   Venter flushed with red or pink 4
2. Thick white spiracular stripe on A1–8; mottled green to brown dorsum; supraspiracular areas black below faint white subdorsal line; subventer unpigmented ..... *E. devia*



FIGS. 10–16. *Eupsilia* genitalia. **10**, *E. sidus* Neotype male. **11**, *E. sidus* Neotype aedeagus. **12**, *E. colorado* male. **13**, *E. colorado* aedaegus. **14**, *E. schweitzeri* Holotype male. **15**, *E. schweitzeri* Holotype aedeagus. **16**, *E. schweitzeri* female (note only the two more well-developed signa are visible).



FIGS. 17–22. *Eupsilia* forewing scales. All images taken in center of forewing with reniform visible at top center. **17**, *E. cirripalea*. **18**, *E. morrisoni*. **19**, *E. schweitzeri*. **20**, *E. sidus*. **21**, *E. tristigmata*. **22**, *E. vinulenta*. Magnification differs among images.

Thin white spiracular stripe often inconspicuous; dorsum concolorous or slightly differentiated in color from supraspiracular area; subventer pigmented or unpigmented 3

3. Dorsum and supraspiracular areas often somewhat contrasting; supraspiracular area usually with deep to smoky wine tints; middorsal and subdorsal lines thin and variously present, former more white in color; ground color usually some shade of brown ……..... *E. morrisoni*

Dorsum and supraspiracular areas usually concolorous; both dorsum and supraspiracular area usually with deep to smoky wine tints; middorsal and subdorsal lines thin, often obliterated; ground color typically velvety black to purplish brown ……..…..... *E. vinulenta*

4. Pink to red on thoracic and abdominal venter (Fig. 29), dorsum dark mottled gray to an olive green brown; supraspiracular area wine colored ……...…….....……..... *E. sidus*

Pink to red confined to thoracic and A1 venter ……5

5. Abdominal subventer usually pink to red above prolegs (Fig. 30) .....…… *E. tristigmata*

Abdominal subventer unpigmented above prolegs (Figs 27, 28) ……..…….... 6

6. Thoracic venter vivid red (Fig. 28); supraspiracular

area often with smoky burgundy hues (Fig. 26) .....*E. schweitzeri*
Thoracic venter pink (Fig. 27); supraspiracular area reddish brown to pink brown ..... *E. cirripalea*

RESULTS AND DISCUSSION

It is easy to understand why this common species, occurring in one of the most densely populated and well-collected regions of North America, went undescribed for so long. Adults are phenotypically difficult to distinguish from those of *E. cirripalea* and *E. sidus*. The genitalia of all seven northeastern *Eupsilia* species are similar, even between species that would easily be separated by wing pattern, coloration, and scale shape, e.g., *E. vinulenta* and *E. schweitzeri*. Where *E. cirripalea*, *E. sidus*, and *E. schweitzeri* co-occur, for some adult phenotypes, the only certain means of identification are genital and larval characters. Female genitalia are indistinguishable from those of *E. sidus*.

Biology and Distribution.

Female *Eupsilia* lay eggs singly on twigs and buds in the early spring. Early instars of *Eupsilia* are believed to feed on a wide taxonomic range of woody plants in the spring, fashioning loose leaf shelters (Fig. 24) or entering those made by other Lepidoptera (Wagner et al. 2011). By the fourth or fifth instar *Eupsilia* abandon their shelters. Where they go is a mystery. In two decades of caterpillar collecting in Connecticut, we have never found a late instar on foliage during the day. Their dark coloration is suggestive that the larvae feed at night. It is our suspicion that the late instars are broadly polyphagous on woody and non-woody plants, and perhaps other organic matter on or near the ground (Wagner et al. 2011). We once found a last instar *Eupsilia* apparently feeding on dog food in a basement bulkhead. It is unclear if larvae ascend trees at night to feed, as is commonly the case with many climbing cutworms, e. g., *Metaxaglaea* Franclemont and related genera. The European *Eupsilia transversa* is recorded from forbs (e.g., dandelion), and a wide range of woody plants; additionally, it is reported to feed on the caterpillars of other Lepidoptera (Manley 2008). *Eupsilia* larvae mature in late spring, and then burrow underground and form a cell where they enter diapause for two to three months, before pupating (Wagner et al. 2011). Adults begin issuing in September in Connecticut.

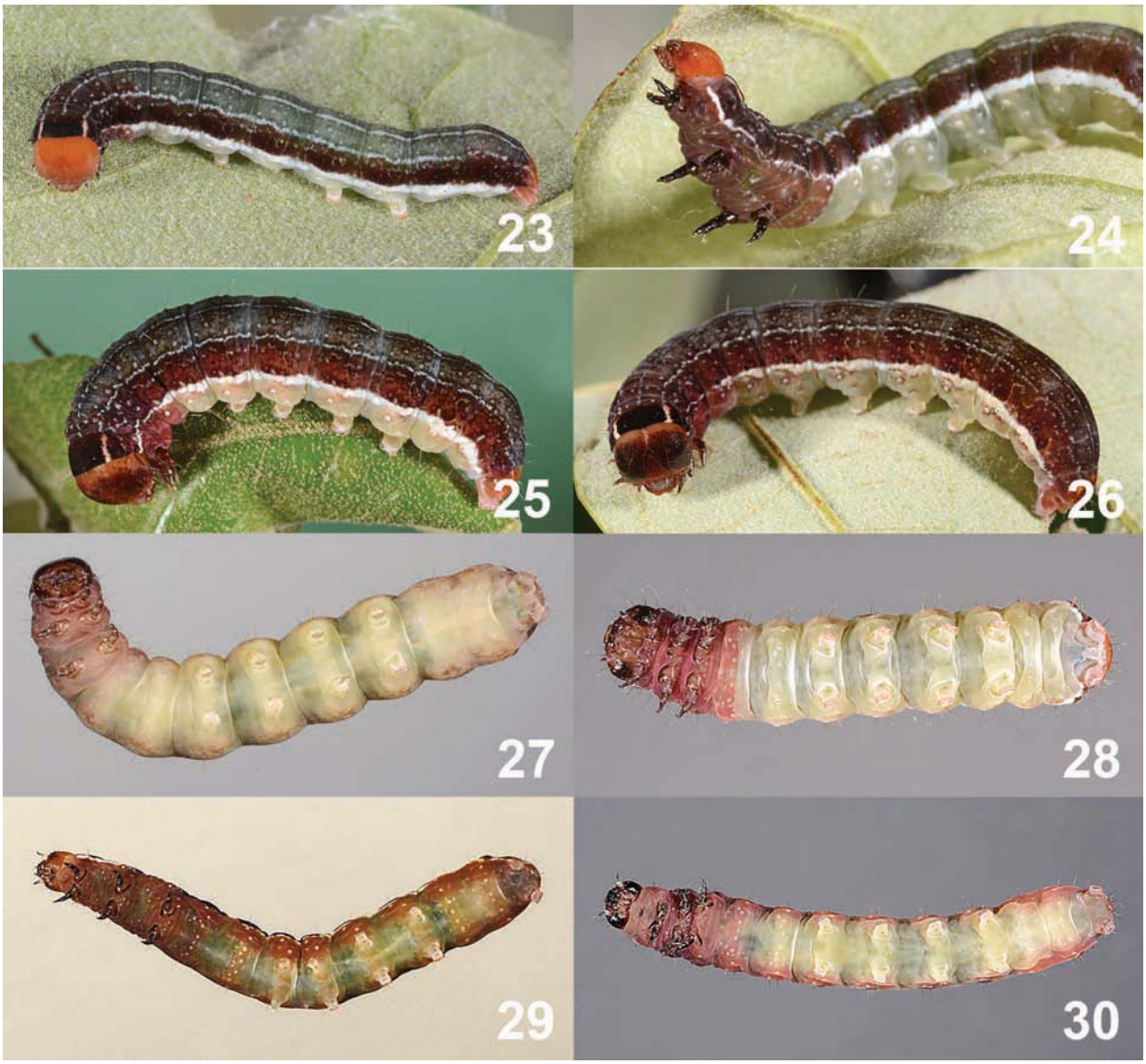
Head capsule measurements for *E. schweitzeri* indicate that there are six larval instars (Fig. 31): 1st: $\bar{x}$ = 0.3 mm (n=6, 0.3 mm); 2nd: $\bar{x}$ = 0.5 mm (n=10, 0.4–0.5 mm); 3rd: $\bar{x}$ = 0.8 mm (n=7, 0.8–0.9 mm); 4th: $\bar{x}$ = 1.4 mm (n=8, 1.2–1.5 mm); 5th: $\bar{x}$ = 2.2 mm (n=8, 2.2 mm); 6th: $\bar{x}$ = 3.2 mm (n=16, 3.1–3.3 mm). The head capsule width ratios between successive instars were generally consistent with those observed by Dyar, which ranged from 1.3–1.7 (Dyar 1890, Berg and Merritt 2009). Observed ratios in *E. schweitzeri* ranged from 1.4 between the last two instars and 1.8 between the 3rd and 4th instars, with a mean, median, and mode of 1.6 across the six instars.

Ex ova lots have been reared on *Carya glabra*, *Juglans cinerea*, *Malus sylvestris*, *Prunus serotina*, and *Quercus ilicifolia*. Our wild larvae of *E. schweitzeri*, have been collected as middle instars in southern New England from *Carpinus caroliniana*, *Cirsium* sp., *Hamamelis virginiana*, *Nyssa sylvatica*, *Prunus serotina*, *Quercus* spp., and *Vaccinium* spp. We suspect that *E. schweitzeri* is a generalist on woody shrubs and trees in early and middle instars, but becomes even more generalized in diet breadth in late instars, consuming a wide variety of plants (e.g., *Cirsium*) and perhaps even other types of organic matter.

Because all *Eupsilia* (across both the Nearctic and Palearctic) are similar in size, we think it likely that head width measurements of any wild-collected *Eupsilia* larva would generally conform to those given in Figure 31. We would be interested to learn of wild larval collections of 4th, 5th, and 6th instars, and upon what substrates they were observed feeding.

Although all seven northeastern *Eupsilia* may co-occur (e.g., in southern Connecticut), *E. sidus* and *E. schweitzeri* are largely allopatric over much of their range. *E. sidus* is usually a denizen of "dry oak forests, savannas, woodlands, ridgetops, and barrens from Wisconsin, southern Ontario, and central New Hampshire south to northern Georgia and Texas" (Wagner et al. 2011) (this range may conflate records of *E. schweitzeri*, e.g., records of *E. sidus* from Ontario may in fact refer to the heretofore unrecognized *E. schweitzeri*). *E. schweitzeri* is more ecologically generalized. It is abundant in both mesic and dry oak woodlands, but also occurs in bottomlands, along the edges of wetlands, in upland woodlands and forests, ridgetops, and other forested communities. In most habitats it is only outnumbered by *E. vinulenta* and *E. morrisoni*.

Wing Scales. *Eupsilia* have derived forewing scales. We confirm the presence of three scale types: lamellar, excelsior, and hooked (Figs 17–22). Lamellar scales, common to Lepidoptera (Scoble 1992, Kristensen & Simonsen 2003), predominate in *E. devia*, *E. morrisoni*, and *E. vinulenta* (Fig. 22) and in the former species, virtually all scales are of the lamellar type. In addition, the lamellar scales of *E. devia* are wider than those found in the six other northeastern species. Excelsior



Figs. 23–30. *Eupsilia* larvae. **23**, *E. schweitzeri*, early instar. **24**, *E. schweitzeri* middle instar making shelter. **25**, *E. schweitzeri*, 5th instar. **26**, *E. schweitzeri*, 6th instar. **27**, *E. cirripalea*, late instar venter. **28**, *E. schweitzeri*, late instar venter. **29**, *E. sidus*, late instar venter. **30**, *E. tristigmata*, late instar venter.

scales are those where the outer teeth of forewing scales tend to be elongated and recurved; in many cases, scale edges are rolled upward to the extent that they almost enclose the dorsal scale surface. Forbes (1954) used the term "shredded wheat" effect to describe the wing surface appearance resulting from the overlap and interlocking of the modified scales.

Excelsior scales occur in *E. cirripalea*, *E. morrisoni*, *E. schweitzeri*, *E. sidus*, and *E. tristigmata*, (Figs. 17–21), and in lower frequency and with an intermediate degree of recurvature of the outer teeth in *E. colorado* and *E. vinulenta*. *E. tristigmata* best represents the scales we describe as hooked; i.e., just the outer (two) teeth are recurved, sometimes as much as 180º (Fig. 21). The outer teeth also tend to be much darker in coloration in *E. tristigmata*.

***Eupsilia walkeri* (Grote, 1864).** With the possibility that *Eupsilia walkeri* might refer to our new species, we compared our type series to Grote's description, which he based on a single specimen from Philadelphia. The type of *E. walkeri* is believed to be lost (Franclemont 1952). Grote's description of *E. walkeri* makes no mention of purple or related violaceous colors (yet he used purplish twice as a

descriptor in his description of *E. vinulenta*, which appeared in the same publication, immediately following the account for *E. walkeri*). Violaceous scaling on the forewings is among the most reliable features for the recognition of *E. schweitzeri* adults, and is usually present basad to the antemedial line. Grote's characterization of the terminal line in *E. walkeri* as "very clearly defined, semi-lunulate," further eliminates *E. schweitzeri* as a possibility (as well as *E. cirripalea* or *E. sidus* and others, a point we return to below). Grote uses ochrecous to describe the forewing color of *E. walkeri* and "rich dull red" to describe the forewing of *E. vinulenta*. Ironically, the only *Eupsilia* in the Northeast that is accurately characterized as ochraceous are the pale (overwintered) forms of *E. vinulenta* (Fig. 9), the most phenotypically variable Nearctic *Eupsilia*. Both *E. schweitzeri* and *E. sidus* are a richer red than Grote's rendering of *E. walkeri*.

In his 1952 treatment of *Eupsilia*, Franclemont regarded *E. walkeri* (Grote) to be a synonym of *E. sidus*:

> "As identified here, *walkeri* Grote is a synonym, and judging from the figure and description it was based on a worn hibernated specimen. Grote's type is also lost, but was probably also from the vicinity of Philadelphia since it was in the collection of the Entomological Society of Philadelphia."

Forbes (1954) and subsequent workers have followed Franclemont. However, based on Grote's original description, remarks made above, and other arguments, we disagree with Franclemont's decision, and treat *E. walkeri* as synonym of *E. vinulenta* (Grote). As noted above, Grote states that *E. walkeri* possessed a clearly defined, semi-lunulate terminal line. Such is absent not only from *E. schweitzeri* but also *E. cirripalea* and *E. sidus*, and is commonly present in *E. vinulenta*. Grote's description of *E. walkeri* states: "transverse anterior [antemedial] line distinct, darker shaded, straight, composed of two narrow lines with a central light shade." Yet in *E. sidus* the antemedial line is poorly differentiated, especially relative to that of *E. vinulenta*. In addition to the character evidence, we note that *E. vinulenta* is among the most common *Eupsilia* in eastern Pennsylvania. *E. sidus* is an oak barrens species, at least at the latitude of Philadelphia, that we associate with scrub oak and low-bush blueberry communities, a habitat absent from the vicinity of present-day Philadelphia. In sum, we are convinced that Grote based his description of *E. walkeri* on a flown (perhaps overwintered) individual of *E. vinulenta*, and could not have been either *E. schweitzeri* or *E. sidus*.

***Scopelsoma colorado* (Smith, 1903), rev. stat.** In his 1952 treatment of *Eupsilia*, Franclemont synonymized *Scopelsoma colorado* with *E. sidus*. The species is known only from the type series evidently collected by William H. Barnes in March and April, 1893–1895, in Glenwood Springs, Colorado. We were able to locate and examine 1 (of the 2 formerly present) specimens in the AMNH, including the lectotype male and its associated genitalic slide; 13 specimens in the USNM; and 2 specimens in the CNC. After examining the above, as well as three additional male genitalic dissections (Franclemont #7574, USNM 38903, USNM 118685) and three female dissections (CNC 16801, USNM 38901 [bearing the notation: (From spem. labeled "♂ cotype") [sic]], USNM 38902), we conclude that *Scopelsoma colorado* is a valid species. The ground color ranges from tan or ochraceous (most) to reddish brown (few); violet scales are absent and any hint of red or orange typical of *E. sidus* is subdued; the antemedial line in *E. sidus* and to a lesser extent *E. schweitzeri* is nearly absent in *E. colorado* (Fig. 4). Most notably the reniform and associated satellite spots common to *E. schweitzeri*, *E. sidus*, and *E. vinulenta* are vague and ill-defined in *E. colorado* save for the dark scales along the lower edge of the reniform spot (Figs 1–9). The forewing margin is less crenulate than that of *E. schweitzeri* or *E. cirripalea*; and especially that of *E. vinulenta*. The forewing scales are intermediate between lamellar and excelsior, and most similar in

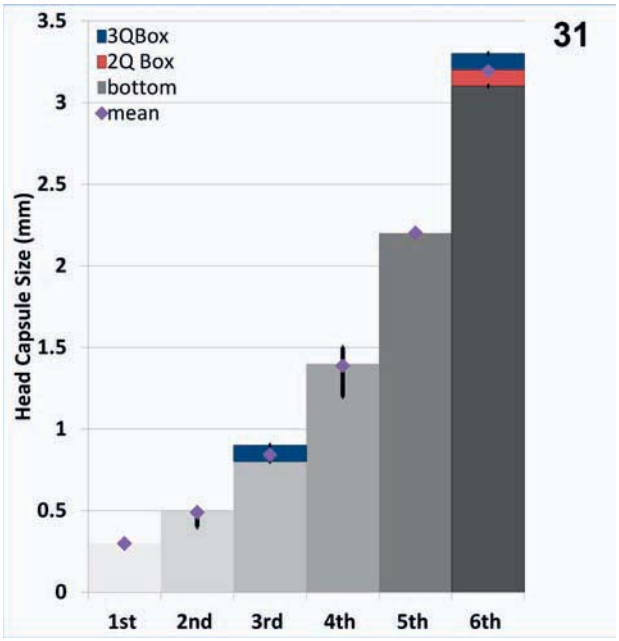


FIG. 31. Head capsule width of *Eupsilia schweitzeri* larvae. Because all *Eupsilia* (across both the Nearctic and Palearctic) are similar in size, we think it likely that head width measurements of other wild-collected *Eupsilia* larva would fall into these same size classes.

character and distribution to *E. vinulenta*. They lack the "shredded wheat" aspect that we (and Forbes) would attribute to *E. schweitzeri*, *E. sidus*, and *E. cirripalea*. Forewing scales in *E. colorado* distad to the postmedial line are lamellar, whereas great numbers of excelsior scales are present in this area in *E. cirripalea*, *E. schweitzeri*, and *E. sidus*. The male genitalia of *E. colorado*, based on dissections from Franclemont (#7574), the USNM material (#38,903), and J. Donald Lafontaine (#118685), are close to those of both *E. sidus* and *E. schweitzeri*, but with important differences. The coronal spines are stouter and fewer in number (14–17) than in other North American *Eupsilia* (Figs 10, 12, 14). The setal rosette at midlength on the vesica (Fig. 13) is like that of *E. schweitzeri* (Fig. 15) with 25–30 fine spines, and but differs from that of *E. sidus* (Fig. 11), whose vesica bears a run of fewer (ca. 20) stouter setae, and these are drawn out along the vesica, rather than pulled into a single rosette. Nor does *E. colorado* have the six small basal pouches, arranged in two groups, as seen in *E. sidus* and *E. schweitzeri*. In our preparations, the most basal set of pouches are much enlarged and give the basal portion of the vesica considerable girth. We note that the lectotype preparation from the AMNH, made by Smith, is badly damaged and distorted.

***Eupsilia sidus* (Guenée, 1852).** The type of *E. sidus* is missing (Franclemont 1952). Given the difficulty of adult identifications, even with dissection, and the taxonomic confusion within the genus, to promote nomenclatural stability we anchor the concept of the name as understood by Franclemont (1952), Forbes (1954), current on-line resources, and institutional collections, by hereby designating a neotype of *E. sidus* from New Jersey, the presumed type locality. (According to Franclemont (1952) "From what we know about the source of Boisduval's material described by Guenée, it seems safe to assume that the type of *sidus* was collected by Say in New Jersey, given to LeConte and sent by LeConte to Boisduval.") The identity of the neotype (Fig. 5) was confirmed by larval characters, the male was dissected, and a leg was submitted for DNA barcoding. Data on the neotype labels are as follows: New Jersey, Cumberland Co., Millville, Poplar Drive ♀ coll. at bait III.11.2010, Dale F. Schweitzer // bred ex ova on *Prunus serotina* at Windham, CT, by Eric L. Quinter, emerge[d]: IX.26-X.15.2010 // *Eupsila sidus* (Guenée), Det. Eric L. Quinter // CNC # ♂ 17072 // Specimen ID CNCLEP 00116372 // Barcode of Life Project // Leg removed, DNA extracted // Photographed J. D. Gill. The neotype has been deposited at the CNC, Ottawa, Ontario, Canada.

Eupsilia is a difficult taxon given the uniformity of the genitalia across species. Likely the new species would have gone undiscovered until the advent of barcoding had not the larval characters revealed that the name *E. sidus* was being applied to two different species. Given the conservative nature of phenotypic evolution in adult *Eupsilia*, it would not be surprising to learn that still other cryptic species of *Eupsilia* remain to be recognized and described from North America. Barcodes are suggestive that *E. tristigmata* represents more than a single entity and perhaps as many as three (Don Lafontaine pers comm.).

Eupsilia colorado has not been collected since 1895—and awaits rediscovery. Efforts to barcode specimens were unsuccessful. The type locality, Glenwood Springs, is noteworthy for its exceptional complex of moths that occur sparingly or nowhere else on the west slope of the Rockies (Don Lafontaine in litt, Terhune Dickel pers. comm.). Other notable lepidopterans known from the area include *Acronicta exempta* Dyar, 1922, *Heterocampa rufinans* (Dyar, 1921), *Hyparpax venus* Neumoegen, 1892, *Orthosia flaviannula* (Smith, 1899), and *Phoberia ingenua* (Walker, 1858). What is special about the conditions in the vicinity of Glenwood Springs remains a mystery; perhaps they relate to a unique paleoclimatological history, specialized edaphic factors, or unique aspects of its flora. Many of the above are oak feeders, and *Quercus* is a known host for many eastern *Eupsilia* (Wagner et al. 2011). *Eupsilia colorado* flies in March and April, when few moth collectors are afoot in the Rockies and weather conditions are especially challenging for nocturnal collecting. Larval sampling, especially on oak in the spring, might prove to be the easiest means to survey for the moth.

Acknowledgements

John G. Franclemont prepared 82 genitalic dissections of *Eupsilia* that were especially helpful over the course of our effort; his preparations were made available by Jason Dombroskie and Cornell University. Tim McCabe also made his genitalic preparations available for study. Eric Quinter supplied reared material of *E. schweitzeri* and *E. sidus* from which the type material was selected. Eric also guided us to relevant literature, specimens in the AMNH, and supplied considerable unpublished information regarding *Eupsilia*. Dale Schweitzer first alerted DLW to the existence of the new species and supplied some of the life history data reported here. Paul Goldstein (USNM) arranged for the loan of specimens of *Eupsilia* (*Scopelsoma colorado*). David Grimaldi provided access to the AMNH collection and the lectotype of *Scopelsoma colorado*. Don Lafontaine and Jocelyn Gill prepared key genitalic preparations and associated images for our manuscript. Bo Sullivan and Don Lafontaine shared relevant barcode data. Annette Evans and Mark Smith took the high resolution images of the forewing scales used in Figs 17–22. Don Lafontaine and Lawrence Gall made helpful suggestions on the submitted version of the manuscript that significantly improved the text. This study was funded in part by USFS Co-op

Agreement 14-CA-11420004-138 and an award from the Richard P. Garmany Fund (Hartford Foundation) to DLW.

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Submitted for publication 16 October 2015; revised and accepted 14 January 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 249–250

A REMARKABLE ELEVATIONAL RECORD OF *METHONA CONFUSA* BUTLER, 1873 (NYMPHALIDAE) IN A HIGH MONTANE AREA OF SOUTHEASTERN PERU

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ABSTRACT. *Methona confusa* has been recorded across its geographic range from low elevations up to around 2,000 m, being rare above 1,500 m. We report herein a new elevational record of *M. confusa* above 3,500 m, the highest ever reported for ithomiines, from upper montane area of Megantoni National Sanctuary and Manu National Park, located on the eastern slopes of the Andes of southern Peru.

Additional key words: Andes, Ithomiini, hilltopping

Methona Doubleday, 1847 (Lepidoptera: Nymphalidae: Danainae) is a small genus of the tribe Ithomiini including seven species (Lamas 2004), distributed from Panama to northern Argentina, Uruguay, and southern Brazil (Neild 2008).

Methona confusa Butler, 1873, is distributed from Eastern Panama to the Amazon basin, with four weakly differentiated subspecies recognized (Lamas 2004). It is encountered commonly in a variety of forest habitats, from primary premontane cloud forest to lowland secondary growth (Neild 2008, Hill & Tipan 2008). In Peru, this species is widespread and common on the eastern slopes of the Andes below 1,500 m.

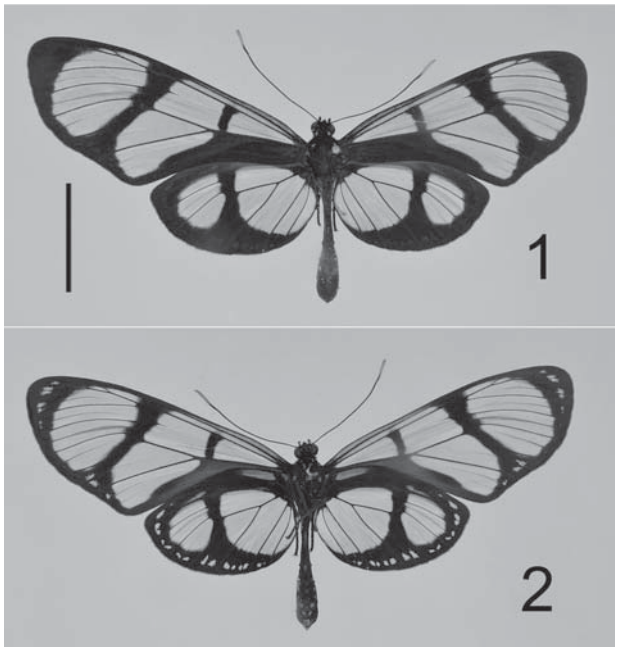
MATERIALS AND METHODS

In September 2012, as a result of a butterfly survey in the upper montane area of the Megantoni National Sanctuary (MNS), on the eastern slopes of the Andes of southern Peru, one female specimen of *M. confusa* (Fig. 1) was collected near the boundary of MNS and the Manu National Park (MNP), Cuzco Department (12°30'25"S, 72°05'20"W), at 3,700 m elevation. The area is an ecotone between open, páramo-like vegetation ('wet puna') and elfin forest. The butterfly was flying at the summit of a small hill, sometimes falling into the ground vegetation and remaining there motionless, but before capture had been cruising "up-and-over" the summit, being blown off the top by strong winds and flying against the wind in approaching summit. In addition, some 200 m downhill, two more individuals of this species were found, heavily damaged and dead on the ground; those individuals may have been killed by the heavy rains falling in the area during the previous days.

Material examined. One female: Peru, Cuzco, Incatambo, 12°30'30"S, 72°05'05"W , 3,700 m, 12–15 September 2012, J. Cerdeña, R. Delgado & E. Huamaní leg. The specimen is deposited in the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM), Lima, Peru.

RESULTS

This is the first Ithomiini species ever reported in a high Andean ecotone between wet puna and elfin forest, as ithomiines normally occur in humid forests from sea level to about 3,000 m (Willmott & Freitas 2006). Indeed, this event would have been less remarkable if a related species, *Methona maxima*



FIGS. 1, 2. Female adult of *Methona confusa* Butler, 1873 collected in September 2012, Cuzco Department, Peru. **1**) Dorsal view; **2**) ventral view. Scale bar 02 cm.

nigerrima (Forbes), which has been recorded up to 2,950 m elevation at sites a few km to the south, in the upper Cosñipata valley (Lamas, unpubl. data), had been found in this particular location. This is also a new elevational record for this group, the highest ever reported for ithomiines.

Discussion

The only reliable hostplant records for species of *Methona* belong to the genus *Brunfelsia* Linnaeus, 1753 (Solanaceae) (Lamas 1973, Plowman 1998, Beccaloni et al. 2008). Five species of *Brunfelsia* (*amazonica* C.V. Morton, 1949, *chiricaspi* Plowman, 1973, *dwyeri* D'Arcy, 1971, *grandiflora* D. Don, 1829, and *pauciflora* (Cham. & Schltdl.) Benth. in DC., 1846) have been reported as larval foodplants for *M. confusa* (Plowman 1998); of them, *B. pauciflora* is almost certainly misidentified as the species is endemic to southeastern Brazil, where *M. confusa* does not occur. Only *B. grandiflora* has been found in the general area where the *M. confusa* specimens discussed herein were recorded, thus it is highly possible that the latter fed as larvae on individuals of that species. Although *B. grandiflora* is often cultivated as an ornamental shrub or small tree (Plowman 1998), there is no evindence of its presence above 2,000 m in southeastern Peru (indeed, no species of *Brunfelsia* have been recorded as occurring above 3,300 m [Plowman 1998]). Furthermore, the area of the MNS where this survey was conducted has no human inhabitants or man-made roads. Therefore, it is reasonable to hypothesize that *M. confusa* has no resident breeding populations in the area surveyed and was not introduced there through human agency.

If the *M. confusa* adults reported here were not part of a resident, breeding population, they may have either been performing long-distance dispersal through unfavorable habitat (the wet puna / elfin forest ecotone) between two separate areas of "normal" habitat (montane forest), or else were exhibiting hilltopping behavior (Shields 1968). At least one species of *Methona* (*singularis* Staudinger, 1884) has been cited as exhibiting summit congregation behavior (Kesselring in Shields 1968), and it may occur in *M. confusa* too. Considering that in the same study site we recorded a skipper (Hesperiidae) specimen which was obviously engaged in hilltopping behavior, and turned out to represent a new country record for Peru (Cerdeña et al. 2014), this highlights the importance of surveying hill summit habitats in order to significantly increase the chances of registering the occurrence of scarce or otherwise elusive butterfly species while performing biodiversity surveys (see also Dolibaina et al. [2012, 2015] and Cerdeña & Farfán [2015] for other remarkable findings made at hill summits).

Acknowledgements

To MBZ Fund Conservation by financing granted to the first author to carry the project titled "High Andean Butterflies of Biosphere Reserve Manu: Diversity, endemism and conservation." Field work was performed under authorization R.J. N° 0010-2012 SERNANP-SNM issued by the Servicio Nacional de Áreas Naturales Protegidas por el Estado (SERNANP), Ministerio del Ambiente, Peru.

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Submitted for publication 19 March 2016; revised and accepted 18 June 2016.

Journal of the Lepidopterists' Society
70(3), 2016, 251–252

ADELPHA COCALA DIDIA (NYMPHALIDAE: LIMENITIDINAE) FEEDING ON *TRIUMFETTA SEMITRILOBA* (MALVACEAE)

Additional key words: Bignoniaceae, butterfly, Papilionoidea, Rhopalocera, Rubiaceae

Adelpha cocala Cramer (Lepidoptera: Nymphalidae: Limenitidinae) has six subspecies (*Adelpha cocala caninia* Fruhstorfer, *Adelpha cocala cocala* Cramer, *Adelpha cocala didia* Fruhstorfer, *Adelpha cocala lorzae* Boisduval, *Adelpha cocala orellanae* Neild, and *Adelpha cocala riola* Fruhstorfer), which are found in Brazil, Bolivia, Colombia, Guatemala, Peru, Suriname, Venezuela, and from Honduras to Panama (Fruhstorfer 1915, Willmott 2003b). The wingspan of these subspecies is around 50 mm (Willmott 2003a). Its larvae feed on *Alseis* spp., *Calycophyllum* spp., *Chimarris* spp., *Chomelia* spp., *Genipa* spp., *Pentagonia* spp., *Psychotria* spp., *Sabicea* spp., *Uncaria* spp., and *Warszewiczia* spp. (Rubiaceae), and *Malania* spp. (Olecaceae) (Beccaloni et al. 2008). *Adelpha cocala didia* occurs in the Brazilian states of Bahia, Espírito Santo, Mato Grosso, Minas Gerais, Santa Catarina, Rio de Janeiro, and São Paulo and in Brasília (Federal District) (Fruhstorfer 1915, Willmott 2003b). The aim of this study was to report a new host plant of *A. cocala didia* in the state of Minas Gerais, Brazil.

Eighty-two first-instar larvae were found defoliating leaves of 18 individuals of *Triumfetta semitriloba* Jacq. (Malvaceae) (around 45 cm in height) on May 2014, in secondary forest, in Viçosa, state of Minas Gerais, Brazil (20º 45' S × 42º 50' W and 655 m above sea level). Some branches were detached from these plants with the larvae and transferred to rearing cages (30 cm width × 30 cm length × 30 cm height) in the Laboratory for Biological Control of Insects (LCBI) of the Federal University of Viçosa (UFV) in Viçosa at 25 ± 1 °C, 12:12 (L:D) h photoperiod and 70 ± 10% R.H., where they remained until adults' emergence. Some females obtained from the collected larvae were sent to the Department of Zoology of the Federal University of Paraná (UFPR) in Curitiba, state of Paraná, Brazil, for identification by O.H.H. Mielke. They have been deposited in the entomological collection of the UFPR.

The number of pupae obtained from the collected larvae and of males and females from these pupae were evaluated. The pupa viability (number of obtained pupae ÷ number of collected larvae) × 100, adult viability (number of obtained adults ÷ number of obtained pupae) × 100, and sex ratio [number of females ÷ (number of females + number of males)] were calculated.

Seventy-two adults emerged from the 78 obtained pupae—44 females and 28 males (sex ratio= 0.61, pupa viability= 95.12% and adult viability= 92.31%). No parasitoids were observed emerging from the larvae or pupae.

This record adds a new plant genus as host of *Adelpha*. This addition is not surprising because *Adelpha* is diverse and widely distributed across North and South America (Aiello 1984, Willmott 2003b). Previously, this subspecies had been reported in the states of Bahia, Espírito Santo, Mato Grosso, Santa Catarina, Rio de Janeiro, and São Paulo, and in Brasília (Federal District) at 1.344, 402, 1.804, 1.340, 355, 645, and 946 km away from Viçosa, respectively. Larvae of *Adelpha* feed on around 116 species of 42 genera of 22 botanical families (DeVries 1987, Brown Jr. 1992, Diniz & Moraes 1997, Freitas et al. 2001, Freitas 2006). *Adelpha cocala* is known to feed on 11 plant genera in Brazil and other parts of South America (Beccaloni et al. 2008). *Adelpha* was found feeding on Caprifoliaceae Juss. (Dipsacales) (Willmott 2003a) and on Rubiaceae Juss. (Gentianales) (Mullen et al. 2011). In Brazil, *Adelpha malea goyama* Schaus feeds on *Arrabidaea mutabilis* Bur. and K. Schum. (Lamiales: Bignoniaceae) in the state of Paraná (Freitas 2006). *Triumfetta semitriloba* is host of *A. cocala didia* in the Brazilian state of Minas Gerais.

ACKNOWLEDGMENTS

To Dr. Marília Cristina Duarte (Universidade de Mogi das Cruzes, Mogi das Cruzes, São Paulo State, Brazil) for identifying the plant and Dr. Olaf Hermann Hendrik Mielke for identifying the butterfly. To "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)", "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)", and "Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)" for financial support.

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Submitted for publication 4 December 2015; revised and accepted 19 March 2016.

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