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Authors: Koi, Sandy, and Daniels, Jaret

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New and revised life history of the Florida hairstreak *Eumaeus atala* (Lepidoptera: Lycaenidae) with notes on its current conservation status

Sandy Koi^{1*} and Jaret Daniels^{2,3}

Abstract

Southeast Florida is considered part of the Caribbean archipelago and a biodiversity hotspot for conservation priorities, with many endangered species precinctive to the Lower Peninsula. The tropical butterfly *Eumaeus atala* (Poey) (Lepidoptera: Lycaenidae) is currently found in Southeast Florida, the Caribbean, Cayman Islands, and Cuba, but was once considered probably extinct in Southeast Florida, where it has made a significant population increase during the past 30 yr. The State of Florida lists *E. atala* as imperiled, based on the species' ephemeral and cyclic abundance, isolated extant populations, reduced and highly fragmented habitat, and vulnerability to stochastic weather events. The objective of this captive-rearing intensive study was to fill gaps related to *E. atala*'s overall biology and ecology in order to assist recovery efforts. Life history was studied under controlled environmental conditions to document the life span, reproduction, and development. Longevity proved to be much greater than previously recorded, reproductive behavior supports increased genetic cross-over, and conservation efforts point toward the need for continued monitoring of fragmented populations. Results may help in the design or improvement of management practices for *E. atala* and other imperiled pine rockland species.

Key Words: butterfly; tropical insect conservation; pine rockland

Resumen

Se considera el sureste de la Florida como parte del archipiélago del Caribe y un *hotspot* (punto clave, gran riqueza de especies) de biodiversidad para las prioridades de conservación, con muchas especies endémicas en peligro de extinción en la parte baja de la península de la Florida. Actualmente, se encuentra la mariposa tropical *Eumaeus atala* (Poey) (Lepidoptera: Lycaenidae) en el sureste de la Florida, el Caribe, las Islas Caimán y Cuba, pero fue una vez considerado probablemente extinta en el sudeste de Florida, donde su población ha incrementado significativamente durante los pasados 30 años. El estado de Florida enumera *E. atala* como una especie en peligro, sobre la base de la abundancia efímera y cíclica de la especie, las poblaciones existentes aisladas, su hábitat reducido y muy fragmentado, y la vulnerabilidad a los eventos meteorológicos estocásticos. El objetivo de este estudio intensivo de criarlas en cautividad fue para llenar los vacíos de datos relacionados con la biología y la ecología general de *E. atala* con el fin de ayudar a los esfuerzos de recuperación. Su historia de vida fue estudiado bajo condiciones ambientales controladas para documentar la duración de vida, la reproducción y el desarrollo. La longevidad resultó ser mucho mayor de lo que fue registrado anteriormente, el comportamiento reproductivo soporta mayores esfuerzos de cruce y de conservación genética apuntan hacia la necesidad de un seguimiento continuo de las poblaciones fragmentadas. Los resultados pueden ayudar en el diseño o mejoramiento de las prácticas de manejo para *E. atala* y otras especies de las áreas aisladas de pinos en terreno rocosos en peligro.

Palabras Clave: mariposa; conservación de insectos tropicales; pino rockland; pinos en terreno rocosos

Lycaenids constitute 30 to 40% of all butterfly taxa and are endemic to often highly specialized and at-risk environments (New 1993; Minno 2012). The hairstreak *Eumaeus atala* (Poey) (Lepidoptera: Lycaenidae) is one such insect. Known hereinafter as the Atala butterfly, it was once called "the most conspicuous insect in semitropical Florida" (Schwartz 1888) but had become rare, perhaps extinct, by the mid-1930s (Klots 1951; Smith et al. 1994). As the anthropomorphic impact in South Florida increased, the butterfly's fragile pine rockland habitat was depleted (Snyder et al. 1990), and its sole host plant, coontie, *Zamia integrifolia* L. f. (= *pumila*) (Cycadales: Zamiaceae), was nearly eliminated from Florida. Coontie is North America's only native cycad, which was overharvested for starch production in the early years of European settlement. It was listed as commercially exploited as recently as 2000 (Coile

2000) and is still considered "Near Threatened" by the International Union for Conservation of Nature and Natural Resources (Stevenson 2012). The Atala occurred sporadically until the mid-1960s (Smith et al. 1994; Cech & Tudor 2005) and was finally deemed extirpated, until a small population was discovered on a barrier island in Miami-Dade County in 1979. As far as is currently known, all extant colonies originated from that population.

Numerous attempts at reintroducing the butterfly have met with varying levels of success (Rawson 1961; Franz 1982; Deyrup & Franz 1994; S. K., unpublished data). Extant Atala populations are monitored by the Florida Natural Areas Inventory (FNAI 2012), the Imperiled Butterfly Working Group (IBWG 2012), which is an assemblage of concerned agencies and organizations, and by the first author. Ongoing

¹9173 SW 72 Ave., M-5, Miami, Florida 33156, USA

²University of Florida, Entomology & Nematology Department, Gainesville, Florida 32611, USA

³Florida Museum of Natural History, McGuire Center for Lepidoptera and Biodiversity, Gainesville, Florida 32611, USA

*Corresponding author; E-mail: sandykoi2009@gmail.com

threats to the Atala include habitat loss and fragmentation, pesticide use, and Florida's stochastic weather events (i.e., hurricanes, tropical storms, flooding, salt-water intrusion, etc.) that could further isolate and splinter colonies. Relocation projects have been developed by the first author in conjunction with Miami-Dade County authorities and other non-governmental agencies to restore Atala colonies in historically occupied pine rockland reserves and in domestic gardens.

Historically, *E. atala* occurred within pine rocklands and tropical hardwood hammock edges of Miami-Dade and northern Monroe Counties. Most of these habitats have been lost to urban and agricultural development. The Atala and its host plant occurred along waterways as well as in the pine rocklands that once flourished along Southeast Florida's Atlantic Coastal Ridge (Land & Cooley undated; Miami-Dade County undated; Smith et al. 1994; Minno et al. 2005). The Atala is a specialist subtropical butterfly with specific floral nectar requirements (Koi 2008) and specialized host plant requirements. It is currently found in small colonies as far north as Martin County, introduced by individuals with little or no scientific reason for doing so; however, the butterfly is found in scattered self-established or reintroduced colonies from Palm Beach to Broward and Miami-Dade Counties (S. K., unpublished data). There has been discussion among scientists both in favor of and in opposition to introducing Atala colonies outside of its historical range. It may prove beneficial to extend its range in the event of a devastating weather event and limited remaining habitat; on the other hand, it may be detrimental to local nurseries that raise and sell cycads, botanical gardens, homeowner landscaping, and local biota. These colonies appear to be part of a larger metapopulation. Individual colonies or subpopulations are highly ephemeral as seasonal or abnormal weather events occur. Overall population dynamics demonstrate periodic and somewhat unpredictable crash-eruption cycles.

Southeast Florida is part of the Caribbean Archipelago and is a hotspot for biodiversity conservation priorities (Myers et al. 2000). A number of South Florida's imperiled taxa are restricted to pine rocklands (Austin undated). According to the Florida Natural Areas Inventory, an ongoing survey of natural ecosystems and biota in Florida, pine rocklands are themselves critically imperiled both in Florida and globally. Hundreds of mammals, plants, and invertebrates endemic to Florida pine rocklands are continually tracked by FNAI, including the Florida panther (*Puma concolor coryi* [Bangs]; Carnivora: Felidae), gopher tortoise (*Gopherus polyphemus* [Daudin]; Testudines: Testudinidae), "Dade County slash pine" (*Pinus elliottii* Engelman var. 'densa'; Pinales: Pinaceae), and shrubs such as *Tetrazygia bicolor* (Miller) Cogniaux (Myrtales: Melastomataceae) or *Acacia pinetorum* F. J. Hermann (Fabales: Fabaceae) that are important food sources for Lepidoptera and other insects. Twenty-seven species of butterflies are currently tracked in South Florida, eight of which occur primarily or exclusively in Miami-Dade County's pine rocklands. Of those species, several are endemic host plant specialists, including the federally endangered Bartram's scrub hairstreak, *Strymon acis bartrami* (Comstock & Huntington) (Lepidoptera: Lycaenidae) and Florida leafwing, *Anaea troglodyta floridalis*, Johnson & Comstock (Lepidoptera: Nymphalidae), both of which are specialists that feed on an increasingly rare host (i.e., pine-land croton, *Croton linearis* Jacquin; Malpighiales: Euphorbiaceae) (Austin undated; Deyrup & Franz 1994; Smith et al. 1994; Worth et al. 1996; Salvato 2003; Salvato & Hennessy 2004; Schweitzer et al. 2011). Of 186 species listed in the "Multi-Species Recovery Plan" by the Florida Fish and Wildlife Conservation Commission in 1977, 30 were located solely in the pine rockland community (Florida Fish and Wildlife Conservation Commission 2005).

Recently published data indicate an alarming decline in these and other pine rockland species (Deyrup & Franz 1994; Worth et al 1996; Schwartz et al. 1996; Schultz et al. 2008; Salvato & Salvato 2010a,b;

Minno 2010; IBWG 2011; Schweitzer et al. 2011). Less than 2% of the original pine rocklands outside of Everglades National Park remain (Miami-Dade County undated; Snyder et al. 1990; U.S. Fish and Wildlife Service 2014), but the Atala also has made use of domestic and botanical gardens that support ornamental landscape plantings of *Zamia*; 100 to 300 ephemeral Atala colonies may exist in natural areas and on private property during any given year (S. K., unpublished data).

There have been several attempts to reintroduce *E. atala* within its historical range, but the reason for failure to establish breeding colonies is unknown (Rawson 1961; Deyrup & Franz 1994; Koi 2013). Although much has been published on *E. atala* (Schwartz 1888; Healy 1910; Dethier 1941; Rawson 1961; Franz 1982; Gerberg & Arnette 1989; Deyrup & Franz 1994; Culbert 1995, 2010; Hall & Butler 2000; Smith 2000, 2002), detailed information on this species' ecology is lacking. Ecological data on survival rates, seasonal population fluctuations, reintroduction, or translocations are needed to assist land managers in conservation of *E. atala*.

The objectives of this study were to document previously unknown parameters of the life history of the Atala butterfly, to clarify poorly understood behavioral factors, and to use these data to help support current conservation and management efforts, increasing the success of reintroduction programs.

Materials and Methods

ORGANISM BREEDING: LIVESTOCK AND PROGENY

A captive breeding population of Atala butterflies was maintained under controlled laboratory conditions in Gainesville, Florida, USA (29° 39' 05" N, 82° 19' 29" W). Comparisons were made with wild colonies previously studied *in situ* by the first author. Atala larvae and pupae were collected, in development stages ranging from neonate larvae to pharate adults (forming adults, not yet eclosed from the pupa), from wild colonies in Southeast Florida according to available stock in those locations. Collection sites varied and were located on publicly inaccessible, sensitive, privately owned properties. Because the Atala is multi-voltine, it was possible to collect approximately 200 individuals (larvae and pupae) 4 times during the 15 mo study (800 individuals in total). The study began in Dec 2011 with wild larval stock from Miami-Dade County, and the colony was replenished with wild larval and pupal stock from 3 different sites located in Miami-Dade, Broward, and Palm Beach Counties in May 2012. Additional larval and pupal stock was collected from a single large population in Palm Beach in Jul 2012, and a final collection of larvae and pupae took place at 3 different locations, again in Miami-Dade, Broward, and Palm Beach Counties, in Oct 2012. Neither eggs nor adults were collected at any time.

The captive breeding population was maintained under controlled laboratory setting with ambient temperature ranging from 24 to 26 °C and relative humidity ranging from 25 to 42%, depending on seasonal weather variation recorded by a Lascar EL-USB-2 data logger (Lascar Electronics, Inc., Erie, Pennsylvania, USA). Insects were kept in a photoperiod of approximately 8:16 h L:D that varied occasionally to 10:14 h L:D. Daylight lamps were programmed for 1:2 h on-to-off increments during the regular day period, and a fan was installed to carry plant and insect chemical signaling volatiles to improve mating and ovipositing activity.

Larvae were fed *Z. integrifolia* leaves *ad libitum* harvested from mature landscape plants located on the campus of the University of Florida (Gainesville, Florida, USA), free of pesticides, or from potted nursery stock maintained in a large outdoor screen house or small glass greenhouse, depending on seasonal weather conditions and the avail-

ability of plant growth. Fresh leaf material was cut bi-weekly, and the stems of the individual leaves were stored in deionized water. Fronds were then placed into a floral aquapic (Aquapic, Syndicate Sales, Kokomo, Indiana, USA) to ensure that fresh leaf material was presented to the larvae for consumption.

Larvae were maintained in open plastic containers measuring approximately 25 × 50 × 10 cm until they entered into the wandering stage, wherein the larvae begin searching for a pupation site, at approximately 10 d of age. At that point, the larvae and boxes were maintained in 61 × 61 × 30.5 cm fabric cages (Castle Cage Size JUMBO, LiveMonarch Foundation, Boca Raton, Florida, USA). Larvae are gregarious feeders (Minno et al. 2005) and were maintained with their brood-mates throughout the life cycle until adult eclosion.

Cages were cleaned and fresh *Zamia* leaves were presented to the larvae every morning. Food was replenished throughout the day if necessary. Plant material was presented to the larvae as appropriate for the larval stage; i.e., fresh new growth was given to neonates and older leaves to older larvae.

Larvae were allowed to complete development and pupate within the screen fabric cages. Pupae were misted periodically with deionized water to help prevent desiccation. Following eclosion, all adult butterflies were transferred to a fabric flight cage of approximately 182 cm² floor area (Greenhouse Castle Cage, Live Monarch Foundation, Boca Raton, Florida, USA) to facilitate courtship and oviposition. Cut *Zamia* fronds were suspended from the ceiling in aquapics for oviposition. Four lamps with 160 Watt bulbs were located at one end of the cage programmed to emit extra lighting on a 1:2 h on-to-off cycle during the day.

Adults were provided with deionized water, to avoid possible chemicals in tap water, and with Gatorade® (The Gatorade Company, Chicago, Illinois, USA) in fruit punch, orange, or watermelon–citrus flavors. The fluids were offered ad libitum from inverted centrifuge tube feeders, modified from the design described by Hughes et al. (1993). Water was also continually provided via a length of dental packing cord (TIDI Products, Neena, Wisconsin, USA) inserted into a 20 mL flask partially sealed with laboratory parafilm and filled with deionized water. Feeders were hung from the ceiling in the same manner as the oviposition leaves, as well as installed on a short “table” for easier accessibility from the floor where older adults of both sexes often congregated during the night. In addition, occasional seasonal native flowers (primarily *Bidens alba* [L.] de Candolle; Asterales: Asteraceae) were provided.

All feeders were replenished daily and cleaned weekly. Cotton balls soaked with deionized water were placed on the ceiling of the fabric cage, and the entire cage was misted twice daily with deionized water from a regular spray bottle. The flight cage was cleaned monthly for disease prevention.

Newly eclosed adults were identified with unique alphanumeric codes using a silver metallic Sharpie® brand permanent paint marker (Newell Rubbermaid Office Products, Oak Brook, Illinois, USA) on both ventral hindwings to allow immediate recognition of individuals. Biological data recorded included sex, dates of ovipositing, larval eclosion, pupal emergence, and day of death. Observed matings, number of ovipositions, and associated behaviors were documented, as were fecundity and fertility. Samples of wing cord length were measured from the basal sclerite and resilin patch of the left forewing attachment to the thorax in a straight line to the apex of the wing (Fig. 1). Male dorsal forewing colors were recorded. Females did not display strong variations in dorsal wing color, although there was a marked difference in the extent of color present. All individual adults observed in both captive and wild colonies displayed unique variations in the described and species-recognized patterns on the ventral wing surface (Fig. 2).

Because adult females were not isolated from one another, egg clutches were not identified per individual females. The adult *Atala*



Fig. 1. Wing cord length on adult *Atala* butterflies was measured from the ventral basal sclerite on the left forewing to the apex of the same forewing using a clear metric ruler.

shows highly aggregated behavior in wild colonies, and females have been observed simultaneously ovipositing on the same leaflet as others (per. obs.). With only 1 or 2 coontie leaves in the flight cage, the females gathered and oviposited together on the leaves without any observed stress or aggression. This is normal behavior in a site with limited host plant availability (S. K., unpublished data). However, because individuals were marked, it was known in many cases which female laid eggs and which male mated with her. Marking also allowed documentation of the time passed between mating and ovipositing and of mating behaviors.

IMMATURE MEASUREMENTS

Eggs were collected from the adult flight cage daily in the afternoon and counted. Eggs were measured with a standard digital microcaliper under a Leica S8APO stereo microscope (Leica Microsystems, Inc., Buffalo Grove, Illinois, USA) while still on the leaf to prevent accidental damage to the egg while measurements were taken.

Larval exuviae were collected and photographed. Measurements of larvae were abandoned soon after recording was started as it was apparent that the size of the larvae did not necessarily correspond to its age and therefore the measurements could not determine age. Weight of larvae was abandoned for the same reasons; however, some measurements and weights were taken for specific parameters and results are discussed below.

Pupal development was categorized from pre-pupal stage to pharate adult. Development time and size were recorded for each life stage, and individuals were followed from egg and first hatch as a larva until time of death. Pupal weights were recorded for variously aged pupae.

ADULT MEASUREMENTS

Adult wing cord length measurement was taken on the left forewing, from the bottom of the basal sclerite and proximal point of resilin where the forewing emerges from the thorax, to the tip of the apex of the forewing in a straight-line measurement to the nearest whole millimeter using a transparent ruler (C-Thru® Ruler Company, Bloomfield, Connecticut, USA). Measurements of newly eclosed 1st generation

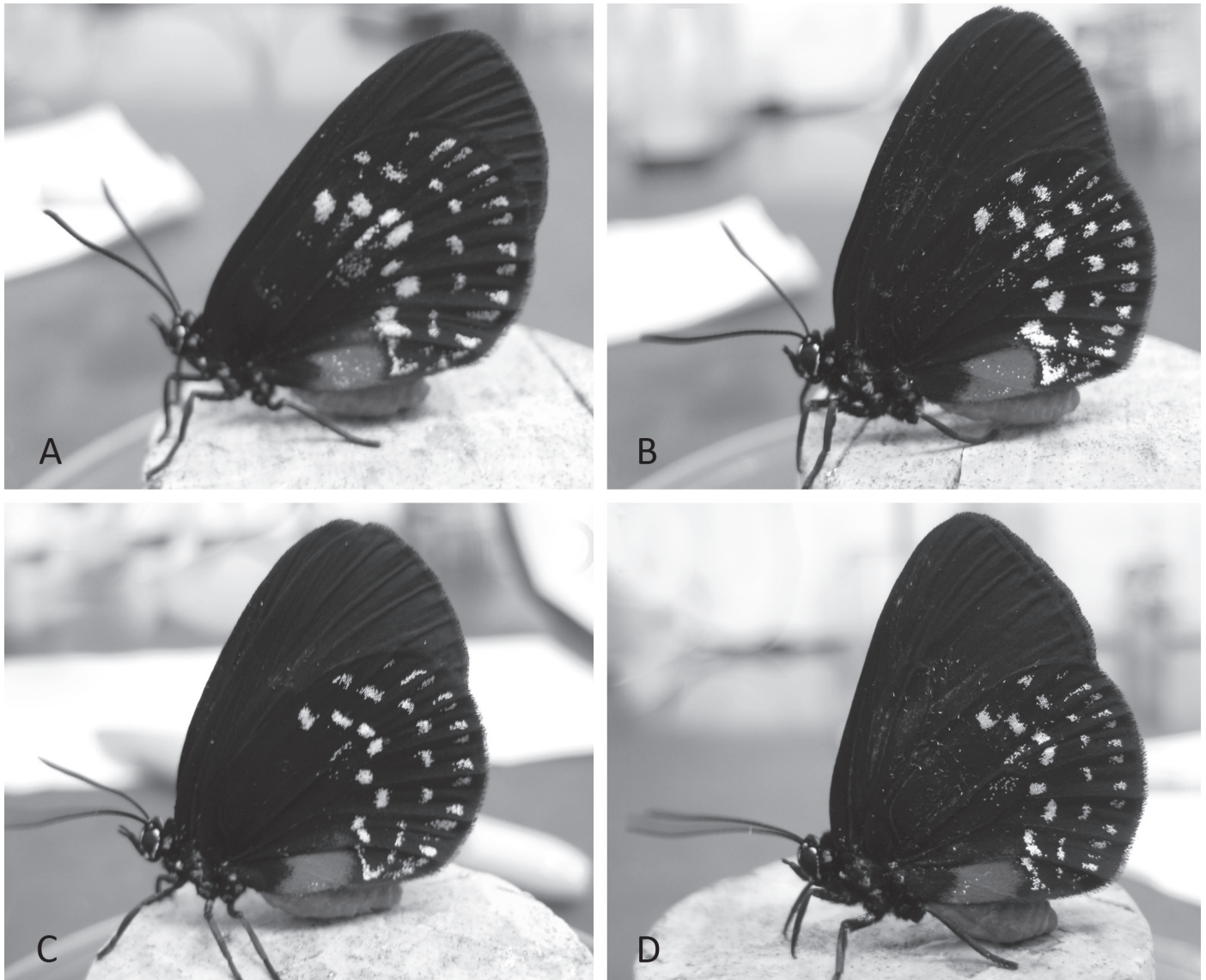


Fig. 2. Individual variations in species-recognized ventral wing patterns were distinct in individuals, but not sexually dimorphic. Four examples of newly emerged adults are presented here. (A and B) Females. (C and D) Males. Note the “sprinkled” iridescence on the anal edge of the hindwing of female B and on the entire wing surface of male D. This iridescence was a frequent observation in both sexes. The forelegs, thorax, and head often had varying degrees of iridescent patterns as well.

adults from wild larval stock that pupated upon arrival were measured for comparison with the captive colony.

Five unmated newly emerged females were euthanized immediately after eclosion to document ovarioles and oogenesis and were dissected to determine average number of ovarioles and oocytes present at eclosion. Subsequent oogenesis, chorion development, egg development and production were determined by counting the numbers of mature and immature eggs in the reproductive tract at death ($n = 141$). The number of eggs remaining in the oviduct was counted at death. In addition to documenting mating behavior, we recorded the age of each individual at mating, the age at which oviposition commenced, the longevity of known mated individuals and unmated individuals, and whether single or multiple matings occurred between individuals.

STATISTICAL ANALYSES

The total number of adults reared and used as breeding stock in the laboratory included over 5,000 individuals. Statistical analysis examined adult longevity; egg size and egg development; adult, larval,

and pupal size range; egg, larval, and pupal maturation rates; fertility and fecundity; sex ratio; and brood size and mortality using R version 2.15.3 (2013-03-01) (R Core Team 2013). Analyses were conducted on varying sample sizes pulled from the total laboratory population based on parameters discussed in the text. For example, of 750 individuals measured for wing cord length, only measurements made by the first author and only measurements associated with adults for which there was a complete life history were used in the analysis. Mean values were presented with the standard deviation (SD).

Results

OOGENESIS, OOCYTE DEVELOPMENT, OVIPOSITION, AND EGG PRODUCTION

New information about female reproduction was discovered. Oocytes were fully formed when adult females eclosed from the pupa,

and oogenesis was continual throughout the lifespan of the female. Females were capable of initiating oviposition by the 3rd day after emergence, when chorions were fully formed around the oocytes, and laid as few as 1 egg up to a cluster of as many as 50 or more eggs, generally using the side of a fiddlehead or the underside of a newly emerged *Zamia* leaf. However, we observed in the field that females also would lay eggs on older leaves, adaxial leaf surfaces, stems, and even reproductive cones of the host plant when host plant availability was limited. Occasionally, females in the captive population laid eggs on non-host substrates such as cage walls, and we had observed newly eclosed females using non-host plants in wild colonies twice (the native host plant was located directly underneath in both cases). Eggs laid on non-host substrates were often fertile, and hatched larvae survived to adulthood within normal development parameters whether reared alone or with other larvae, or reared on the native host or experimentally on non-native *Zamia vazquezii* D. W. Stev., Sabato, A. Moretti & De Luca (Cycadales: Zamiaceae).

Ovipositing occurred on the same day as mating, or within a day afterwards, and occurred before or without mating. The average age of 1st-time female oviposition in the captive colony was 19.36 ± 7.85 d ($n = 234$) (Table 1).

Eggs were chosen haphazardly for measurement from fronds suspended in the adult flight cage, from 5 different broods over a 2 mo period to assure a robust sample size ($n = 600$). Eggs ranged in size from 0.75 to 1.25 mm (mean 1.00 ± 0.15 mm) and showed a bimodal distribution, with the modes at 0.99 and 1.11 mm (Fig. 3). We observed a marked visual difference in the size of eggs from individual dissected females, indicating that size variability is an inherent part of their biology (Fig. 4). Egg development time varied greatly, ranging from 4 to 13 d in the captive population, with an average development time per egg of 6.6 ± 1.1 d ($n = 10,582$). There was a 36% successful hatch rate from eggs in the captive population ($n = 3,809$ larvae) (Table 2).

Eggs had a 3-leaved micropyle and highly sculptured chorion surface, typical of Lycaenidae (Fig. 4). We noted that the underside of the egg was smooth and flat and adhered to the leaf surface with proteins exuded by the females' accessory glands. The undersurface was clear, allowing yolk or the embryo to be seen when the egg was removed from the substrate and inverted upside down. Larvae chew through the micropyle area and did not eat the shell, even avoiding it entirely when neonates congregated to scrape the surface of the leaves en masse with their mandibles during the 1st instar; older larvae also avoided eggs by eating around them.

LARVAL AND PRE-PUPAL DEVELOPMENT

Larval development time was highly variable, ranging from 14 to 28 d in the laboratory ($n = 1,021$). The average development time was 18.4 ± 3.6 d after emergence from the egg (Table 2). Neonate larvae were capable of releasing silk lifelines to aid in locomotion, such as descending from a higher leaflet to another, or to adhere to a leaf.

Larval broods were reared with siblings; individuals were reared alone when they had hatched from only 1 ovum in a clutch. Five newly hatched larvae that were weighed within 2 h of hatching and reared on *Z. integrifolia* had an average weight of 7.2 ± 0.9 mg, ranging between

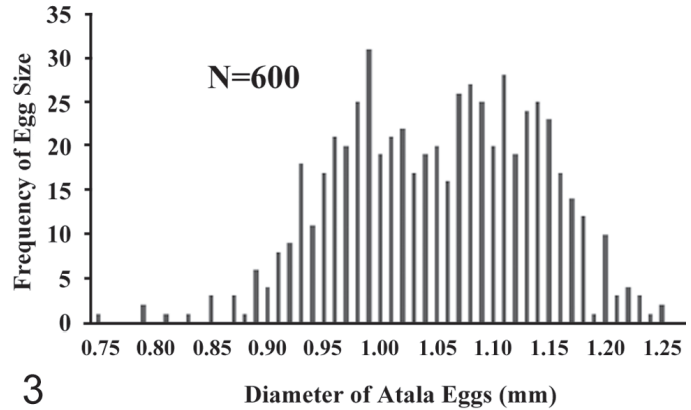


Fig. 3. Frequency and mean size of Atala egg distribution ($n = 600$).

0.4 and 2.5 mg. By day 5, they had reached an average weight of 385.3 ± 34.7 mg with a range between 7.6 and 85.8 mg. From day 5 to day 11, larvae increased their weight to 537.4 ± 95.0 mg, ranging between 470.2 and 604.6 mg. Larvae showed marked differences in feeding robustness.

Newly hatched larvae measured between 0.5 and 15 mm, but after the 1st molt it became difficult to standardize a measurement protocol

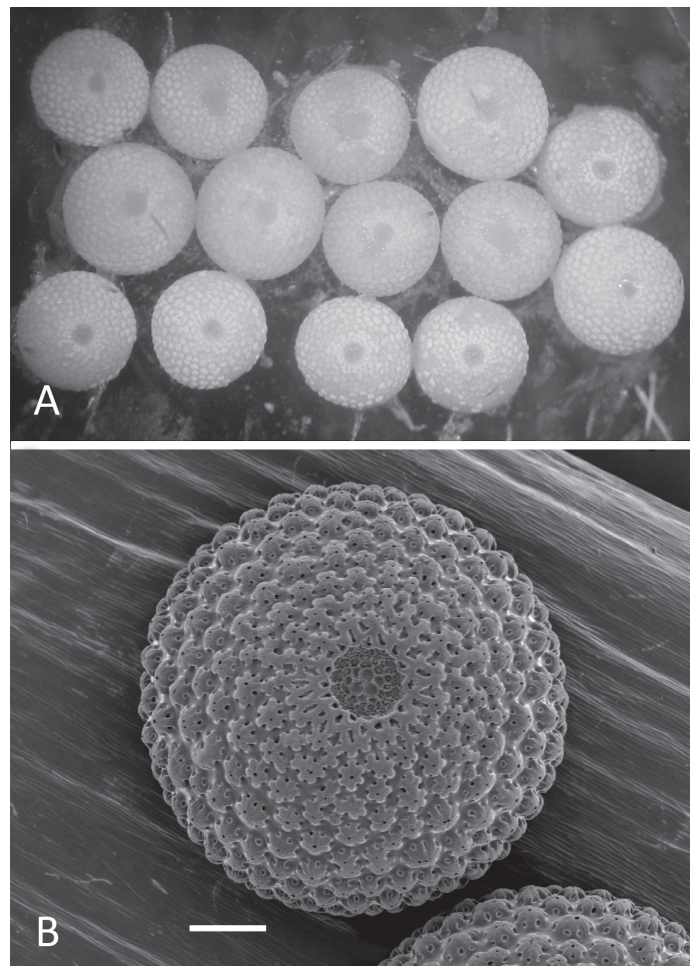


Fig. 4. (A) Visually apparent size differences in eggs from a dissected female. (B) Scanning electron microscopy photograph of an Atala egg showing highly sculptured chorion ultrastructure and micropyle. Bar measures 200 μ m.

Table 1. Mean \pm SD lifespan (d) and mating and ovipositing ages (d) of individuals after eclosion from the egg.

Parameter	Female ($n = 117$)	Male ($n = 117$)	t-test
Lifespan (mated individuals)	32.96 ± 8.83	34.38 ± 11.03	$P = 0.99$
Mating age	15.45 ± 8.94	11.98 ± 7.63	$P = 0.38$
Ovipositing age	19.36 ± 7.85	not applicable	not applicable

Table 2. *Atala* development time and survival from ovipositing to adult emergence ($n = 10,582$).

Parameter	Eggs	Larvae	Pupae	Egg to adult
Range (d)	4–13	14–28	11–19	30–57
Average development time \pm SD (d)	6.6 \pm 1.1	18.4 \pm 3.6	14.4 \pm 2.4	39.3 \pm 3.7
Survival from eggs (n)	10,582	3,809	2,209	2,077
Survival from one stage to the next	not applicable	36%	58%	94%

partly because of the high variability in the growth and weight of the larvae regardless of age, and the extreme plasticity of the larval body, so the endeavor was abandoned after the 1st instar (Fig 5A). Larger larvae (1.0–1.5 mm) fed more aggressively and developed more rapidly than the smaller larvae.

This report is the first record of larval instars in the *Atala*, which present a 3 d, 5 d, 7 d, 9 d, and sometimes 11 d molt (Fig. 5C). Being variable in almost every stage of development, occasionally there was a 4 d, 6 d, 8 d, or 10 d molt evident in a clutch, likely because the individual was not as robust a feeder, and perhaps started out smaller than the other larvae in the brood and therefore developed more slowly than its cage mates (Fig. 5B). Exuviae were not ingested by larvae in any instar and were avoided in much the same way as were the eggs and egg chorions.

Additionally, although the larvae were fed *ad libitum*, they often also completely devoured the rachis of their host plants as well as the foliage. Occasionally, the larvae would ingest parts of the silicone caps on the waterpics used to hold the host material, and would cannibalize conspecifics, which dropped the survival rate to as low as 14% in some broods. In several instances, a brood that started with small numbers could be reduced to a single larva by the time it entered the wandering stage if one of the larvae was a particularly aggressive feeder.

The wandering stage of development occurred at approximately day 10 of larval life. By day 12 or 13, most larvae became “pre-pupae” in preparation for the final molt (Fig. 6A and B). Larvae would migrate en masse to the pupation site within the fabric cage, which could be the walls of the shoebox housing or the walls or ceiling of the fabric cage. One or two larvae in the brood would rarely migrate away from cage mates to pupate alone; larvae that were slower in development usually migrated to a pupation site previously established by other larvae.

Pre-pupae formed large areas of silk mats underneath their aggregation, anchoring the cluster to the substrate and to each other (Fig. 5D). Larvae that were under stress from food limitations due to aggressively feeding brood mates, handling (such as being moved from wild stock), larval competition for space, type of food, or other unknown factors were capable of pupating after the 3rd instar, forming exceptionally small pupae (see below). These individuals survived to adulthood and were often successful in mating, particularly the small males (those with wing cord lengths <2.0 cm). The lifespan of these small individuals was not adversely affected by size or sex. The average lifespan of females with wing cord lengths measuring under 2.0 cm was 19.06 \pm 12.17 d ($n = 32$). The average lifespan of males with wing cord lengths measuring under 2.0 cm was 23.03 \pm 12.62 d ($n = 32$), and the average lifespan of individuals with wing cord lengths measuring under 2.0 cm was 21.05 \pm 12.64 d ($n = 64$).

Juvenile mortality was high in the larval stage, and cannibalism was substantial in broods even when continually provided with high-quality, green, fresh host material. Cannibalism is common in otherwise herbivorous lepidopteran species (Richardson et al. 2010). There was an increase in survival to pupation in smaller groups, but it was not found to be as significant with *Atala* as it has been documented in other species, such as *Euselasia chrysippe* (Bates) (Lepidoptera: Lycae-

nidae) (Allen 2011). Of 90 broods, the number surviving to the next life stage increased as the life stage progressed from egg to larva to pupa and finally to adult (Table 2 and Fig. 7). The survival rate from larva to pupa in a sample of the captive population was 58% ($n = 3,809$; Table 2).

PUPAL DEVELOPMENT

Pupal development time was recorded as the time of the final larval molt to eclosion as an adult and ranged from 11 to 19 d ($n = 1,021$). The average development time was 14.4 \pm 2.4 d (Table 2). There was a substantial difference in size and weight of pupae, but the results were similar to individuals from wild colonies, which also had smaller than normal pupae under stress (Fig. 8A). Pupae were weighed as available and whenever time and equipment use permitted.

Pupae were sexed under a dissecting scope. Females had 2 small bulges on the last abdominal section and a pointed apex at the anal opening. On males, the last abdominal section and the anal opening were rounded. Weights of male and female pupae indicated that even though female pupae weighed more than male pupae (Table 3), there was no significant difference in development time ($t = 1.3659$, $df = 48$, $P = 0.1783$).

Pre-pupal stages occurred over several days, beginning between 12 and 14 d of larval development and usually in the 4th instar. In the 1st stage, the larvae “bunched up” in a vermiform position and anchored firmly to the substrate, although they were still capable of re-locating before final stages began. In the 2nd stage, the last larval integument began to separate from the pharate adult forming internally and the skin had a semi-translucent and mottled appearance. The body was firmly anchored to the substrate, the silk girdle was released from the thorax to support the body, and by the 2nd or 3rd day, the larva made the complete transformation to a pupa. The head and caudal region turned a soft yellow color in the newly formed pupae but soon changed to a darker mottled brown. Pupation took place within 1 or 2 d after the pre-pupal stage, indicated by the shedding of the last larval skin with associated mouth parts and exuviae. Subsequent experiments showed that cooler temperatures caused darker pigmentation in the pupa (S. K., unpublished data).

There was 91 to 98% survival from pupa to adult in the laboratory ($n = 2,077$) (Table 2). Pupal size and weight varied significantly, but there was no correlation between sex and longevity of the resulting adults. Small pupae eclosed as small adults of both sexes, with wing cord lengths ranging from 1.4 to 1.9 cm. Small adults with wing cord lengths measuring less than 2.0 cm of both sexes lived on average as long as adults that had an average wing cord lengths of 2.1 cm or more ($n = 32$). Small males often successfully mated with larger females.

TOTAL DEVELOPMENT TIME

Total development time from egg to adult ranged from 30 to 57 d ($n = 1,261$). Average total development time was 39.3 \pm 3.7 d. Adults successfully eclosed from 94% of pupae in the captive population (Table 2). Development time for all life stages varied even

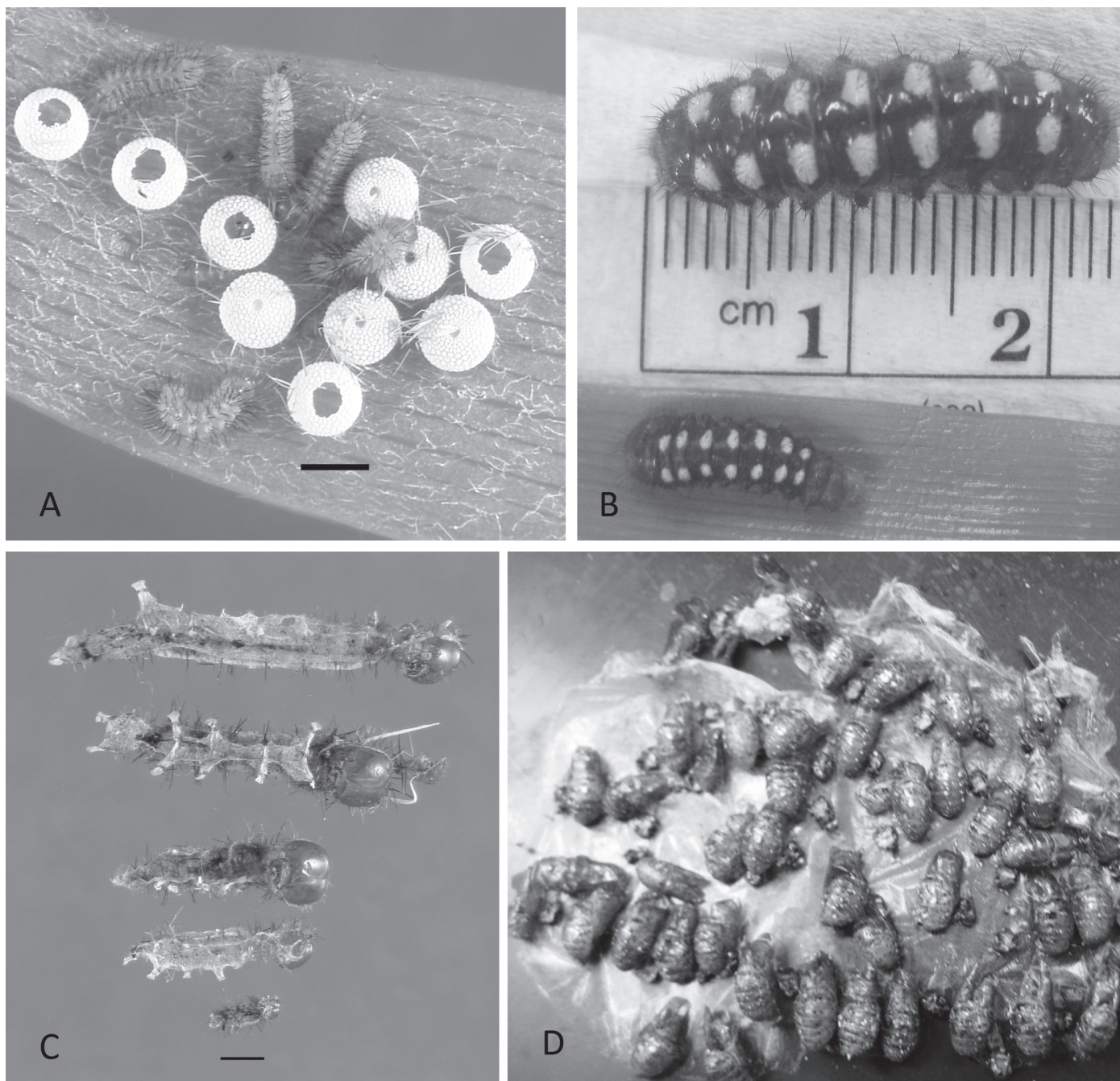


Fig. 5. Life stages of *Eumaeus atala*. (A) Automontage photograph of 2-h-old newly hatched larvae that measured between 0.5 and 1.5 mm in length. (B) Nine-day-old larvae from the same brood, and which hatched on the same day, showed high variability in size. The smaller larva successfully pupated 3 d later than its brood mates but developed into a smaller pupa and subsequent adult. (C) Automontage photograph of 5 normal instar exuviae. Stadia were variable, between 2 and 4, with most larvae pupating in the 4th instar. The 1st instar occurred at 3 d. (D) Silk mats may form an extensive anchor to the substrate beneath an aggregation of *Atala* pupae. Bars measure 1 mm.

more widely in environmental chambers programmed for seasonal experiments (S. K., unpublished data).

ADULT MATING, MEASUREMENTS, AND LONGEVITY

The average age of female mating was 15.45 ± 8.94 d ($n = 117$) and ranged from newly eclosed to 41 d of age. The average age of male mating was not significantly different ($P = 0.38$) at 11.98 ± 7.63 d ($n = 117$) and ranged from newly eclosed to 38 d of age (Table 1). Of

those observations, the mean longevity of observed mated females was 32.96 ± 8.83 d ($n = 76$) and the mean longevity for observed mated males was not significantly different ($P = 0.99$) at 34.38 ± 11.03 d ($n = 76$) (Table 1). Average adult wing cord length did not significantly differ between the sexes ($t = 1.0765$, $df = 31$, $P = 0.2900$) ($n = 600$), but the shortest cord lengths were measured in males and the longest in females. In the captive population, the average wing cord length was 2.2 ± 0.19 cm for females and 2.1 ± 0.15 cm for males. In the captive population, the shortest measured wing cord length was 1.4 cm (male)

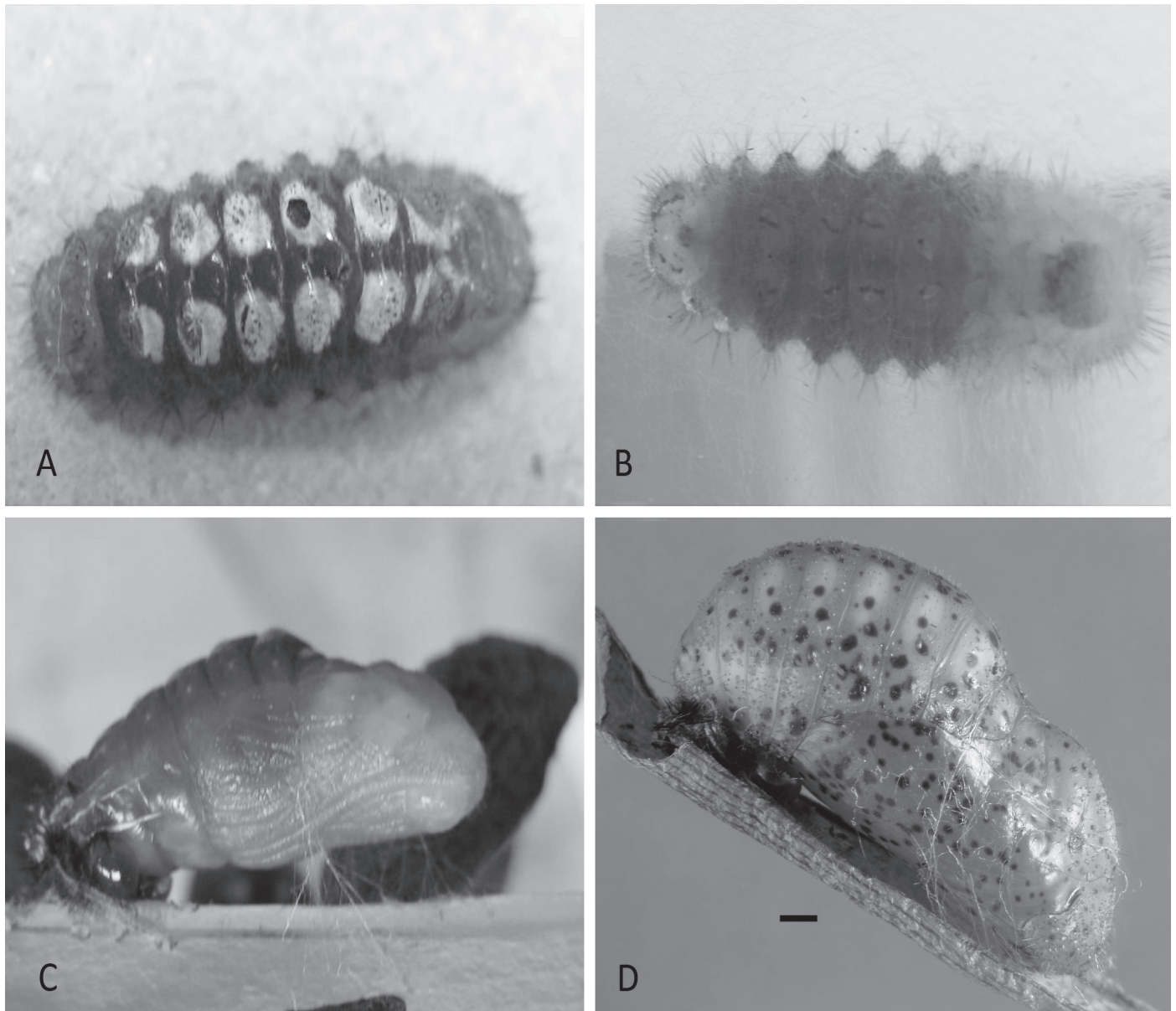


Fig. 6. Development of *Atala* pupal stages; the head region is on the right. (A) The dorsal surface of the larva begins to disengage from the larval integument, leaving a mottled appearance. (B) The ventral surface displays extensive webbing, and the head region and caudal end turn yellow by the 2nd day. (C) On the 2nd or 3rd day, the pupa is fully formed. (D) Automontage photograph of a 4-d-old pupa showing silk girdle around the thoracic segment and mat attached to the host plant leaf, with cast-off larval skin at the caudal end of the pupa. It is shown in a head-down position. Bar measures 1 mm.

and the longest was 2.5 cm (female), although one individual female measured 2.7 cm in a controlled seasonal experiment. Although no measurements were taken in wild colonies in prior years, photographs of deceased individuals in both captive and wild colonies placed beside common U.S. coinage allowed a visual comparison to be made (Fig. 8C and D).

Measurements were taken of adults that eclosed from wild larval stock collected in May 2012 (Fig. 9). Females in that brood had an average wing length of 2.37 ± 0.13 cm, whereas males had an average wing length of 2.22 ± 0.11 cm. Results from *Atala* reared in controlled environmental chambers by the first author will be forthcoming and show significant differences between seasonal growth rates and size.

Our observations challenge previously recorded lifespans, as the average lifespan of adults was 23.6 ± 13.4 d ($n = 1,021$) in the labora-

tory. In addition, an outdoor colony was maintained in a shade house in Gainesville, Florida, USA, for several months, receiving only flowers for nectar and *Zamia* plants for oviposition and for feeding the larvae (no Gatorade or additional water was provided); individuals in this colony lived on average 14.2 ± 6.2 d ($n = 36$). One male in the outdoor colony lived 31 d. There was no correlation between smaller adult size (wing cord length <2.0 cm) and longevity, and no statistically significant difference between males and females ($t = 1.0765$, $df = 31$, $P = 0.2900$). One male with a wing cord length of 1.9 cm lived 53 d, and 1 female with a wing cord length of 1.6 cm lived for 26 d.

Adults often exhibited a strong startle or warning response when first handled (to be marked with identification numbers) by quickly flashing open the ventral forewings to expose the dorsal wing colors (bright blue or blue-green) and raising the bright red-orange abdo-

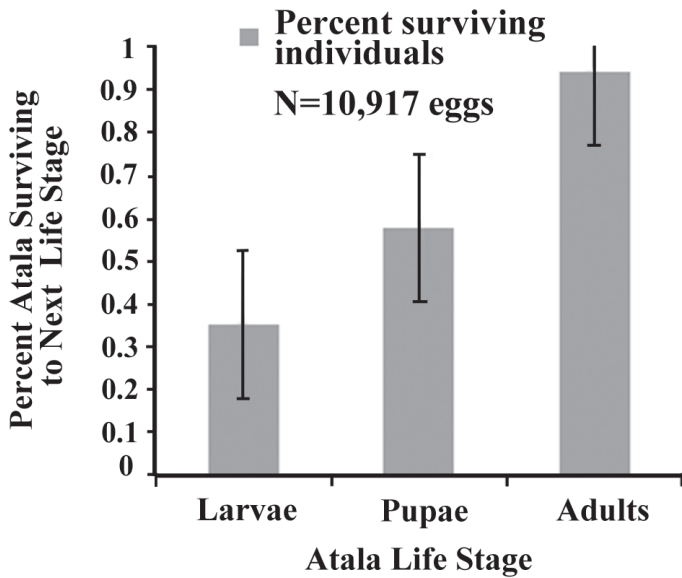


Fig. 7. Mean proportion of survival of Atala per life stage ($n = 10,917$ eggs).

men in protest, possibly to advertise its toxic nature via its aposematic colors. Evasive tactics employed by flying adults included dropping suddenly to another level and changing flight direction erratically and unpredictably.

ADULT DISSECTIONS AND EGG DEVELOPMENT

Newly emerged females had no developed eggs with chorions, but did possess 4 to 15 fully yolked oocytes, with a mean of 9.6 oocytes ($n = 5$). Chorions were developed between the 3rd and 7th days even in unmated females, and oogenesis was continual throughout the females' lifetime. Females had 6 ovarioles, and eggs from the same female often varied in size. As many as 51 eggs were dissected from 1 female (15 d old) that was not observed ovipositing prior to death. One 80-d-old female contained no eggs and an 86-d-old female contained 29 eggs. Female continuous egg production and variability was evident at all ages ($n = 128$; Fig. 10). Egg maturation with chorion also varied between 3 d (1 egg) to 7 d (21 eggs). A 9-d-old female contained 1 ovum, but a 4-d-old contained 24 eggs.

LIFESPAN AND SEX RATIO

Adults in captivity lived an average of 23.6 ± 13.4 d ($n = 1,021$) (Fig. 11), with a very wide range from immediate death because of eclosion failure (0 days) to a female that lived for 86 d. The oldest male in the captive population (Fig. 12A) survived to 71 d and originated as a late instar from the Miami-Dade County summer stock, perhaps indicating that wild individuals are also capable of a much longer lifespan than previously recorded. For instance, a 1-mo-old Atala was photographed as part of a wild colony observed daily by the first author in 2008 in Broward County (Fig. 12B).

The sex ratio was 1:0.95 (female to male; $n = 600$). Lifespans were not significantly different between females and males ($t = 1.5863$, $df =$

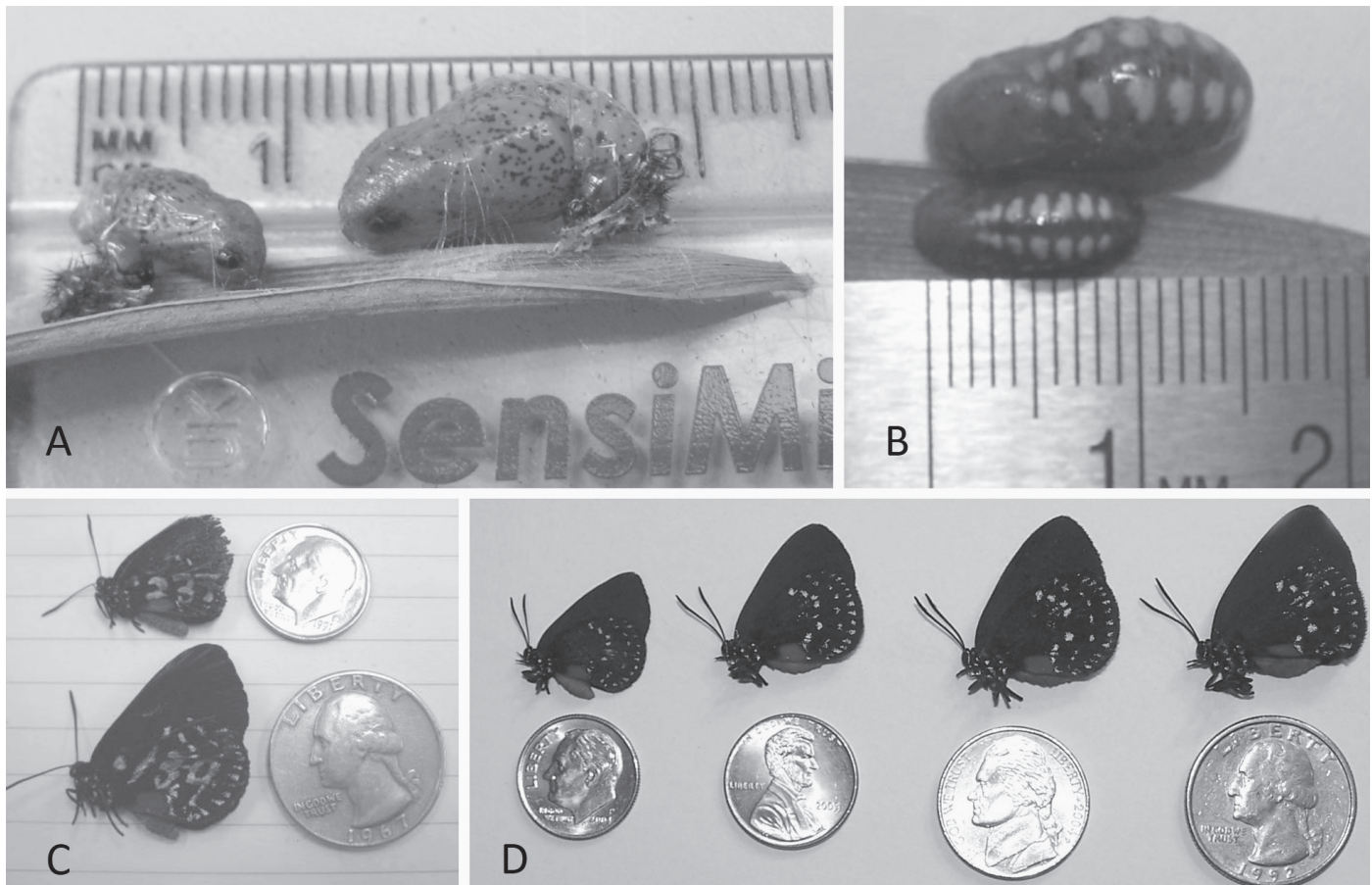


Fig. 8. There was a significant difference in size and weight of pupae not correlated with sex. (A) Pupae from the captive population, approximately 4 d old. (B) Compare with a similar photo of pupae from a wild colony in Fort Lauderdale, Florida, taken in 2006, approximately 2 d old. (C) Photograph of deceased adults from captive populations placed beside common U.S. coinage. (D) Adults from wild stock collected in 2006 from a Broward County site in Florida and placed above common U.S. coinage displayed similar size differences.

Table 3. Weight of *Atala* pupae by age and sex.

Age of pupa (d)	Sex	Sample size (n)	Weight (g)	
			Mean ± SD	Range
5	Female	6	0.4251 ± 0.0387	0.3626–0.4648
	Male	1	0.2679	not applicable
7	Female	5	0.41842 ± 0.0515	0.3850–0.4414
	Male	10	0.34827 ± 0.0773	0.2441–0.4580
9	Female	4	0.4124 ± 0.0108	0.4026–0.4276
	Male	1	0.3422	not applicable
11	Female	5	0.4049 ± 0.0196	0.3865–0.4345
	Male	2	0.3049 ± 0.1312	0.2121–0.3976
13	Female	8	0.3617 ± 0.0205	0.3349–0.3940
	Male	5	0.3461 ± 0.0514	0.2841–0.4052
15	Female	3	0.3469 ± 0.0386	0.3910–0.3307
	Male	1	0.3202	not applicable

49, $P = 0.1191$), with females (22.57 ± 11.84 d) living an average of 2 d less than males (25.14 ± 14.86 d).

Adult *Atala* lifespans were not significantly different between small individuals with wing cord lengths measuring less than 2.0 cm and large individuals with wing cord lengths measuring 2.0 cm or more, regardless of sex. Small and large females lived an average of 17.84 ± 12.02 d and 19.13 ± 9.34 d, respectively ($t = 0.3356$, $df = 30$, $P = 0.7695$). Small and large males lived an average of 23.03 ± 12.62 d and 18.03 ± 7.48 d, respectively ($t = 1.9022$, $df = 31$, $P = 0.0665$).

ABNORMALITIES AND PATHOGENS

Less than 1% of individuals presented an eclosion failure, pupation or wing abnormality, or other aberration in the captive population (n

$= 1,282$). In observations of pooled data from wild colonies in Palm Beach, Broward, and Miami-Dade Counties between 2004 and 2005, the first author documented that 4% of the wild populations had wing abnormalities, such as an inability to fully expand wings, and 8% of the population did not successfully eclose ($n = 3,430$).

An entomopathogenic *Paecilomyces* fungus (Eurotiales: Trichocomaceae) was found occasionally in eggs, larvae, and pupae, which likely was contracted from the substrate as larvae crawled from place to place. One experiment with our captive population indicated that the fungus is contagious, but more experimentation would need to be conducted to determine if it has a deleterious effect on wild colonies.

Discussion

Whereas previous studies had provided the initial details on the species development times, longevity, fecundity, and numerous other aspects of *E. atala* ecology (Schwartz 1888; Healy 1910; Dethier 1941; Rawson 1961; Franz 1982; Gerberg & Arnette 1989; Deyrup & Franz

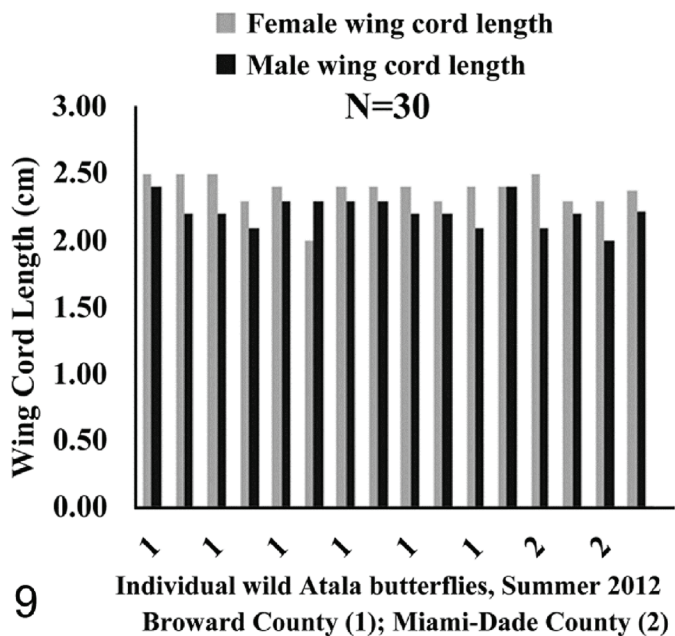


Fig. 9. Graph of wing cord length of emerged adults that were collected as wild late larval stock and that completed final instar in the laboratory, pupating immediately after relocation. Numbers refer to site locations (1 is in Broward County and 2 is located in Miami-Dade County, Florida).

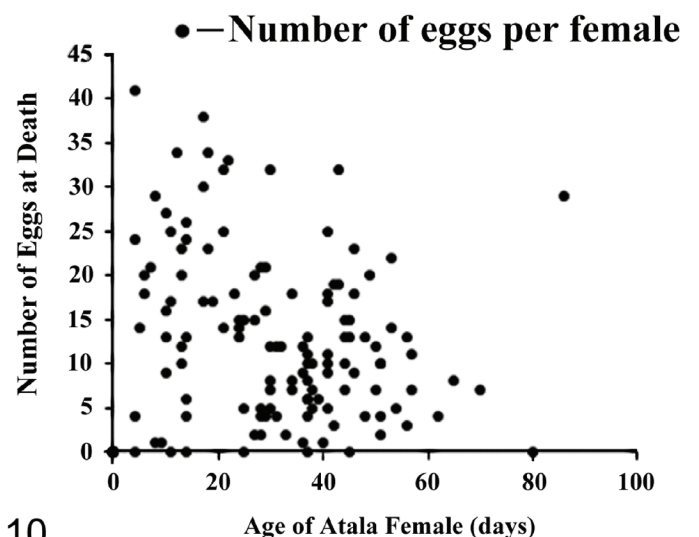


Fig. 10. Graph displaying number of eggs found per age in females dissected at natural death ($n = 128$). Note the 86-d-old female that still had 29 eggs internally in contrast to the 80-d-old female with no eggs.

Hawkins 1995; Hawkins et al. 1997; Queiroz 2002; Canto-Silva & Romanowski 2003).

The size of the eggs may influence the development time of larvae and their hatch time. The development of the embryo in the egg varied greatly (4–13 d) and may reflect, besides seasonal variation, how well the eggs are provisioned (Dethier 1959; Daniels 1999; Trager & Daniels 2011). A large egg with a diameter of 1.25 mm, compared with a smaller egg, will presumably contain more yolk nutrients for the embryo and may influence the embryo's ability to remain in the egg; it would have no need to eclose to search for food until the egg yolk is depleted. In comparison, a small egg with a diameter of 0.85 mm would have less yolk provision, and the larva would be forced to hatch in search of food sooner than a well-provisioned larva.

Daily observations indicated that ovipositing varied greatly between females, and some females were not observed ovipositing. Of 99 observations, the average age of ovipositing females was 16.27 ± 6.79 d, and the oldest female observed ovipositing was 41 d old. The viability was not known because the eggs were inadvertently destroyed. It is noteworthy that some larvae were larger upon hatching, were much more aggressive feeders, and developed into larger juveniles much faster than brood mates. A potential advantage to females depositing eggs in larger clusters is that larvae often hatch within a few hours of each other and line up "sardine style" to begin eating simultaneously; doing so likely allows them to break down the tough cuticle of the coontie leaves, thereby skeletonizing the leaves and facilitating feeding.

During late winter, when host plants were not sending up new growth, the surfaces of older leaves were scraped with a surgical blade and the mash served to neonates in a Petri dish, allowing the larvae to survive until they were large enough to handle tough leaves as a group. Individuals that hatched and were reared alone chewed the surface in tiny single bites and developed much more slowly than those in groups. Newly hatched larvae took a bite immediately after emerging but before lining up with siblings. Smith (2000) reported a survival rate of *Atala* eggs to larvae in an introduced wild colony in Key Biscayne, Florida, USA, as 47.4% ($n = 369$), similar to our captive colony's survival rate of 58% ($n = 2,209$).

Another survival advantage to depositing eggs in large clusters is that aggregation of larvae increases advertisement of their toxic nature by multiplying the effect of the larval aposematic coloring (Stamp 1980; Bowers 1993; Osborn & Jaffe 1998). This may help warn potential predators of the sequestered neurotoxins, thereby providing increased safety (Stamp 1980; Rothschild et al. 1986; Bowers & Larin 1989; Nash et al. 1992; Rothschild 1992). There are only a few reports of predators (Smith 2000, 2002) other than many ant species and assassin bugs (Hemiptera: Reduviidae).

Pupal development varied widely in this study, ranging from as short as 6 d to as long as 29 d, regardless of stable environmental conditions. Interestingly, if the larval state was longer in duration, with 5 instars, pupal development took less time. Pupa-to-adult survival was very high at 94% ($n = 2,077$), and Smith (2000) recorded the same survival rate in the introduced wild Key Biscayne colony ($n = 175$).

The extensive pupal mats most likely acted as cushioning, protection, and additional security against winds and possibly helped avoid potential predators because the stickiness may make ingestion difficult. The silk may potentially be chemically protected via the cycasins and other neurotoxins found in the host plant (Bowers & Larin 1989; Nash et al. 1992; Rothschild 1992; Osborn & Jaffe 1998; Schneider et al. 2002).

Total development time from egg to adult emergence was 28 to 57 d. *Atala* reared in controlled environmental chambers showed an increased range of development in all life stages partially associated with seasonal factors (S. K., unpublished), as has been shown in other

Lepidoptera (Blau 1981; Boggs 1981; Abrams et al. 1996; Fischer & Fielder 2002).

Wing cord length in this study cannot be compared with wingspan in previously published reports. In wild colonies, as well as in captive colonies, seasonal availability of vigorous nectar and host plant resources may affect size; insects grow faster and larger following summer precipitation, along with increased temperature and day length, factors that positively affect nectar and host plant growth and availability (Abrams et al. 1996). Later laboratory experiments that we performed under controlled conditions indicated that season also affects adult size (S. K., unpublished).

Lifespan showed arguably the greatest discrepancy between published literature and this report. Although it could rightly be argued that the captive laboratory population was living in optimal conditions that affected the butterflies' lifespan in a positive manner, forthcoming results will demonstrate that the insect is capable of a much longer lifespan than previously known.

Although the *Atala* butterfly is capable of flying very fast on occasion, and males use elaborate aerial displays to attract females, in general it is mostly sedentary. The flight pattern has been described as "deceptively slow and lazy-looking" (Klots 1951) and *E. atala* often flutters rather moth-like (Smith et al. 1994); the neurotoxins in its host plant may affect the insect's flight muscles, as it is known that the bodies of the larvae and adults contain high concentrations of cycasins and other toxic chemicals (Rothschild et al. 1986; Bowers & Larin 1989; Nash et al. 1992; Rothschild 1992).

We occasionally observed an entomopathogenic *Paecilomyces* fungus in eggs, larvae, and pupae of *E. atala*. In wild populations, the fungus would be contacted in mulch, leaf material, or other organic debris with which the mobile larvae may have contacted. In the captive population, it was most likely contracted via the daily introduction of new host material (as rearing containers were disinfected between every brood). Introduced new stock as larvae or pupae from wild sites could have introduced the fungus, and in the egg, it would have been contracted via the female, which would have contracted the fungus as a larva. The fungus has been recorded in one *Eumaeus* species in South America and in several other arthropod orders including Hemiptera, Coleoptera, many species of Lepidoptera, and Diptera (Norstog & Fawcett 1989; Fargues & Bon 2004; Torres-Barragán et al. 2004; van Munster et al. 2005; Ríos-Velasco et al. 2010).

The widely variable physiological life history adaptations that *E. atala* displays may serve to benefit an insect that lives in a highly stochastic environment such as South Florida. Direct effects of seasonal weather impacts include potential mortality in all life stages of the insect and loss of the host plants due to severe defoliation followed by flooding, drought, or salt-water intrusion. Prolonged drought affects host quality, and good environmental conditions may lead to eruptive events in which the insect takes advantage of the optimal resources available. In addition, the butterfly may disperse into previously unoccupied habitats that are less optimal for long-term survival (i.e., residential areas).

In addition to a harsh natural environment, more than half of this butterfly's extant populations now live in domestic gardens or remnant natural areas that are small and fragmented (S. K., unpublished data), surrounded by a highly urbanized matrix, filled with non-native ornamental plants that provide no nectar, or non-native invasive plants and animals (ants, reptiles, and amphibians) competing with *E. atala* over host plant and natural nectar sources (Hanski 1999; Hardy & Dennis 1999; Smith 2000, 2002; New & Sands 2002; Schultz et al. 2008; IBWG 2011). Furthermore, these urban populations likely are exposed to herbicides, unnatural fertilizers, pollution, and mosquito control adulticide applications (Hoang et al. 2011; Bargar 2012).

However, there are good aspects about urbanization for *E. atala*, such as increased availability of its host resources in both domestic and public botanical gardens, which are a vital link in the survival of this butterfly (Smith 2000; Koi 2008). Continued support of educational programs, re-introduction endeavors, and monitoring of wild and semi-wild colonies are other important steps in maintaining the viability of a species (Rawson 1961; Covell & Rawson 1973; Kremen 1992a,b; Emmel & Minno 1993; New 1993; Deyrup & Franz 1994; Cornell & Hawkins 1995; Hanski 1999; Hardy & Dennis 1999; Schultz et al. 2008; Algar et al. 2009; IBWG 2011).

There is increasing awareness of the need to protect cycads worldwide (Hubbich 1991; Oberprieler 1995, 2004; Chemnick et al. 2002; Schneider et al. 2002; Pérez-Farerra et al. 2006; Nagalingum et al. 2011; Calonje et al. 2013), and the economic value of ornamental non-native cycads in urban environments can be exponential. Control of herbivory, by culling eggs or removing larvae to another plant or location, in an integrated pest management manner (Hubbich 1991; Culbert 1994) is vital to sustaining urban populations to protect both the cycad and the butterfly. Eggs can fairly easily be removed from the slick waxy surface of most *Zamia* plants with a fingernail or metal spatula, which is helpful for those who wish to remove the eggs to reduce the population to control eruptions or who wish to protect their cycads from herbivory damage. Detached eggs should be frozen overnight to humanely dispatch of unwanted future generations, although eggs that land in the substrate will be found quickly by predatory ants or anoles. Neonate larvae that hatch in the substrate are so small that they will be unlikely to survive long enough to find the host plant again before predators find them (especially because they do not harbor toxins until they have ingested plant material).

The information presented here on *E. atala* ecology is designed to assist biologists, conservationists, and homeowners in managing and recovering this rare endemic species. Conserving all elements in ecosystems is ultimately mandatory for human existence, and recognizing the value of all of the earth's biota is one of the most important steps in bringing the balance back to humanity.

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