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A Butterfly Picks Its Poison: Cycads (Cycadaceae), Integrated Pest Management and *Eumaeus atala* Poey (Lepidoptera: Lycaenidae)

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Abstract

The imperiled Atala hairstreak butterfly, *Eumaeus atala* Poey 1832 (Lepidoptera: Lycaenidae) is a specialist species historically living in southeast Florida's endangered pine rockland ecosystems. Until relatively recently, the butterfly used North America's only native cycad *Zamia integrifolia* L. (Zamiaceae: Cycadales), commonly called "coontie," as the host plant for its offspring. With the introduction of many non-native and valuable cycads into botanical and domestic gardens, the butterfly has expanded its choices to include these exotic species. Conservation of both the plant and insect is complex as herbivory can damage the plant, but control of the larval damage may be detrimental to the fragile populations of the butterfly. Larval and adult host plant choice tests were implemented to compare larval survival, development rates and subsequent adult choice between *Z. integrifolia* and non-native *Zamia vazquezii* L. (Zamiaceae: Cycadales), a popular garden cycad that is critically endangered in its native Mexico. Results indicate that both adults and larvae chose native more often than non-native; larval survival decreased, but development time increased, as did adult lifespan when utilizing *Z. vazquezii*. Adult survival to successful mating and fecundity occurred with either host choice. Integrated pest management techniques are discussed for avoiding pesticide use to control larval herbivory on valuable cycads.

Keywords: Coontie; Herbivory; Pine rocklands; Pesticides; Urban landscaping

Introduction

Botanists have recently established global networks to monitor the highly endangered populations of cycads worldwide [1-4], now recognized as the most highly endangered plant species on earth [5]. These severe global declines mirror the losses of many other species recognized as part of the "sixth great extinction" on our planet [6-8]. Causes of cycad decline include legal and illegal collection, removal of seed heads and root calyxes for food and/or "bush medicines," religious ceremonies, and overexploitation, including the two biggest on-going threats: unsustainable trade and habitat loss [1,9]. Another possible reason for declining colonies of these ancient plants is the possibility that the specialist pollinators associated with an individual cycad species have been extirpated, or is extinct [1,2,10]. Notwithstanding legislation and laws designed to protect them, both legal and illegal trade negatively impact global cycad colonies, many of which are isolated and highly vulnerable [1-3]. In Colombia, González [10] indicated that the native cycad, *Zamia encephalartoides*, its pollinator and the herbivores are all in danger.

Found historically from southern Georgia to the Florida Keys, North America's only native cycad, *Zamia integrifolia* L.f. (Zamiaceae: Cycadales), commonly known as "coontie," is still listed as "Threatened" in wild natural areas [11]. Although different cycad species contain various mixes of neurotoxins, the roots of the cycads plants can be harvested and washed to remove the water-soluble toxins in order to make mildew-resistant flour [11,12]. In south Florida, the original resident Calusa Indians processed coontie root for flour; ensuing inhabitants, from the Seminole and Miccosukee Indians to the European settlers, followed suit. The first industries in south Florida were the starch industries of the last century, which depleted wild populations of the cycad, almost to the point of state-wide extirpation. In many undeveloped and underdeveloped nations, cycad-root flour is still a staple [1,2,4] and often, the reproductive cones are removed as well [4,13], hindering seed recruitment for new plants.

Nevertheless, during the past twenty years, native North American coontie has been recovering due to the Florida landscaping industry, as nurseries and homeowners discovered how well-adapted the luxurious leafy plant is to Southeast Florida's diverse ecosystems and stochastic weather cycles [14,15]. More exotic non-native cycads have been added to the repertoire available to landscape planners, increasing urban use and world nursery trade. The coontie plants are not increasing their populations in wild colonies, however, partially because the slow-growing plants are located in habitats with very thin nutrient-poor soils resting on limestone outcrops (Figure 1).

Two species of native lepidopteran herbivores attack cycads in North America: the Echo Moth (*Seirarctia echo* Smith 1797) and the Atala butterfly (*Eumaeus atala* Poey 1832). The Echo moth range covers Georgia south to the Florida Keys, and west to Mississippi, which is wider than the range of the cycad. The moth is widely polyphagous, utilizing Sabal Palmetto (*Sabal palmetto*), and various species of crotons, lupines, oaks and persimmons, as well as coontie and other plants.

Called "destructive" in the early part of this century, the Echo moth was lamented as being a possible competitor of the other lepidopteran cycad-herbivore, the Atala butterfly, at a time when the butterfly was thought to be extinct or nearly so [16-18].

Historically, the Atala hairstreak butterfly was only found in southeast Florida, in the now endangered pine rockland ecosystems and tropical hardwood hammocks. Unlike the Echo moth's eclectic choice of foods, the Atala is a specialist butterfly that used our only native cycad, *Z.*

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Figure 1: Native *Zamia integrifolia* (coontie) growth habit varies greatly. A) In the oolitic limestone substrate of south Miami-Dade County coontie struggles to survive. B) A domestic garden provides high nutrients. C) A ruderal area provides slightly more organic material in the substrate, but it is still compact rock. D) Coontie is found in scattered pockets in its native pine rockland ecosystem (Photo credit: Koi S).

integrifolia as its sole host plant. The decline of native cycads caused the radical decline in the butterfly's population and by 1951 it was thought to be extinct [16]. Unbeknownst to almost everyone, the butterfly had survived in isolated refugia (safe havens) far from the increased habitat destruction as the cities in southeast Florida grew.

Chemoecology of cycads and butterfly associates

Each species of cycad contains unique mixes of neurotoxins, such as cycasins and macrozamins in different concentrations and amounts. Butterflies recognize and respond to chemical volatiles released by their host plants, and by larval herbivory, to help them locate potential oviposition sites [1,2,15,19-21].

The Atala has utilized as many as 38 additional cycad species in southeast Florida [22-28], all of which contain similar chemoecology [1,2,15,21].

Integrated pest management

Integrated Pest Management (IPM) is a multifaceted methodology developed to prevent or control pest insect damage to economically valuable plants, including ornamentals that may be expensive, rare and/or aesthetically significant to botanical gardens and homeowners. Various methods for detecting, identifying, controlling and managing insect pests are integrated by combining biological, cultural, physical and chemical means to keep insect damage at an acceptable level. IPM controls damages caused by insects in the most economically balanced way, preventing potential health hazards to humans and animals [29-31]. It is one of the safest methods for controlling infestations of unwanted insect pests, while conserving beneficial insect species, as well as other imperiled or endangered biota sharing the same habitat.

The four systems of IPM are: Cultural control, physical control, biological control and chemical control. The ultimate goal of IPM is to use the first three to prevent using the fourth. Cultural control is the foundation for everything else; it includes a thorough understanding of the biology of the plant and the pest insects' life, sanitation practices (such as removing sick plants promptly) and both planting and harvesting times.

Physical control includes proper pruning methods, mulching appropriately for the plant species, and may include mixed plantings that encourage beneficial insects and discourage pests. Biological controls may include releasing commercially propagated beneficial insects into a garden and/or providing the plants and cultural practices that promote naturally occurring beneficial insects. Chemical means are used as a last resort for severe infestations, to target specific pest species, maximizing effectiveness while minimizing hazards to humans and other animals [29-31].

One of the valuable non-native cycads being used by the Atala butterfly as an alternative host plant is *Zamia vazquezii* D. W. Stev, Sabato and DeLuca (Zamiaceae: Cycadales) [23-27], a critically endangered cycad in its native Mexico [27,32] and a highly employed cycad in upscale urban developments, and botanical and domestic gardens in southeast Florida.

Herbivory of *Z. vazquezii* is a pest management concern of economic importance not only because it is considered endangered in its native Mexico (The IUCN 2013), but also because it is one of the exotic cycad species grown in highly specialized south Miami-Dade nurseries; it is considered a valuable addition to a prestigious landscape (i.e., it is not generally utilized along highway median strips or shopping plaza parking lots). However, *Z. integrifolia*, our native cycad, has shown itself to be hardy and resilient enough to be successfully utilized along such heavily trafficked or polluted sites.

Both of these cycads are readily used by the Atala butterfly in these 'semi-natural' urban environments, and often sustain considerable herbivory damage (Figure 2), sometimes to the extent that the cycad is unable to recover. Pesticides have been used repeatedly in urban arenas to control the Atala caterpillar damage [24-29], thereby triggering both an economic impact in chemicals as well as potentially detrimental environmental costs [29-31].

Host plant choice tests with the Atala butterfly

Larval and adult host plant choice tests were implemented to compare larval survival, development rates and subsequent adult choice

Figure 2: Atala larvae completely consuming a non-native *Zamia erosa* at a botanical garden in south Miami-Dade County (Photo credit: Koi S).

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between native *Z. integrifolia* and non-native *Z. vazquezii*. Oviposition choice by offspring has been shown to be at least partially affected by the adults' oviposition choices [31-34]. Understanding host preference, egg distribution, heritability of oviposition sites [33-40], colonization of non-native host plants [35], larval feeding strategies and choices and adult oviposition site selection [35-41] for the Atala butterfly and larvae will help lepidopterists, botanists and landscape managers develop feasible management protocol to protect the butterfly colonies while controlling herbivory by focusing on cultural practices and reducing pesticide use.

Materials and Methods

Biological livestock and rearing

Atala larvae and pupae were collected from wild colonies in several southeast Florida stable colony sites that had been previously identified during on-going long-term surveys [24-26,42]. These choice test experiments were performed from 7 November 2012 to 30 January 2013.

All tests were completed under the same environmental conditions in a laboratory at the University of Florida in Gainesville, FL, USA (29° 39' 05" N, 82° 19' 29"W) in approximately 8:16 h photoperiod and stable ambient temperatures between 24°C (nocturnal) and 26°C (diurnal). Cages were sprayed twice daily with water misters to maintain normal relative humidity between 25-42%. Adult butterflies of both sexes were housed together in a large walk-in adult flight cage (LiveMonarch Foundation, 1.78 m^2, Greenhouse Castle Cage, Boca Raton, FL, USA) to approximate a natural environment and to encourage mating. Cages were provided *ad libitum* with a nectar substitute (Gatorade®, The Gatorade Company, Chicago, IL, USA) in fruit punch, orange or watermelon-citrus flavors, hanging approximately 46 cm from the ceiling in a modified feeder (Figure 3) [43]. Water was also provided in feeders; host plants for larval consumption or adult oviposition were provided as described herein, depending on test. All feeders were cleaned and replenished daily and larval cages disinfected with 1% bleach solution weekly for disease control.

Larvae from all trials were reared with their brood mates on the host plant of adult or larval choice in square plastic boxes (19.05 × 19.05 × 10.16 cm); individuals that chose differently than brood mates were reared singly or with their congeners, depending on their choices. The box tops were covered with 1 mm tulle netting to prevent larval escape; the food of the larvae's choice was replaced daily as needed *ad libitum*. The rachides of all food fronds were housed in 15 cm floral aqua tubes (Aquapic, Syndicate Sales, and Kokomo, Indiana) to maintain plant freshness for the larvae (Figure 4). The larvae in all tests were maintained as described above until pupal emergence, when the eclosed adults were transferred to flight cages measuring approximately 30 × 30 × 61 cm (LiveMonarch Jumbo Cage, Boca Raton, Florida).

Adult Choice Test I

For adult oviposition choice tests, one frond each from both *Z. integrifolia* and *Z. vazquezii*, closely matched in size, were installed in the flight cage. The host plants for ovipositioning were each encased in an aqua tube (Aquapic, Syndicate Sales, Kokomo, Indiana) to keep the leaves fresh, and were hung approximately 46 cm from the ceiling to make them more apparent to the adults (Figure 5). One leaf of each species was installed about two feet apart on the same side of the cage so that they were not touching. Leaves were collected daily from the previous day and number of eggs counted on each frond. Fresh leaves were installed and the positions of the fronds in the cage alternated daily to avoid position effect preference by the females. Trials were executed for forty-two uninterrupted days. Because the adults were free-flying in

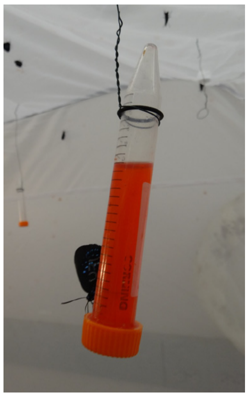

Figure 3: An economical, easily made liquid feeder adapted for butterflies (Photo credit: Koi S).

Figure 4: The larval box cage, with plant frond housed in an aqua tube, and tulle netting lid secured with a rubber band to prevent larval escape (Photo credit: Koi S).

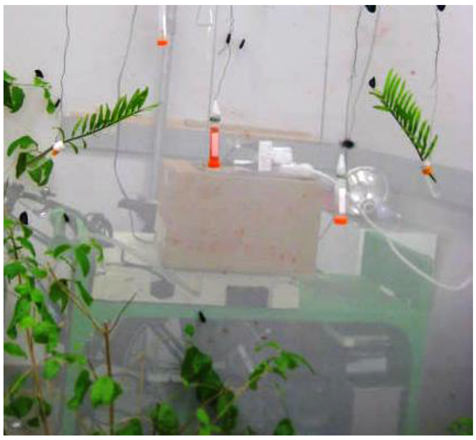

Figure 5: The adult breeding cage showing the host plant (and feeder tubes) suspended from the ceiling, where "Adult Host Plant Choice I" took place (Photo credit: Koi S).

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the flight cage, and both sex multiple mate [24-26], parentage was not followed for this test.

The hatched larvae from the adult-choice test were maintained in larval boxes with chosen host plant as described above until pupal emergence. Those eclosed adults became First Generation Adult Test (Figure 6 for experiment design).

First generation Adult Choice Test I

Larvae that successfully pupated and emerged as adults from "Adult Choice Test I" were housed together as they emerged in the smaller flight cages measuring approximately 30 × 30 × 61 cm (LiveMonarch Jumbo Cage, Boca Raton, FL, USA). Broods were housed together as much as possible, but because of the variable hatch rate and high mortality, the sample size was small. The cages were supplied with nectar and water feeders as previously described, as well as two fronds of host plant, one each *Z. integrifolia* and *Z. vazquezii*. Fronds were hung approximately 46 cm from the ceiling and installed about 15 cm apart. The position of the fronds was not alternated because the adults of both sexes were set free in the large flight cage as soon as the females chose an oviposition site (Figure 6). Only one cage had more than one female.

First generation Adult Choice Test II

Larvae that successfully pupated and emerged as adults from "Larval Choice Test II" were housed together as they emerged in the smaller fabric flight cages as previously described. Clutches were housed together as much as possible but because of the variable hatch rate and high mortality, the sample sizes are small for this test as well. The cages were supplied as described above and adults released to the larger flight cage as soon as the females made ovipositing choices (Figure 6).

Larval Choice Test I

Eggs were collected from any non-plant substrate, such as the wall, floor, nectar feeders or roof, from inside the large flight cage. Multiple parents were most likely represented by the eggs in this test because of the nature of the free-flying adults in the flight cage. Sanitized 9 cm plastic petri dishes were prepared as choice test arenas by trimming Whatman filter papers to fit inside the petri dishes. A center line was drawn with a graphite pencil (Figure 7). The top of each half of the arena paper was labeled either "N" (designating native *Z. integrifolia*) or "NN" (designating non-native *Z. vazquezii*). The labels and plants were alternated in each arena to reduce possible side-preference effects by the larvae (i.e., 1="N-NN", 2="NN-N", 3="N-NN" etc.). One leaflet from each cycad species was pinched off at the petiole where it connected to the main stem of the compound leaf, and placed inside the arena on the appropriate side (Figure 7). Just before eggs were placed into the arena, the filter paper was dampened with deionized water and the dates of collection recorded so that expected hatch dates were known. Hatches were monitored immediately after eclosure and larval eclosure, timing, behavior and choice were photographed.

A single ovum from the day's collected eggs was placed in the center of each prepared petri dish and lightly sealed with string to prevent larvae loss, in case eclosure occurred earlier than expected. The arena and egg were allowed to rest undisturbed until the larvae hatched. On the expected day of larval hatch, the lid and string were removed from the petri dish, and fresh single leaflets were pinched off at the stems of each of the two host plants. The individual leaflets were installed on opposite sides of the center line approximately 1 cm from the center (Figure 7). If the larvae did not hatch when expected, the leaflets were changed daily until the larva did emerge.

The larvae were monitored to record behavior and choice immediately during and after eclosure. Choice was recorded when the larvae climbed onto the plant it chose and commenced feeding, i.e., "settling in" (Figure 8a) If the larvae did not remain on the leaf to continue feeding after an initial "natal test-bite," it was not recorded as a choice (Figure 8b). The time from eclosure to choosing its host plant was recorded. Larvae were reared singly on the host plant of their choice in standard square plastic boxes as described above.

Larval Choice Test II

The same basic protocol for this test was followed, with two changes: instead of one egg per arena, an entire brood of eggs from the same location was deployed in the arena. These small clusters were likely from the same female. Eggs were lined up on the center line in haphazard order. This test explored how and if siblings influenced each other in their choice of host plant (Figure 9a-9d). There were only 23 larvae successfully hatched in this experiment.

The second change in this test involved trimming both ends of the leaves with scissors (rather than pinched off at the petiole), to both simulate herbivory and release volatile plant chemical clues.

Larvae were reared with their brood mates on the host plant of their choice as described; individuals that chose differently than brood mates were reared singly or with congeners who chose likewise and maintained as described above until pupal emergence. The eclosed adults became First Generation Adult Test II (Figure 6 is a graphical representation of the experimental design).

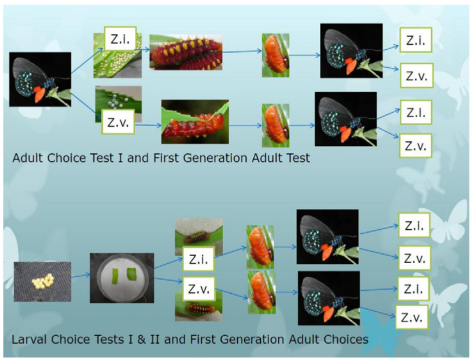

Figure 6: Graphic showing the adult choice test I-larval choice test I & II and first generation adult choice test design (Photo credit: Koi S).

Figure 7: In Larval Host Choice Test I, a solitary egg was placed on the center line in the arena and the larva monitored to record choice and timing. The larva in this photograph is just beginning to hatch (Photo credit: Koi S).

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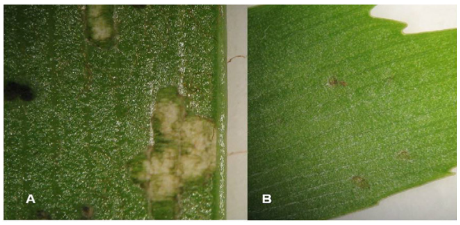

Figure 8: A) The native *Zamia integrifolia* leaflet has numerous natal test-bites indicating that the larva has "settled in" to consuming the plant. This kind of mark was counted as a choice (note the frass). B) The non-native *Z. vazquezii* shows shallow nibbles but no decisive bites and were not counted as choices (Photo credit: Koi S).

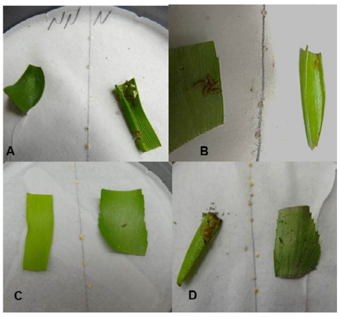

Figure 9: The arena for Larval Test II trials utilized the entire brood. A) Five larvae of five chose native *Zamia integrifolia*. B) The first eclosed larva chose non-native *Z. vazquezii,* but the remaining six eggs did not hatch. C) Five larvae in the brood chose non-native and one larva chose native. D) All five larvae chose native, but there was evidence of frass and nibbles on the non-native plant (Photo credit:Koi S).

Results

Adult choice test

Eggs were collected from the leaves of the two cycads in the flight cage daily and counted. A total of 9055 eggs were laid during the forty-two day trial. There was a significant difference in female host plant choice, with 8094 eggs laid on *Z. integrifolia* and 961 eggs laid on *Z. vazquezii* (p-value <0.000). Native *Z. integrifolia* (*Zi*) received 89% of the eggs and 11% were laid on *Z. vazquezii* (*Zv*). Although it is possible for all life stages to be completed successfully while feeding on the non-native plant *Zv*, the mortality rate of the larvae was high and many larvae feeding on both plants died before reaching pupation (Figure 10). Development times was not significantly different for any life stage between the two groups (in either the Adult or Larval Choice) or between the two plants (p>0.05), although there were obvious differences in sizes (Figure 11). The proportion larvae surviving on *Z. vazquezii* was lower, but not significantly so. Egg-to-adult survival was 0.021 for larvae consuming *Zv* and 0.153 for larvae feeding on native *Zi*. Pupa-to-adult eclosion survival was 0.666 for larvae that had consumed *Zv*, and 0.732 for larvae that had consumed *Zi*. Survival was markedly lower for larvae reared on *Zv*, but not significantly so (Figure 12).

In another example of the differences, the average number of days from oviposition to adult emergence in Adult Choice Test I was 46.80 days on non-native *Zv* and only 38.69 days on native *Zi* (Table 1). Only 2% of eggs laid on non-native *Zv* hatched and only seven out of fifteen adult butterflies reared on *Zv* successfully emerged from the pupa (66%). Only 15% of eggs laid on native *Zi* hatched, but 254 adults successfully emerged from 347 pupae (73%).

Immature development time and duration of life stages was not significantly different for larvae reared on either plant, but there was a significant difference between the number of eggs laid on *Zv* and *Zi* (χ^2=df14, 0.05, 169.00) (Table 2).

Although adults reared on *Zv* (Adult Choice) lived longer than other adults in the trials, longevity of the adults did not vary significantly between individuals reared on either plant; there were no significant differences between immature-to-adult development rates and lifespans (Table 1).

First generation adults from "Adult Choice" Test

First generation adults that eclosed from "Adult Host Plant Choice Test" pupae chose to oviposit on native *Zi* in 100% of the trials (n=32, 11 females, 4 trials).

Larval Host Plant Choice Test I

Most larvae chose native *Zi* (77%), but many nibbled the non-

Figure 10: Many of the larvae feeding on non-native *Zamia vazquezii* died before reaching pupation. This larva developed a fissure in the midgut caused by an erupted gut, likely associated with the sharper, tougher leaf structure of *Z. vazquezii* (Photo credit: Koi S).

Figure 11: Comparisons of 6-day-old larvae in the Larval Choice Test I show obvious differences in development size; the larger larvae consumed native *Zamia integrifolia* while the smaller larvae ate the non-native *Z. vazquezii.* Note the surface scraping on the non-native, which has a much thinner epidermis than native plants (Photo credit: Koi S)

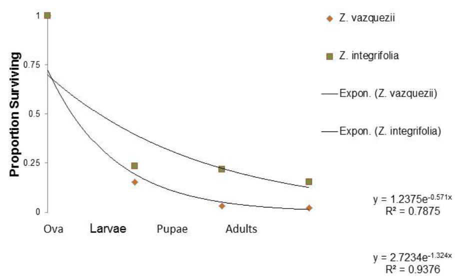

Figure 12: Proportion of Atala surviving to the next life stage was lower on non-native *Zamia vazquezii* host plant than on the native *Z. integrifolia* (Adult Choice Test I) (Photo credit: Koi S).

	Ova development (days)	Larval development (days)	Pupal development (days)	Total development (days)	Adult lifespan (days)	n
Larval choice: *Zamia integrifolia*	7.47	16.47	15.21	39.16	27.68	19
St. deviation	± 0.70	± 1.74	± 1.18	± 1.30	± 13.47	
Larval choice: *Zamia vazquezii*	7.00	14.00	19.00	42.75	25.75	4
St. deviation	± 1.15	± 2.00	± 2.06	± 3.69	± 16.32	
Adult choice: *Zamia integrifolia*	8.44	14.25	16.00	38.69	33.44	16
St. deviation	± 0.51	± 1.18	± 1.21	± 1.96	± 12.28	
Adult choice: *Zamia vazquezii*	8.87	22.20	15.73	46.80	22.13	16
St. deviation	± 0.35	± 2.76	± 3.65	± 4.83	± 15.32	

Table 1: Development times and life span were both different depending on whether the adult or larva made the choice of host plant, as well as which plant was chosen, but the differences were not statistically significant.

	Ova observed	Ova expected	Chi-square	p-value >0.05
Zamia vazquezii	582	1122.50	260.26	3.841
Zamia integrifolia	1663	1122.50	260.26	
			520.52	

Table 2: Although nearly twice as many ova were laid on native *Zamia integrifolia* than on non-native *Zamia vazquezii*, the difference was statistically significant.

native plant first. Of 126 ova, 47 hatched to complete the choice trial. One neonate eclosed from an egg that had been laid on a non-host plant, Florida Keys Blackbead (*Pithecellobium bahamense)* Britton ex Britton & Rose, a southeast Florida native that occurs in coastal areas where there are extant Atala colonies. The plant had been installed in the flight cage as a roosting tree, and was not originally intended to be used for this experiment. However, it was an opportunity to observe any behavior or other changes that may take place if or when eggs are laid on non-host plants. When this larva eclosed, it moved toward the petri-dish sides and circled consistently, wandered to the native leaflet but left it again without feeding. It then wandered to the non-native leaflet in the same manner but did not commence feeding. After four hours without choosing a host, this larva was counted as the only "no choice" in all of the trials (the larva was removed from further testing in this trial but was placed with congeners in another cage). A third short trial within this test used 112 eggs which developed into 42 larvae, 93% of which chose native.

First generation adults from "Larval Host Plant Choice" II

First generation adults that had been reared on both native and non-native plants in "Larval Host Plant Choice Test II" chose to oviposit on native *Z. integrifolia* 100% of the time (n=32 ova; 11 females).

Larval Host Plant Choice Test II

The sample size was small (n=23) as individual mortality was high reared on *Zv.* Larvae from the eggs appeared to make independent choices, as not all of the larvae chose the same host plant. A higher percentage chose native over non-native (83%) than in the first trial and 17% chose the non-native host plant. Total development time from egg-to-adult (Larval Choice II) showed 42.75 days for larvae reared on *Zv*, and 39.16 days for larvae reared on *Zi*.

First generation adults from "Larval Host Plant Choice" II

First generation adults that had been reared on both native and non-native plants in "Larval Host Plant Choice Test II" chose to oviposit on native *Z. integrifolia* 100% of the time (n=169 ova; 23 females).

Discussion

All cycads contain variations of the same basic chemicals (cycasins, macrozamins, etc.) and are most likely recognized by the Atala butterfly as basal host plants. Some of the chemicals in cycads, such as cycasin and macrozamin, have been analyzed in some cycad species and in a few herbivorous insects, but there is a lot still unknown about the complex relationship between the insects and the plants. Many of the cycad species have not yet been chemically analyzed and the analyses of others have been performed using different methods or testing different parts making comparisons difficult to interpret.

Beta-methylazoxymethanol (BMAA) is stored in the idioblasts of the leaves of some cycads [20,44]; herbivory by insects has been shown to have beneficial effects regarding enhanced growth patterns as an agonist of glutamate receptors which induces increased hypocotyl growth, as evidenced in *Arabidopsis*. Teas (1967) [45] was the first to record the metabolic pathways in *Seirarctia echo* by which the neurotoxic Methylazoxymethanol (MAM) in the coontie host plant was transformed into less toxic cycasin via beta-glucosidase activity, thereby avoiding autotoxicity. It is thought that the same metabolic detoxification methods are used by other the cycad insect associates. There is a question as to whether the BMAA neurotoxin may have evolved before it developed as an herbivore deterrent [44]. Brenner et al. [44] questions if the idioblasts may have evolved to protect the other cells because of the high concentrations of the chemical.

Azoxyglucosides are not released until leaf tissue injury occurs, which may provide a clue as to why the neonate larvae appeared to seek out the chemical volatiles released when the cycad leaflets were cut to simulate herbivory. This release of volatile organic chemicals from herbivory may in fact serve as a field signal for female Atala that are searching for a suitable host plant. This may also help explain why Atala larvae must often be introduced several times into new colony sites before the adults persist at the new location (Koi unpublished). Methylazoxyglucosidemethanol (MAM) is released via herbivory or as enzymes in the digestive tract of herbivores [21], but BMAA contents have not been analyzed in any of the herbivores yet [28]. Castillo-Guevara and Rico-Gray (2003) [46] conjectured that macrozamins are the most primitive of azoxyglucosides, and may have evolved as a primeval method of storing nitrogen. They mention that because the plants are very slow growing, this added protection would have proven a beneficial to prevent competition for space in crowded tropical regions where many cycads grow. The sarcotesta of the *Z. integrifolia* seeds contain a growth inhibitor (Taylor 1999) [47] and must break down over time, usually germinating the next season unless the flesh is consumed by wildlife (some animals are capable of utilizing the toxic flesh). Taylor also indicated that the butterfly larvae associated with the cycads may break down the germination-inhibiting sarcotesta on the female seed cones, helping the seeds sprout faster than they do otherwise, and are therefore helpful herbivores.

Castillo-Guevara and Rico-Gray show a negative relationship between the types of herbivory and azoxyglucoside content of the plants, regardless of whether the herbivore was a butterfly or moth, a beetle, weevil, or midge, a mealy bug or scale insect. It does not matter what kind of herbivory occurs, it does not predict the neurotoxin concentration in the cycad.

However, both larvae and adults preferred native *Z. integrifolia* over non-native *Z. vazquezii*. Growth and survival were arrested to some extent on the non-native cycad, and development was influenced as well, although there was no significant difference in the growth rates. There was a significant difference in the number of eggs laid on the different plants, indicating that females choose the native plant more often than non-native. It was interesting that the first generation adult females from the larvae that had chosen the non-native cycad as its larval food chose the native cycad for ovipositing.

In general, the butterflies reared on *Z. vazquezii* lived longer than those reared on the native host, although not significantly so. It could be that insects that are tough enough to survive on the non-native plant simply have a more robust constitution. The surface epidermis of *Z. vazquezii* is much thinner than that of native Z. *integrifolia*, requiring larvae to feed longer and consequently it took the larvae longer to acquire enough nutrients to successfully complete their life cycle to adult emergence (Figure 13).

There were two unsuccessful attempts to rear individuals from eggs that had been laid on native and non-native plants by removing the eggs from their substrate and switching them to the other plant. However, because the hatch rate was very low, these trials were not used in any analyses.

Switching from the pinched-off leaflet in Larval Test I to a clipped leaflet in Larval Test II was made based on observed behavior exhibited by the larvae in Larval Choice Test I that included apparent larval attempts to garner these volatiles from the atmosphere in the arena by rearing up on their prolegs and swaying back and forth with their "nose in the air," and then returning repeatedly to the egg, circling it and touching it (ostensibly looking for clues that it did not have because the eggs had been laid on non-plant substrates).

Beneficial insect associations with Cycads

There are some probable beneficial effects of insect-associated activity with cycads. Each cycad species has a mutualistic relationship with a specialist, sometimes obligate, pollinator weevil. Like butterflies, moths or other insects, including pests such as scale, they each play a part in the total ecology of the plants [1,2,15,19-21]. The co-relationships between the specialized insect herbivores and pollinators may be more recent than previously thought, as new molecular phylogenies show the relationships have co-evolved since the late Miocene [48].

Nagalingum et al. [48] notes that this may be one of the reasons for the low genetic diversity in the genera, which has made identification of the cycad species difficult [3,20,44,49-51]. It is thought now that the diversification of the six cycad genera may have first been triggered when the current continents were moved into their present locations as the tectonic plated shifted during the Miocene [48], although there is still some controversy [50]. A recent paper indicates that the seeds may have been ingested by large birds, mammals and other megafauna, and dispersed in clusters along with large amounts of 'fertilizer' in places some distance from the mother plant [52], explaining the growth habit of the plants, usually adapted to growing in high density. The large volume of frass left behind by small herbivores, especially butterflies and moths, acts as a fertilizer as well. Hall and Gimme [52] determined that opossums were primarily responsible for consuming the fleshy sarcotesta and dispersing the seeds of the Australian cycad *Macrozamia miquelii* (Zamiaceae). They found that few seeds were dispersed more than a meter from the maternal plant and that most juvenile seedlings did not establish within a few meters of the parent, even though most seeds fell beside the maternal plant. In Florida, squirrels are known to ingest the sarcotesta, but opossums and other wildlife may be responsible for dispersal of the seeds as well. Because of this growth habit, *Zamia* plants may be locally abundant, but rare; this is also true of *Z. integrifolia* in wild lands in Florida.

Conservation concerns: encounters between wildlife and humans

Both plants are pest-resistant under optimal conditions, but show signs of stress, such as scale infestations or sooty mold, when too crowded or poorly maintained. Recovery of the Atala butterfly from near-extinction is associated to the increased use of *Zamia* plants in landscaping, as well as increased conservation and restoration projects by biologists, botanists, park managers, scientists and concerned citizens. In 2008, Everglades National Park, for instance, grew additional coontie for the park from seed harvested four years prior in the park by volunteers, to prevent possible dilution of genetic material (Perry, pers. comm.). Most restoration endeavors include replanting native vegetation as well as removing non-native invasive plants.

Although it is generally accepted as good that the Atala has increased its range and distribution because of increased use of cycads for landscaping [24-27,42], the adult butterflies have expanded their oviposition choices during the past twenty years as well. That expansion includes many of the non-native introduced ornamental cycads found

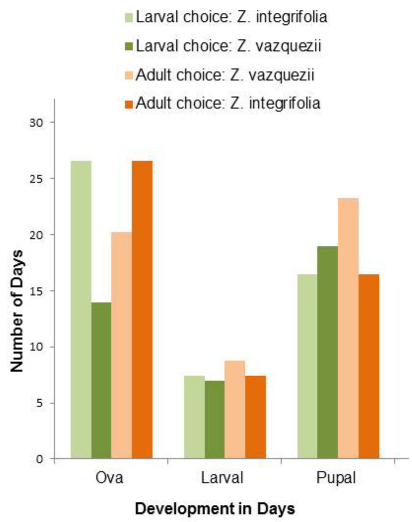

Figure 13: Comparisons of development time per life stage and adult lifespan between larval and adult choice trials. Difference was dependent on whether the adults or larvae chose the host plant, as well as which host plant was chosen (Photo credit: Koi S).

in south Florida's botanical and domestic gardens [22-27,42]. The larvae are able to successfully complete their life cycle on many, if not most, of these non-native plants [22-26].

This expansion into exotic Cycadales, many of which are extremely rare and valuable, has in turn increased potential struggles between home-owners, botanists, property managers and city planners with park managers, biologists, entomologists and conservationists as the Atala butterfly larvae attack these urban cycads [1,14,22,24-26]. Fortunately there have been no records of the Echo Moth using any of these non-native cycads as host plants at this time, although the moths do find their way to urban gardens occasionally.

Conservation methods are complicated because the often severe herbivory caused by the Atala larvae may damage the plant, but control of the larvae may impact the fragmented populations of the butterfly. The butterfly, like the coontie plant in the wild, is locally abundant but rare. The Atala is also currently listed as imperiled by the State of Florida, and the two natural ecosystems where coontie grows, the pine rocklands and tropical hardwood hammocks, are both now recognized as endangered (MDC undated; Myers;). The question of how to address one endangered species' natural but possibly destructive interactions with another endangered species is a dilemma faced by conservationists worldwide [1,14,22].

In Eastern Oregon and Washington, for example, forestry managers face similar conflicts between balancing pest insect species, such as mosquitoes, while protecting scores of threatened insects that live in the tracts of forested area [53]. The paper points out that many insect species are ephemeral, and/or diminutive; that they are therefore difficult to monitor, challenging to find, may live in scattered fragmented habitats, but are nonetheless vital to overall health of the forest. Areas bordering urban localities are especially vulnerable; LaBonte et al. [53] point out the need for mosquito control spraying in recreational areas, controlled burns to maintain forest ecology, road building for access to locations within the forest, changes in soil chemistry from compaction and erosion, herbicide use to control unwanted invasive plant species; the possible extirpation of the *Polites mardon* butterfly and a flightless beetle, *Agonum belleri*, may have been caused because of these challenges.

Urban interface areas such as this become classic fields of controversy when conflicts between human wishes and endangered species are involved, in any country, in any state. The environmental impacts of pesticide use, herbicides and other chemical means of controlling pests while managing wildlife is extremely complex to manage [29-31].

Conclusion

Hand-Management and IPM Practices for Atala Butterflies

Hand management practices must evolve as we learn more about the requirements of the Atala butterfly, including landscape architecture, specific nectar sources and host plants [42]. Recommendations involve a number of protocols, some of which have not yet been defined for this species. Pesticides to control unwanted insect pests such as mealy bugs should be used sparingly in butterfly gardens as non-target insects, such as Lepidoptera, are often impacted adversely by their use [30,54-56].

Planting native and non-native together may be another possible management protocol for protecting valuable cycads, as intercropping has been shown to be an effective pest management for conservation [40]. Because the Atala prefers native to non-native cycads, utilizing more native plants would be beneficial. One of the tricks to any successful butterfly garden is to move highly attractive host plants to areas where they will not be scrutinized by the public because complaints from people about herbivory is one of the biggest reasons the host plants get torn out of public venues [24,26,42]. It is a shame that rather than using the herbivory to teach the public about the insects, the plants are removed (often replaced by undesirable non-native vegetation). Atala butterflies use these pockets of coontie that are planted in public areas as stepping stones for dispersal [24,26,27,42,57], and it is certain that other butterflies need some form of connectively between fragmented suitable patches embedded within a concretized urban matrix.

Cultural management methods could be used to prevent herbivory, such as covering the most valuable plants while adult butterflies are active. A tomato cage covered with tulle mesh and buried into the ground an inch or so will prevent female ovipositioning on the plants. The adult Atala females will flatten their wings 'airplane style' to crawl into tight places, so the mesh must be securely closed on all sides and along the bottom.

Hand-removal of eggs on either *Z. integrifolia* or *Z. vazquezii* is easy with a thumb nail as they are not strongly adhered. The eggs can be frozen to humanely dispatch the embryos or simply left in the substrate where the ants and other insects will eat them. Although it can be time-consuming, hand-removal is certainly less expensive than buying a new cycad, especially since some valuable plants can cost thousands of dollars! If larvae hatch, they will most likely be unable to find food before dying as the larvae must find food within a few hours after eclosure or they will die.

Because it requires more time to develop on non-native *Z. vazquezii*, there is additional time for owners to remove the larvae from the plant. Contacting the local chapter of the North American Butterfly Association in southeast Florida, or the local Institute of Food and Agricultural Services (IFAS) Extension office Master Gardeners program will help find new 'foster homes' for unwanted or excess Atala larvae or pupae. Contacting the parks and natural areas management staff may welcome hosting this beautiful butterfly and be willing to take excess Atala. It is highly suggested not to carelessly introduce this butterfly outside of its native range (southeast Florida, south of Lake Okeechobee and east of the Everglades) [24,25]; the consequences of introducing even native species outside their normal range could have economically devastating results (a very destructive new herbivore attacking valuable nursery plants, for example). However, the butterfly is surviving in self-established colonies as far north as Martin County now, undoubtedly in response to changing climatic conditions as average temperatures increase.

And lastly, a thought from the world-renowned cycad botanist, Rolf Oberpreiler [1]: The natural insect fauna of cycads should not be brazenly dismissed as 'pests.' These insects are a natural and mostly vital component of the environment of the cycads, and their destruction can have severe impacts on the survival of the plants. The most obvious examples in this regard are the pollinators, but other insects may also play important roles in e.g., the disintegration of the cones and release of the seeds, the decomposition of old cones, leaves and stems, the recycling of nutrients, etc., their survival is so inextricably attached to that of the plants that extinction of their host plants will inevitably lead to the extinction of these insect species.

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