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Research article

Selaginella and the Satyr: *Euptychia westwoodi* (Lepidoptera: Nymphalidae) Oviposition Preference and Larval Performance

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Abstract

Members of the plant genus *Selaginella* (de Beauvois 1805) have few known insect herbivores even though they are considered by some to be 'living fossils', with extant taxa virtually indistinguishable from 300 Mya fossils. Butterflies are well-known herbivores, and the satyrs are among the most speciose of them despite having radiated ~35 Mya ago. Nearly all satyrs feed on grass or sedges, but members of the Neotropical genus *Euptychia* Hübner 1818 feed on *Selaginella*; little is known about the degree to which this butterfly favors this ancient plant over those that its close relatives utilize. To advance our knowledge of *Euptychia* natural history, we conducted a series of experiments to examine oviposition preference and growth rates across a series of potential host plants on a *Euptychia westwoodi* population in Costa Rica. We found that *Euptychia westwoodi* Butler 1867 exhibit a strong preference to oviposit on *Selaginella eurynota* over the sympatric *Selaginella arthritica*, though they perform equally well as larvae on both plants. We did not observe oviposition on a sympatric grass that is commonly consumed by close relatives of *E. westwoodi*, and when larvae were offered the grass they refused to eat. These results suggest that *E. westwoodi* in Costa Rica exhibit a strong preference for *Selaginella* and may have lost the ability to feed on a locally abundant grass commonly used by other Satyrinae.

Key words: Lepidoptera, herbivory, specialist, larvae

Butterflies of the family Nymphalidae underwent a period of rapid diversification during the late Cretaceous period, ~90 Ma, resulting in ~6,000 extant species in ~540 genera (Wahlberg *et al.* 2009). The timing of the nymphalid radiation is highly correlated with the diversification and spread of the angiosperms (their primary host plants), such that this pattern is considered a classic example of coevolution (Ehrlich and Raven 1964, Crane et al. 2005). The Satyrinae is the most speciose subfamily within the Nymphalidae, containing ~2,200 species in ~200 genera (Hamm and Fordyce 2014), and experienced a burst of diversification in the Oligocene (~25 Ma [million years]) (Peña 2009). Consistent with the coevolutionary hypothesis, this expansion is associated with diversification of the satyr's primary host plants, which are graminoids, such as Poaceae and Cyperaceae (Strömberg 2005, Peña 2009, Spriggs et al. 2014). Graminoid feeding is likely an ancestral condition in the Satyrinae as this regime is found in the vast majority of satyrs (Ackery 1988, Peña 2007).

The satyrine genus *Euptychia* Hübner 1818 is found in the Neotropics but does not follow the dietary trends of its relatives (e.g., other members of the subfamily or tribe Euptychiini). Although there are records of *Euptychia* feeding on Poaceae (Beccaloni et al. 2008, Janzen and Hallwachs 2009), species within this genus primarily feed on two plant lineages, the Selaginellaceae (Lycopsidophyta) and Neckeraceae (Bryophyta) (Singer et al. 1971, Singer and Mallet 1985, DeVries 1987, Mound et al. 1994). These are interesting host plants for *Euptychia*, not only because these are not graminoid plants, but also because they are two of the oldest plant lineages known (Finet et al. 2010).

Members of the Lycopsidophyta reached peak diversity during the Carboniferous period (~310 Ma) and are among the ancestral taxa to the gymnosperm/angiosperm radiation (Nickrent et al. 2000, Banks 2009). The genus *Selaginella* (de Beauvois 1805) is typically considered a 'relict' species as it has exhibited very little phenotypic change over the last 300 Ma, yet few insects feed on it (Mound et al. 1994, Banks 2009). Only 16 species from 6 orders of insects have been reported to feed on *Selaginella*, yet there are multiple accounts of *Euptychia* spp. feeding on *Selaginella* (Singer et al. 1971, DeVries 1987, Mound et al. 1994, Janzen and Hallwachs

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Fig. 1. *E. westwoodi* larva feeding on *S. eurynota* at the La Tirimbina Biological Reserve. Photo by Philip J. DeVries.

2009). It is intriguing that *Euptychia* would make the apparent switch to *Selaginella* and mosses considering these plants typically grow in nutrient poor soils and are thought to have less nutrient content relative to the more derived plant graminoids (Scriber and Slansky 1981, Egorov 2007).

Despite this apparent transition to feed on what are considered nutrient poor and very old plant lineages, little is known about the oviposition and feeding ecology of *Euptychia* on *Selaginella*. We set out to address outstanding questions in *Euptychia* ecology, such as what is the oviposition preference for different plants, what is the degree of feeding specialization on different *Selaginella* species, and at what rate do larvae add mass under different feeding conditions? To this end we conducted a series of 'buffet' style oviposition experiments and no-choice feeding using *Euptychia westwoodi* Butler 1866, a species of *Euptychia* common to the lowland forests of Costa Rica that has only been reported feeding on one species of *Selaginella*, *S. eurynota* Alston 1935 (DeVries 1987) (Fig. 1).

Materials and Methods

Oviposition Preference

A series of buffet style oviposition experiments were conducted at La Tirimbina reserve between 1 and 14 May 2015. Sprigs of *S. eurynota*, *Selaginella arthritica*, and the grass *Lasiacis ruscifolia* (Kunth) Hitchc. 1911 were collected in the field and immediately placed in water. This grass was chosen because it is host plant to other Euptychiini species and is commonly found with *S. eurynota and S. arthritica* (DeVries 1987, Janzen and Hallwachs 2009). At the same time, female *E. westwoodi* were collected in the field and then all specimens were returned to the lab and experimental chambers prepared. Each experimental chamber consisted of a 16 oz. plastic cup with perforations at the base that allowed plants stems to be laced through and a lid made of a coffee filter with perforations to allow air flow. This apparatus was then set in water so the plant stem-ends were submerged in water and the samples could retain turgor pressure, while the bottom of the chamber itself was dry. One sprig of each plant species (of approximately the same size) was placed in the chamber. A single *E. westwoodi* female was then placed in the chamber and the lid was placed to prevent escape. We sprayed the filter paper with a sugary electrolyte solution *ad libidum* to provide a carbohydrate source for the butterfly. Each replicate ($n = 13$) was run for 48 h, after which time the female was removed and eggs were counted on each plant.

Following the oviposition experiments we analyzed the data using a hierarchical Bayesian framework, as implemented in the R package *bayespref* (Fordyce et al. 2011). This framework uses the count data (number of eggs deposited on a plant by individual) and allows direct estimation of parameters of interest, in our case the preference for putative host plants. In order to generate a reasonable posterior distribution density from the MCMC, we sampled the 1,000th step from each of 1,000 MCMC runs that we conducted.

Larval Performance

Larval feeding experiments were conducted at both the La Selva Biological Research Station and the Tirimbina Biological Reserve (10° 22′ 0N, 84° 7′ 60W) in Costa Rica between 18 and 23 May 2010. These sites are located in lowland tropical wet forest of Sarapiqui County, Heredia province. Larvae of *E. westwoodi* (third and fourth instars) were collected from *S. eurynota*, a locally abundant species. We were restricted to using larvae of this size because the balance at the field station could not reliably measure smaller larvae. In total, 54 *E. westwoodi* larvae were collected in the field. Once in the laboratory, each individual larva's mass was measured to the nearest 0.01 mg using a SM-50 semimicro balance (Curtiss-Wright Corp.). Larvae were then haphazardly placed into one of three treatment groups, each with an initial $n = 18$.

Individual larvae were placed into 2 oz. sealable containers with the respective food plant for that treatment. Treatment group S_1 was offered *S. eurynota*, the host plant the larvae were collected on; treatment group S_2 was offered *S. arthritica*, another *Selaginella* species found sympatrically with *S. eurynota* (indeed, right next to); and treatment group G_1 were offered the grass *L. ruscifolia*. All treatment groups were offered fresh, undamaged plant material *ad libidum* in the morning and again in the evening. The experiment was maintained in an open-air laboratory under ambient conditions (~25°C, 80% RH) with containers placed haphazardly relative to the room's window. Larvae were allowed to feed for 48 h., after which time the mass of all larvae was measured.

We asked if there were difference in final mass among treatment groups using ANOVA followed by Tukey's post hoc and implemented in R 3.2.2 (R Core Team 2015). All data and the code necessary to reproduce the results presented here are freely available on FigShare (https://dx.doi.org/10.6084/m9.figshare.3083320.v1). All comparisons were considered statistically significant at $\alpha = 0.05$. Because we used third and fourth instars in the feeding trial, we also considered that mass gain might covary with initial mass. Therefore, we also modeled final mass as explained by treatment with starting mass as a covariate.

Results and Discussion

We observed 113 eggs deposited during the course of our experiment, of which 111 were deposited on *S. eurynota*. Plotting the posterior densities for each individual indicated a strong preference for *S. eurynota* (Fig. 2). After 48 h of no-choice trials, the final experimental group sizes were: *S. eurynota* $n = 18$, *S. arthritica* $n = 17$, and *L. ruscifolia* $n = 17$. *E. westwoodi* larvae offered either *Selaginella* were observed feeding, and most gained mass over the course of the experiment (Fig. 3-S1, S2). One individual from the *S. eurynota* group lost mass and expired shortly after the conclusion of the feeding trial. Necropsy revealed what appeared to be fungal mycelia, which filled the body cavity. Additionally, one individual from each of the *Selaginella* groups maintained mass during the experiment. In these cases, the individuals molted (and eventually

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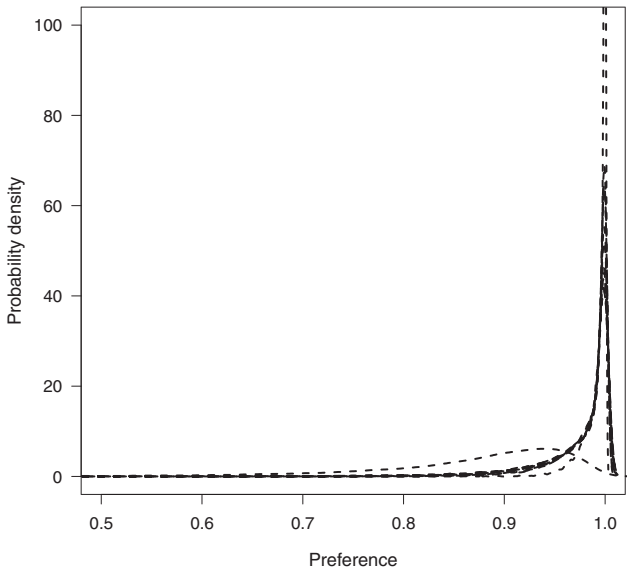

Fig. 2. Host plant preference of *E. westwoodi*. Each curve indicates the posterior density of individual-level preference for *S. eurynota*

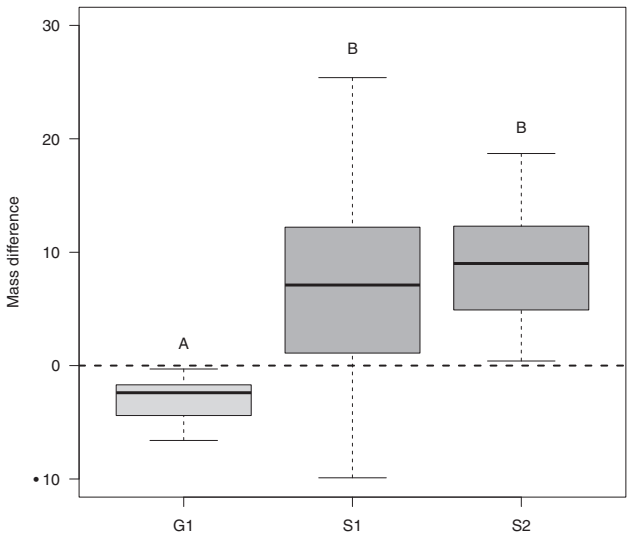

Fig. 3. Boxplots of representing the difference in mass from the start to the end of the larval *E. westwoodi* feeding experiment, dashed line indicates no difference between starting and ending mass. *E. westwoodi* larvae were fed: G_1 = *L. ruscifolia*, G_1 = *S. eurynota*, G_2 = *S. arthritica*. Letters indicate statistically significant differences ($P < 0.001$, Tukey's HSD).

pupated), which suggests they that each would have continued to gain mass had the experiment continued.

Contrary to expectations, larvae offered *L. ruscifolia* were never observed feeding and all individuals in this treatment lost mass during the trial (Fig. 2, G1). This was surprising given that *L. ruscifolia* is a common host plant of close relatives of *Euptychia* (DeVries 1987, Beccaloni et al. 2008, Janzen and Hallwachs 2009), and because grasses are generally considered to have low levels of allelochemicals relative to other angiosperms (Ackery 1988). Behaviorally, larvae placed with *L. ruscifolia* would crawl over leaf plant material but were never observed feeding; in contrast to *Selaginella* groups, which appeared to feed continuously. When fresh food was exchanged for old, we microscopically examined *L. ruscifolia* leaves for damage and observed none.

Given these observations, it was not surprising that mass gain varied among host plants ($F_{2,49} = 19.37$, $P < 0.001$). The average mass change (± SE) over the 2 days the experiment were as follows: *S. eurynota* = 7.74 mg (±2.20), *S. arthritica* = 8.91 mg (±1.277), *L. ruscifolia* = −3.54 mg (±0.67). The model using final mass as a response and initial mass as a covariate approach gave similar results ($F_{5,46} = 40.01$, $P < 0.001$). Tukey's post hoc-tests on both modeling approaches indicated that both *Selaginella* diets resulted in greater mass gain compared with the *L. ruscifolia* diet, and that there was no difference between the *Selaginella* diets ($\alpha = 0.05$). Once the no-choice trials were concluded, half of the larvae in the *L. ruscifolia* treatment group were offered *S. eurynota*, or maintained on *L. ruscifolia*. All larvae switched to *S. eurynota* immediately resumed feeding and eventually completed metamorphosis, while individuals that remained on *L. ruscifolia* expired within 96 h.

Our data suggest that *E. westwoodi* in the Heredia Province of Costa Rica exhibit a strong preference for oviposition on *S. eurynota* while retaining the ability to feed on at least one other species of *Selaginella*. Even though *E. westwoodi* laid eggs almost exclusively on *S. eurynota*, larvae retained the ability to feed on *S. arthritica*. We did not observe *E. westwoodi* oviposition or feeding on the grass *L. ruscifolia*. These data suggest that *E. westwoodi* in the Heredia Province of Costa Rica are specialized on *Selaginella*, it is premature to make that claim for the species as a whole for a number of reasons. There are many instances of local herbivore populations evolving some degree of host specificity that the species as a whole does not exhibit. This phenomenon has been referred to as the 'mosaic pattern of coevolution' (Thompson 1994, 2005) and is commonly recognized in butterflies. Furthermore, we know from different regions that other *Euptychia* feed on both grasses and *Selaginella* (Janzen and Hallwachs 2009). Additionally, it is possible that *E. westwoodi* is capable of feeding on grasses other than *L. ruscifolia*, and was not offered a suitable alternative.

Another possible explanation for the refusal of *L. ruscifolia* was the use of third and fourth instars. Other researchers have demonstrated *Pieris rapae* (Lepidoptera: Pieridae) larvae will reject host plants if switched during later instars (Karowe 1989, Renwick and Lopez 1999). We cannot discount this, but note that transferring *E. westwoodi* larvae from *S. eurynota* to *S. arthritica had no* apparent effect on mass; however, the magnitude of difference between *Selaginella* and *Lasiacis* could be much greater than the differences among *Selaginella*. In contrast, other Lepidoptera are capable of switching host plants at later instars and still complete development (Scriber 1979, 1982). A longer experiment using neonate caterpillars and additional alternative host plants are needed to further understand the degree of host specialization present in *E. westwoodi*.

Given these results and the apparent specialization of *E. westwoodi*, what is the diet breadth of *Euptychia*? This is not an easy question given the high level of divergence among *Euptychia*'s three host plants. Using a metric such as Faith's Phylogenetic Diversity (PD) index (Faith 1992, Symons and Becalloni 1999) and functions in the 'picante' package in R (Kembel et al. 2010), *Euptychia* has an unrooted PD of ∼0.578 (branch lengths from Finet et al. [2010]). This value is strongly influenced by the phylogenetic distance between Neckeraceae, Selaginellaceae, and Poaceae. For example, this influence is observed when analyzing the genus *Adelpha*, which has a PD of 0.576, but feeds on 22 host plant families. Other methods that consider host breadth, such as the ordinated diet breadth (ODB) (Hamm and Fordyce, 2014, Fordyce et al. 2016), may provide a more intuitive answer to the question of diet breadth. ODB asks, based on insect diets, what is the relative diet breadth of a taxon compared with all other taxa in the data set (Fordyce et al. 2016)? Using this metric,

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Euptychia occupies 1.4% of the total potential diet space, whereas *Adelpha* occupies 13.2%, which seems more in line with the number of host families that these genera are known to feed on.

To the best of our knowledge, these are the first data reporting insect performance on *Selaginella*. These data are useful but indicate other experiments should be performed. Future directions for research on *Euptychia* diet breadth include: beginning the experiment with eggs, conducting the experiment for a longer period, and comparing final adult dry mass and size among experimental groups. In addition, it would be useful to investigate other *Selaginella* feeding insects and butterflies, such as the two Oriental satyr genera, *Acrophtalmia* and *Ragadia*. Both have been reported to feed on *Selaginella*, and would provide an important comparative framework for future work (Igarashi and Fukuda 1996).

Conclusions

Females exhibited a strong preference to oviposit on *S. eurynota*, with 111 of 113 eggs being deposited on this species and none on the grass *L. ruscifolia*. Larval (third and fourth instar) *E. westwoodi* feeding on *Selaginella* gained an average of $\geq$10 mg during a 2 day no-choice feeding experiment, while larvae offered a common grass refused to feed and lost $\sim$3 mg during that same time. Larvae that were initially offered grass, but were later switched to *Selaginella*, resumed feeding and eventually completed metamorphosis. A number of issues warn against a broad interpretation of these results, and calling *E. westwoodi* a specialist based on these results would be over reaching. However, it appears that third and fourth instar *E. westwoodi* from the Sarapiqui region of Costa Rica were adapted to *Selaginella* and incapable of feeding on a common grass.

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