

## Research Article

# Ant-Related Oviposition and Larval Performance in a Myrmecophilous Lycaenid

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We experimentally assessed ant-related oviposition and larval performance in the Miami blue butterfly (*Cyclargus thomasi bethunebakeri*). Ant tending had sex-dependent effects on most measures of larval growth: female larvae generally benefitted from increased tending frequency whereas male larvae were usually unaffected. The larger size of female larvae tended by ants resulted in a substantial predicted increase in lifetime egg production. Oviposition by adult females that were tended by *C. floridanus* ants as larvae was similar between host plants with or without ants. However, they laid relatively more eggs on plants with ants than did females raised without ants, which laid less than a third of their eggs on plants with ants present. In summary, we found conditional benefits for larvae tended by ants that were not accompanied by oviposition preference for plants with ants present, which is a reasonable result for a system in which ant presence at the time of oviposition is not a reliable indicator of future ant presence. More broadly, our results emphasize the importance of considering the consequences of variation in interspecific interactions, life history traits, and multiple measures of performance when evaluating the costs and benefits of mutualistic relationships.

## 1. Introduction

For egg-laying animals that do not provide parental care, oviposition location is among the most important maternal decisions affecting subsequent offspring performance [1, 2]. Consequently, when growth and survival of immature stages are strongly influenced by other species—either positively or negatively—spatial patterns of oviposition often reflect these interactions [3–7]. In mutualistic relationships, we might expect preferential oviposition and improved performance when and where mutualists are present, but tradeoffs and constraints in both oviposition choices and immature growth strategies may make such simple correlations rare.

Most lycaenid butterfly species interact with ants, and many of these relationships include substantial benefits for lycaenid larvae [8]. However, the costs and benefits of ant tending for larval growth of lycaenids vary substantially among systems. For example, larvae of *Jalmenus evagoras* and *Glaucopsyche lygdamus* pupate at a greater mass when

untended by ants [9–11] but are dependent on ants for protection under natural conditions. By contrast, larvae of *Paralucia aurifera* and *Hemiargus isola* pupate at a greater mass and developed faster when reared with ants [12, 13]. Other studies have found that the consequences of ant tending on larval performance are conditional upon the sex of the larvae [14] or the identity of the tending ant species [13, 15]. In a comparative study of five lycaenid species, Fiedler and Saam [16] suggested that compensatory or slightly overcompensatory growth in response to the costs of nectar production for ants may be common among facultatively myrmecophilous lycaenids.

Although interactions with ants influence lycaenid growth decisions, they are also subject to many of the same environmental pressures and life history tradeoffs experienced by other immature insects. In particular, the size and age at which metamorphosis occurs are important life history traits for organisms with complex life cycles, particularly for taxa in which most or all growth occurs during immature

development [17, 18]. Large phenotypes often confer substantial advantages, but theoretical and empirical studies suggest that physiological limitations, tradeoffs in resource allocation, and variation in environmental conditions may constrain both large size per se and the growth rates required to attain larger sizes [19, 20]. For many insects, the primary benefit of larger size is higher potential reproductive output and the primary risk of prolonging the immature growth period to accommodate the larger size at maturity is increased exposure to predators and parasitoids [17, 21, 22]. Behavioral and physiological adaptations to deal with this tradeoff include altering the location and timing of feeding to minimize predation and the evolution of phenotypically plastic growth rates that allow rapid growth when resources are abundant or risks associated with growth are low [23–26].

Ant-related oviposition by lycaenids is somewhat less studied but appears to be at least as variable as ant-related larval growth. Atsatt [27] proposed that ovipositing females should evolve searching behaviors for ants when ant abundance and predictability are high and should be accompanied by obligate and usually species-specific larval relationships with ants. Pierce and Elgar [28] suggested a more continuous relationship in which the degree of preference demonstrated by ovipositing lycaenids for host plants where ants are present should correspond with the importance of the ant interaction for larval survival and growth. Several individual studies and comparative empirical data support this hypothesis [29–31], but relatively few lycaenid species have been shown to preferentially oviposit on host plants with ants present in experimental settings.

Oviposition decisions are complex and may not be correlated with offspring performance for a variety of reasons. For example, in stochastic environments ovipositing females may not discriminate among potential oviposition sites because there are no reliable indicators of future offspring performance at the time of oviposition [32]. Alternatively, variation in natal experiences (e.g., habitat or diet) may affect oviposition decisions later in life [33–35]. Additionally, for short-lived species or older females, optimal oviposition preferences may be superseded by the evolutionary imperative to distribute eggs prior to death [36]. Therefore, we would only expect strong oviposition preference for mutualists in systems that meet the following criteria: (1) the interaction is very important for immature performance, (2) the conditions at the time of oviposition accurately indicate mutualist presence, or (3) the immature organisms have low likelihood of encountering mutualists if they are initially absent [1].

We experimentally tested effects of ant interactions on larval performance and adult oviposition for the Miami blue butterfly (*Cyclargus thomasi bethunebakeri*). This lycaenid engages in facultative mutualistic interactions with several ant species [37], but preliminary laboratory studies suggested that ant tending had no effects on the timing of larval development or size at pupation [38]. However, based on sex-dependent effects of size on reproductive performance [39], production of nutritional rewards for ant defenders, and nearly constant tending by ants [37], we expected frequency of ant tending to influence larval development in a more detailed study. We then tested the effects of interaction with



FIGURE 1: *Camponotus floridanus* workers tending two fourth instar *C. thomasi bethunebakeri* larvae. Note the everted tentacular organs in the upper larva and the ant consuming secretions from the dorsal nectary organ of the lower larva.

ants during the larval stage and female age on oviposition preference for host plants with and without ants present. We interpret the results of these studies in the context of oviposition and larval growth theories, with particular attention to how and why this system deviates from a simple positive correlation between oviposition preference and larval performance.

## 2. Materials and Methods

**2.1. Study System.** The Miami blue butterfly is a small lycaenid native to southern coastal Florida and the Florida Keys, USA, with other *C. thomasi* subspecies occurring elsewhere in the Caribbean. Formerly abundant in suitable habitats, the Miami blue butterfly declined in the 20th century due to a variety of anthropogenic and natural factors; at the time this study was conducted, the subspecies occurred in small populations on Bahia Honda in the middle Keys and several islands in Key West National Wildlife Refuge [40]. All butterflies used in this research were part of a captive colony originating from wild stock collected at Bahia Honda State Park, where the larvae apparently feed exclusively on gray nickerbean (*Caesalpinia bonduc*).

Females oviposit on leaf or flower buds or young leaves, usually singly as they walk along the plant. The first and second instar larvae feed cryptically in flower or leaf buds and are not usually tended by ants. However, after molting into the third instar, Miami blue butterfly larvae are commonly tended by ants while feeding on the host plant (Figure 1); *Camponotus* species are the most frequent ant associates [37, 38, 41]. Saarinen and Daniels [37] reported that all late-instar larvae found in the wild were tended by ants, but this may be due to the high detectability of larvae when surrounded by large and active ants. Larvae reared with *Camponotus floridanus* in laboratory conditions are rarely untended and regularly evert paired tentacular organs and secrete nectar from their dorsal nectary organ in response to ant presence [38].

**2.2. Larval Performance Experiment.** To generate variation in ant tending frequency, we reared Miami blue butterfly

larvae in plastic trays (27 × 19 × 9.5 cm) in which we placed 0, 10, 20 or 30, workers of *Camponotus floridanus*, one of the ant species that most commonly tends larvae in the wild [37, 38]. We placed two larvae in 10 replicate trays for each ant abundance treatment for a total of 80 larvae. At the beginning of the experiment the larvae were all the same age (8 d) and similar in wet mass (1.43 mg ± 0.04 SE,  $n = 80$ ). Each larva was placed in an open 150 mL plastic vial with cuttings of new growth from the host plant, gray nickerbean (*Caesalpinia bonduc*), to allow access by the ants but reduce larval wandering. We replaced the host plant and vial daily and transferred larvae from the old to the new cuttings with a sterilized paintbrush. We measured the mass of each larva every morning with a digital analytic balance accurate to 0.01 mg (Denver Instruments SI-215D). We recorded ant tending of each larva twice each day (~900 h and ~1600 h). Behaviors recorded as tending included antennating the larvae, consuming secretions from the dorsal nectary organ or running on or around the larvae. Upon pupation, we measured pupal mass and placed the pupae in individual closed vials without ants in the laboratory until adult emergence. When the adult butterflies emerged, we calculated the duration of the pupal stage, measured the forewing length, and noted the sex of each adult.

We tested the effects of ant abundance treatment (10, 20, or 30 ants), larval age, and time of observation (morning or afternoon) on frequency of ant tending with a generalized linear mixed-effects model with a binomial error and logit link that included larva within tray as a random variable. The age-specific probability of tending by ants for each of the three ant abundance treatments was estimated with the lmer function in the R package lme4 [42].

We tested the effects of ant tending frequency (measured as the overall proportion of observations in which tending was observed) and sex of larvae on the following growth parameters: maximum larval mass, age at maximum larval mass, percent loss of mass from the final larval instar to the pupa, pupal mass, age at pupation, duration of the pupal stage, and total combined duration of the immature stages. For the analysis of duration of the pupal stage, we included pupal mass as a predictor variable, and the analyses that tested sex as a predictor variable only included the 73 individuals (38 males and 35 females) that survived to adulthood. These relationships were tested with linear mixed-effects models in the R package nlme [42] and  $P$  values were evaluated against a table-wide false discovery rate of 0.05 for each predictor variable to control for multiple comparisons [43]. Although this approach resulted in multiple related tests, controlling for the false discovery rate allowed us to make inferences about stage-specific differences in growth due to sex and ant tending without overinflating Type I error.

Our previous work found a strong positive relationship between forewing chord length and egg production for females but found no effect of size on male reproductive performance [39]. To test the explanatory power of pupal mass for adult size, thereby linking larval growth to a correlate of female fitness, we analyzed a linear model testing the effects of pupal mass and sex on forewing chord length. For this

analysis, we included all of the individuals from this study and those from an earlier experiment [38].

**2.3. Oviposition Preference Experiment.** The oviposition experiments were conducted in small screen flight cages in which we placed two plastic trays containing cuttings of the host plant either with or without *C. floridanus* ants. Cages were placed under incandescent lights on a timer set to a 14 hr light/10 hr dark cycle with some intermittent dark periods during the day to stimulate activity and prevent overheating. Each day, we replaced the host plant cuttings, counted the previous day's eggs, alternated the position of the ant treatments, and provided fresh nectar sources for the female butterflies. Preliminary trials and observations showed that *C. floridanus* did not consume or damage Miami blue butterfly eggs.

We first tested the effects of ant interactions during the larval stage on ant-related oviposition preferences of adult females. Four mated female Miami blue butterflies from one of the larval treatments (i.e., tended by *C. floridanus* or not) were placed in each of 6 screen cages and eggs were counted on host plant cuttings with and without *C. floridanus* ants every morning for 7 days. We then conducted a similar oviposition preference trial to test if butterflies responded to ant pheromones rather than the physical presence of ants on the host plant. For this experiment, we tested oviposition preference between one control cutting of the host plant and one cutting that had been exposed to *C. floridanus* for 30 min prior to placement in the flight cage but from which ants were absent during the trial. We conducted this experiment in 5 flight cages, each containing 4 naïve female Miami blue butterflies, for 5 days.

The number of eggs varied substantially among days and cages within experiments, so we used a standardized measure of preference (the number of eggs on the control plant minus number of eggs on the plant with ants) as the response variable for analysis. We calculated this daily difference in egg number for each cage every day that eggs were present on at least one plant. To evaluate the effects of natal experience (i.e., naïve or ant tended) on oviposition preference, we tested a mixed-effects model with larval experience with ants and day as fixed effects and cage as a random grouping variable. For the test of the effects of ant pheromones on oviposition preference, we analyzed a mixed-effects model with day as a fixed effect and cage as a random grouping variable. We complemented these more detailed linear mixed-effects analyses with chi-squared tests on the cumulative egg distributions for the entire experiment.

### 3. Results

**3.1. Larval Performance Experiment.** The probability of tending by ants increased with larval age, and among treatment contrasts showed that tending was less frequent in the 10-ant treatment than in the 20- and 30-ant treatments throughout larval development (Figure 2, Table 1). Over the course of the study, larvae in these three treatments were tended during 49% (±5% SE), 79% (±2% SE), and 74% (±3% SE) of

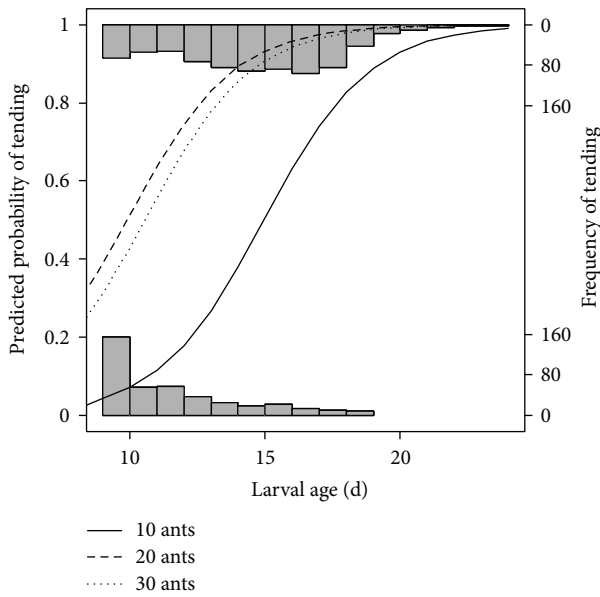


FIGURE 2: Fitted logistic regression curves showing the predicted probability of ant tending (left  $y$ -axis) as a function of larval age for the three ant abundance treatments in this study. The histograms show the frequency of observations in which tending occurred (upper right  $y$ -axis) or did not occur (lower right  $y$ -axis) each day for the three ant abundance treatments combined. Results of statistical analyses on the effects of ant abundance treatment and larval age are in Table 1.

TABLE 1: Results of generalized linear mixed-effects model testing the effects of ant abundance treatment (10, 20 or 30 ants), larval age (d) and time of observation (morning or afternoon) on the probability that ants would be tending Miami blue butterfly larvae.  $\beta$  is the coefficient describing the shape of the logistic curve (Figure 2). The table below presents the tests of contrasts among levels of ant abundance (the  $P$ -value of the 10 ant treatment is from a test against the null of  $\beta = 0$  and the  $P$ -values associated with the 20 and 30 ant treatments indicate the difference between those treatments and the 10 ant treatment). The interaction term between ant abundance treatment and age of larvae was not significant so was removed to more accurately assess the main effects.

| Variable                | $\beta$ ( $\pm$ SE) | $z$ -value | $P$     |
|-------------------------|---------------------|------------|---------|
| Ant abundance treatment |                     |            |         |
| 10 ants                 | -7.69 (0.59)        | -13.10     | <0.0001 |
| 20 ants                 | -5.09 (0.45)        | 5.84       | <0.0001 |
| 30 ants                 | -5.43 (0.45)        | 5.02       | <0.0001 |
| Age of larvae           | 0.51 (0.034)        | 15.16      | <0.0001 |
| Time of observation     | 0.065 (0.16)        | 0.41       | 0.68    |

observations, respectively; larvae in the no ant treatment were obviously not tended.

The results of models testing ant tending frequency and sex on larval growth parameters are in Table 2. Note that many of the larval growth parameters were highly correlated, so the tests for sex and ant tending as predictor variables at a given stage are also related to those for other growth responses. For example, maximum larval mass strongly

TABLE 2: Results of linear mixed-effects model testing the effects of ant tending frequency and sex on growth parameters of Miami blue butterfly larvae. Non-significant interaction terms were omitted from some models to better estimate the effects of tending frequency and sex of larvae on growth responses. Uncorrected  $P$ -values are presented; test results lower than the table-wide false discovery rate for the corresponding predictor variable are indicated in bold italic font.

| Source of variation                     | Growth parameter tested |            |               |
|---|-------------------------|------------|---------------|
|   | df                      | $F$ -value | $P$           |
| Age at maximum larval mass              |                         |            |               |
| Tending frequency                       | 1, 32                   | 0.83       | 0.37          |
| Sex                                     | 1, 32                   | 0.13       | 0.72          |
| Tending frequency * Sex                 | 1, 32                   | 4.55       | 0.041         |
| Maximum larval mass                     |                         |            |               |
| Tending frequency                       | 1, 32                   | 15.30      | <b>0.0004</b> |
| Sex                                     | 1, 32                   | 12.61      | <b>0.0012</b> |
| Tending frequency * Sex                 | 1, 32                   | 4.54       | 0.041         |
| Percent mass lost during prepupal stage |                         |            |               |
| Tending frequency                       | 1, 32                   | 1.71       | 0.20          |
| Sex of larvae                           | 1, 32                   | 0.084      | 0.77          |
| Tending frequency * Sex                 | 1, 32                   | 6.55       | <b>0.015</b>  |
| Pupal mass                              |                         |            |               |
| Tending frequency                       | 1, 32                   | 5.55       | 0.025         |
| Sex                                     | 1, 32                   | 7.09       | <b>0.012</b>  |
| Tending frequency * Sex                 | 1, 32                   | 7.52       | <b>0.0099</b> |
| Age at pupation                         |                         |            |               |
| Tending frequency                       | 1, 32                   | 1.10       | 0.30          |
| Sex                                     | 1, 32                   | 0.022      | 0.88          |
| Tending frequency * Sex                 | 1, 32                   | 4.52       | <b>0.048</b>  |
| Duration of pupal stage                 |                         |            |               |
| Sex                                     | 1, 33                   | 4.64       | 0.039         |
| Pupal mass                              | 1, 33                   | 0.32       | 0.57          |
| Total duration of immature stages       |                         |            |               |
| Tending frequency                       | 1, 33                   | 1.45       | 0.24          |
| Sex                                     | 1, 33                   | 0.53       | 0.47          |

predicted pupal mass regardless of other variables but testing both responses showed that the interaction of tending frequency and sex affected the percent mass lost between these stages.

Male and female larvae both reached a maximum larval mass of approximately 70 mg when untended, but as tending frequency increased the maximum mass of female larvae increased whereas the maximum mass of males decreased. Tending frequency had a similar effect on the age at maximum larval mass: untended females required approximately 1 d longer than untended males to attain their maximum mass (16.31 d v. 15.4 d, resp.) but tending frequency decreased female development time (difference between never tended and always tended = -0.55 d) whereas it increased male development time (difference between never tended and always tended = +1.61 d). Across all ant abundance treatments, Miami blue butterfly larvae lost 20.8% ( $\pm$ 0.66 SE) of their mass during the prepupal stage (time from maximum

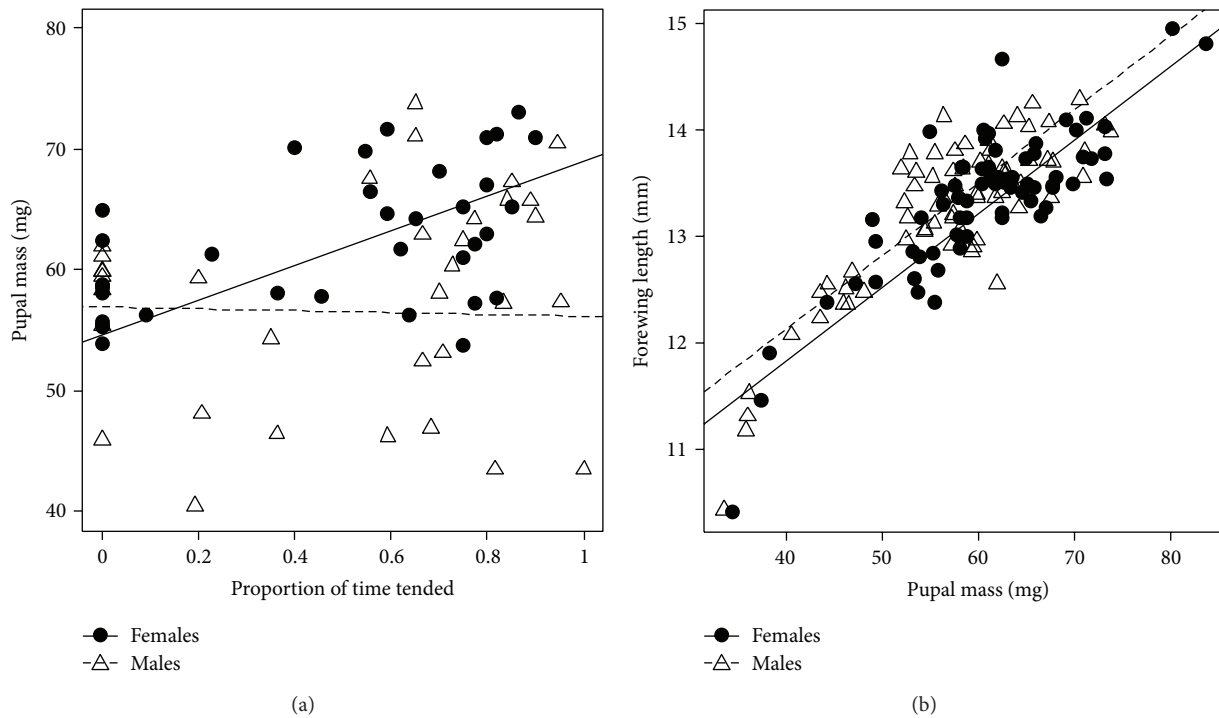


FIGURE 3: (a) Frequency of ant tending had sex-dependent effect on pupal mass, with female larvae pupating at a higher mass as tending increased whereas male larvae were unaffected by tending frequency. (b) Pupal mass and sex strongly predicted adult forewing chord length for Miami blue butterflies.

larval mass to pupation). This loss was influenced by sex and frequency of tending: the percent mass lost with no ant tending was significantly higher for females than for males ( $22.5 \pm 1.7$  SE and  $17.4 \pm 2.2$  SE, resp.;  $t = -2.27$ ,  $df = 32$ ,  $P = 0.03$ ) with the opposite relationship at very high levels of tending.

Female pupae were significantly larger than male pupae overall ( $61.9$  mg  $\pm 1.4$  SE and  $56.6 \pm 2.0$  SE, resp.;  $t = 2.6$ ,  $df = 71$ ,  $P = 0.011$ ), but pupal mass of males and females was differentially affected by frequency of ant tending during the larval stage in a pattern similar to that for maximum larval mass. The mass of female and male pupae was not significantly different when they were untended as larvae ( $54.6 \pm 2.5$  SE and  $57.0 \pm 3.3$  SE, resp.;  $t = 0.73$ ,  $df = 32$ ,  $P = 0.47$ ); however, tending frequency increased pupal mass for females whereas the pupal mass of males was unaffected (Figure 3(a)). The frequency of ant tending also had sex-dependent effects on the age at which larvae pupated: females pupated earlier in response to higher tending frequency whereas males pupated later as tending frequency increased. Despite the numerous sex-specific larval growth parameters, the total time spent in the immature stages was invariant with respect to either sex or the frequency of ant tending ( $29.66$  days  $\pm 0.17$  SE).

Both pupal mass and sex significantly predicted forewing length of adult butterflies ( $F_{1,135} = 344.3$ ,  $P < 0.0001$  and  $F_{1,135} = 18.9$ ,  $P < 0.001$ , resp.). There was no difference in the slope of the relationship between the sexes (i.e., no significant interaction term), but males had a longer forewing chord length for a given pupal mass (Figure 3(b)).

**3.2. Oviposition Preference Experiment.** Naïve female Miami blue butterflies raised without ants as larvae laid  $8.03$  ( $\pm 2.44$  SE) fewer eggs per day per cage on plants with ants present ( $df = 9$ ,  $t = 2.47$ ,  $P = 0.035$ ). However, for females tended by ants as larvae, the mean difference between the number of eggs on control plants and plants with ants was only  $1.99$  ( $\pm 2.65$  SE), which was not significantly different from no preference ( $df = 41$ ,  $t = 0.75$ ,  $P = 0.46$ ). Although neither treatment produced an absolute preference for ovipositing where ants were present, females reared with ants as larvae laid significantly more eggs on plants with *C. floridanus* ants present than naïve females (Table 3). Ant-related oviposition patterns did not change as the butterflies senesced. The cumulative frequency of eggs across all days and cages was consistent with the results of the linear mixed effects model analyzing the daily results: naïve female Miami blue butterflies deposited only 30.5% of their eggs on host plant cuttings with *C. floridanus* present, whereas female Miami blue butterflies that were tended by *C. floridanus* as larvae laid 45.2% of their eggs on host plant cuttings with *C. floridanus* present (Figure 4;  $\chi^2 = 15.26$ ,  $df = 1$ ,  $P < 0.0001$ ).

There was no effect of ant pheromones on oviposition preference when ants were not physically present. The mean difference between the number of eggs on control plants and those on plants exposed to ant trail pheromones was  $1.13$  ( $\pm 15.53$  SE) eggs per cage per day, which was not significantly different from no preference ( $df = 16$ ,  $F = 0.042$ ,  $P = 0.84$ ). This result was corroborated by the cumulative test: of 772 eggs laid throughout the course of this experiment, 371 (48.1%) were on plants exposed to *C. floridanus* pheromones

TABLE 3: Results of linear mixed-effects analysis testing the influence of larval experience with ants and day on mean daily difference in egg number between host plants without and with *C. floridanus* ants present.

| Source of variation       | df    | F    | P     |
|---------------------------|-------|------|-------|
| Ant interaction as larvae | 1, 9  | 5.93 | 0.038 |
| Day                       | 1, 41 | 0.22 | 0.65  |

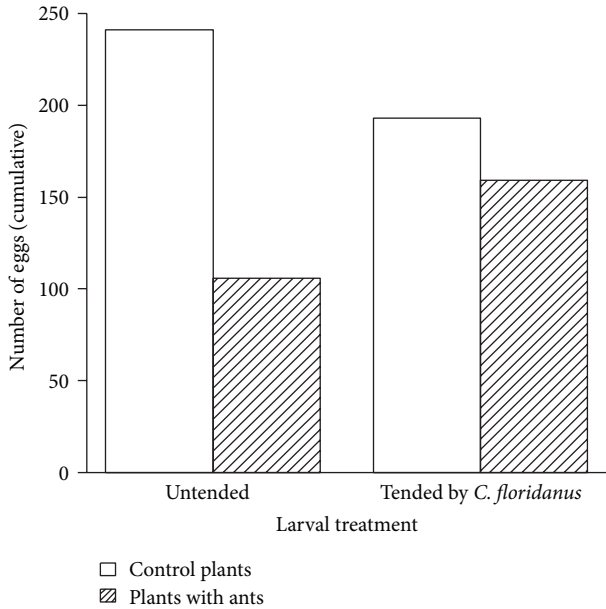


FIGURE 4: The total number of eggs laid by untended (naïve) females and females tended by *C. floridanus* as larvae on host plants with ants either present or absent. Although neither category of females preferred to oviposit on plants with *C. floridanus* present, the oviposition preference of those raised with ants did not differ from random egg distribution whereas the naïve females laid significantly fewer eggs on plants with ants.

and 401 (51.9%) were on control plants not exposed to ants ( $\chi^2 = 1.17$ ,  $df = 1$ ,  $P = 0.28$ ).

#### 4. Discussion

Identifying the costs and benefits of mutualistic relationships and investigating the conditions under which they manifest is invaluable for understanding the consequences of behavioral variation for life history strategies and fitness [44]. In the Lycaenidae, ant association may have a range of direct and indirect effects on development and survival [8, 45], and even relatively small differences in life history parameters due to interactions with ants can affect the evolution of lycaenid-ant mutualisms [46].

Our previous research demonstrated that wing length was positively related to higher lifetime fecundity for female Miami blue butterflies [39]. The present study showed that pupal mass increased with frequency of ant tending for female Miami blue butterfly larvae (but not for males) and that pupal mass and sex were reliable predictors of wing

length. By combining these results with previous findings, we estimate that always tended female larvae would pupate at 14.4 mg higher mass than untended larvae, a difference that would result in an additional 1 mm of wing length, which in turn would predict an increase in lifetime fecundity of 53 ( $\pm 17.8$ ) eggs. This provides clear evidence that ant-mediated variation in female larval growth has direct consequences for an important component of fitness. Patterns in the other size parameters (i.e., maximum larval mass, mass lost during the prepupal stage) largely supported the conclusion that female larvae benefited from ant tending, whereas ants had neutral or slightly negative effects on male development.

If interacting with ants increases growth of female Miami blue butterfly larvae, then they should generally engage in behaviors that increase the probability of attracting and retaining ants. However, the proportion of observations in which tending occurred did not differ between male and female larvae. This apparent contradiction may have a relatively simple explanation in the broader perspective of the mutualistic relationship between the Miami blue butterfly and ants. If the primary benefit for lycaenids associating with ants is protection from predators, male and female Miami blue butterfly larvae may be equally likely to elicit ant tending. Attaining higher pupal mass is more beneficial for females than for males, so perhaps female larvae have evolved overcompensatory feeding behavior when tended by ants that results in faster growth and larger size [16, 47]. This explanation is consistent with the hypothesis that selection should reduce the costs of mutualism, particularly when the cost reduction mechanism (i.e., altered larval feeding and movement) does not directly conflict with the benefit gained by the other partner (i.e., dorsal nectar secretions that ants consume) [44].

Maximizing the larval growth objectives of large size and fast development generally involves substantial tradeoffs [15] or requires relaxation of other limitations such as food resources or predation risk [48]. We did not examine predation and antipredator protection in this study, but anecdotal evidence suggests that *C. floridanus* ants are effective at deterring natural enemies of Miami blue butterfly larvae [38]. Together with the sex-specific effects of ant tending on pupal mass, it appears that female Miami blue butterfly larvae largely mitigate the common tradeoff between size and age at pupation by interacting with ant mutualists.

Pierce and Elgar [28] proposed a suite of traits correlated with ant-related oviposition among lycaenid butterflies, but the Miami blue butterfly only meets some of the criteria. First, although the interaction with ants is beneficial for larval performance in this system, it is facultative rather than the obligate relationships found in some ant tended lycaenids and the first two instars feed within host plant buds and are not tended by ants. That said, later-instar larvae in the wild are usually guarded by presumably mutualistic ants, most commonly *C. floridanus* and *C. planatus* [38]. Second, there are both predictable and unpredictable components of the ant environment that could affect females' perception of site suitability for oviposition. Ants do not constantly patrol the host plant in large numbers and *C. floridanus* is primarily nocturnal, so presence at any given time may not

be a reliable indication of whether ants will tend Miami blue butterfly larvae at that potential oviposition site. Third, field surveys suggest that ants are likely to find larvae on the host plant regardless if they are present or absent at the time of oviposition [37, 38]. In short, the interaction between larvae of the Miami blue butterfly and ants features strong association with ants in the later instars but does not meet any of the other characteristics listed by Pierce and Elgar [28] as traits common among lycaenids thought to use ants as oviposition cues. Taken together, these aspects of Miami blue butterfly ecology suggest that there may be little selective pressure for ovipositing females to select sites based on ant presence.

The results of our study were only partially consistent with this prediction. We found no evidence that Miami blue butterflies preferentially oviposited on host plants where mutualistic ants were present. On the contrary, naïve females oviposited less frequently on plants occupied by the ant species that most commonly tends Miami blue butterfly larvae. It is not uncommon for ants that tend lycaenid larvae to behave antagonistically toward ovipositing females of the same species [28, 29, 49, 50], so there may be benefits to adult survival from avoiding interactions with potentially aggressive ants. However, it is not clear from our study why female butterflies tended by ants as larvae would respond differently to ant proximity or potential aggressive behaviors during oviposition.

The most provocative result of the oviposition experiment was that Miami blue butterflies tended by *C. floridanus* ants as larvae were significantly more likely to oviposit on plants with ants than were naïve females. This suggests that larval history of ant interaction can reduce the apparent avoidance of ants at potential oviposition sites, although it resulted in a relative preference rather than an absolute one. Natal habitat preference induction has been convincingly demonstrated in relatively few systems [33, 51, 52]. However, both behavioral and neurological studies have shown that larval exposure to odors or suboptimal foods can induce preference for or reduce aversion to otherwise deterrent chemical stimuli among both herbivorous and parasitoid insect taxa [34, 35, 53, 54]. We did not evaluate potential mechanisms for conditioning for ants by Miami blue butterfly larvae, but high-quality mutualists can have substantial positive effects on survival and performance, so it is not surprising that there may be selection for such behavior in ant-lycaenid mutualisms.

Previous research on lycaenid butterflies has suggested ant-associated oviposition for dozens of species with the presumed benefit of increased larval performance [8, 28]. Oviposition preference for host plants where ants are present has been subject to rigorous experimentation in some systems (e.g., *Jalmenus evagoras* [28, 55], *Anthene emolus* [29, 50], and *Ogyris amaryllis* [31]). However, other studies suggesting ant-associated oviposition have probably actually measured the correlation between ant presence and suitable host plants rather than strictly demonstrating oviposition preference for ants [56–58].

The results of our study lead to three suggestions for developing and testing hypotheses related to ant-related

oviposition and larval growth among lycaenids. First, it is important to recognize that relative preference may be adaptively significant even if butterflies do not show an absolute preference for ovipositing in sites associated with ant mutualists. Second, researchers would benefit from considering the broader literature on oviposition preference [1]. Most tests of ant-related oviposition among lycaenids note the importance of the relationship with ants but do not consider the reliability of cues at the time of oviposition for future interaction or the likelihood that larvae will encounter ants even if they are initially absent. For example, oviposition on a high-quality host plant may be more important for many lycaenids than ant presence at the time of oviposition, particularly when larvae are likely to encounter ants later in life as is true for the Miami blue butterfly. In such cases, we would not expect females to use ants as oviposition cues even if ant tending has substantial effects on fitness. Third, identifying the mechanisms that allow ovipositing females to distinguish among host plants with and without ants should be a priority for future work [59, 60]. Other studies on lycaenids have suggested that visual [28, 29, 50], tactile [31], and chemical [57] cues associated with ant presence may stimulate oviposition. Further examination of the physiological and behavioral components of such mechanisms would greatly enrich the existing theory and empirical evidence of ant-related oviposition among lycaenids.

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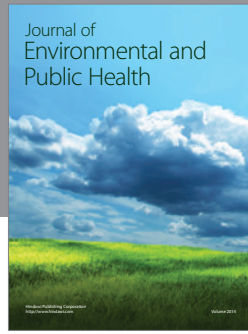
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