

## ANT TENDING OF MIAMI BLUE BUTTERFLY LARVAE (LEPIDOPTERA: LYCAENIDAE): PARTNER DIVERSITY AND EFFECTS ON LARVAL PERFORMANCE

MATTHEW D. TRAGER<sup>1,2</sup> AND JARET C. DANIELS<sup>1,2</sup>

<sup>1</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611

<sup>2</sup>McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History,  
University of Florida, Gainesville, FL 32611

### ABSTRACT

The larvae of many lycaenid butterflies (Lepidoptera: Lycaenidae) are tended by ants that protect them from natural enemies in return for sugar-rich secretions that the larvae produce to attract and retain their ant guards. We investigated the relationship between larvae of the endangered Miami blue butterfly (*Cyclargus thomasi bethunebakeri* Comstock & Huntington) and potentially mutualistic ants. We observed 10 ant species interacting with Miami blue larvae in the field, and raised larvae successfully in captivity with 3 of these and 6 other ant species that are not known to tend larvae in the wild. In an experimental assessment of ant effects on larval performance, we found no differences in age at pupation, pupal mass, length of pupation, total time as an immature or ratio of time as a larva to time as a pupa among larvae raised with *Camponotus floridanus* Buckley, *Linepithema humile* Mayr, or in a no-ant control. Larvae raised with *C. floridanus* were significantly more likely to pupate in the ant harborage than larvae in the other treatments. We did not observe ants behaving antagonistically toward Miami blue larvae in field, laboratory, or experimental conditions; even ant species previously identified as potential predators tended larvae. Our results demonstrate that Miami blue larvae can elicit typical tending behaviors across diverse ant taxa and that ant tending does not substantially alter larval development, findings that may have implications for conservation and population restoration of the Miami blue butterfly.

**Key Words:** *Camponotus floridanus*, *Cyclargus thomasi bethunebakeri*, *Linepithema humile*, lycaenid, mutualism, myrmecophily

### RESUMEN

Las orugas de las mariposas en la familia Lycaenidae a menudo son atendidas por hormigas que las protegen de enemigos naturales a cambio de secreciones ricas en azúcares producidas por las orugas para atraer y retener sus hormigas guardias. Investigamos la relación entre larvas de la mariposa Miami blue (*Cyclargus thomasi bethunebakeri* Comstock & Huntington), un especie en peligro de extinción, con las hormigas posiblemente mutualistas. Observamos 10 especies de hormigas interactuando con las orugas de la Miami blue en el campo, y criamos satisfactoriamente las orugas en cautiverio con 3 de éstas y con 6 otras especies de hormigas no observadas al atender orugas en el hábitat natural. Hicimos un ensayo experimental para observar los efectos de las hormigas para el desempeño de las larvas, en que medimos los variables de respuesta siguientes: edad a la pupación, masa de la pupa, duración del estadio pupal, plazo total como inmadura, y relación de los plazos larva:pupa. No encontramos distinciones en ninguno de los variables entre las orugas criadas con *Camponotus floridanus* (Buckley) o con *Linepithema humile* (Mayr) o en un ensayo control sin hormigas. Sin embargo, hallamos que las larvas criadas con *C. floridanus* lograron significativamente más probable hacerse en pupa con las hormigas que en los otros tratamientos. No observamos comportamiento antagonista por las hormigas hacia las orugas, ni aún por especies previamente identificadas como posibles predadores de estas larvas, que de hecho atendieron las orugas. Nuestros resultados demuestran que las orugas de la Miami blue provocan comportamientos típicos de atención por diversas especies de hormigas y aunque el cuidado de las hormigas no altera significativamente el desenvolvimiento larval, hallazgo que puede tener implicaciones para la conservación y la rehabilitación de poblaciones de la mariposa Miami blue.

Translation provided by the authors.

---

The larvae of many lycaenid butterflies (Lepidoptera: Lycaenidae) interact with ants in relationships ranging from brood parasitism in ant colonies to mutualism in which both species benefit. Approximately 60% of the ant-associated lycaenid species are facultative myrmecophiles that are tended by several different ant species, usually on the larval host plant (Pierce et al. 2002). Lycaenid larvae in such relationships generally emit semiochemicals from a pair of tentacular organs and secrete a sugar-rich solution from a dorsal nectary organ to attract and retain their ant guards (Axén et al. 1996; Axén 2000; Daniels et al. 2005), which then protect the larvae from predators and parasitoids (Pierce & Mead 1981; Fiedler et al. 1996). Additionally, larvae of some lycaenid species pupate inside ant nests, where the ants presumably protect them until adult eclosion (Wagner 1995; Dejean & Beugnon 1996). Thus, these associations entail an exchange of goods and services that usually results in net benefits for both partners (Pierce et al. 1987; Cushman et al. 1994). However, there is often substantial variation in quality among potential partners in facultative, diffuse mutualistic systems that may result in negative or neutral consequences for the survival and performance of the interacting species (Bronstein 2001; Miller 2007).

Evaluating partner quality in purportedly mutualistic relationships is necessary to understand the ecological conditions and evolutionary processes that influence the outcome of the relationship (Bshary & Grutter 2002; Ness et al. 2006). A central question in research on lycaenid-ant mutualisms is how interactions with different ant species affect larval performance (Axén 2000; Fraser et al. 2001; Weeks 2003). The physiological cost of provisioning ants with a sugar-rich exocrine secretion leads to reduced growth of ant-tended larvae in some systems (Pierce et al. 1987; Baylis & Pierce 1992), but growth of ant-tended larvae in other systems is similar to or greater than that of untended larvae (Fiedler & Hölldobler 1992; Cushman et al. 1994; Wagner & Del Rio 1997; Fraser et al. 2001). Because pupal mass is often correlated with adult reproductive output in Lepidoptera (Gotthard 2008), the growth consequences for larvae of ant tending could have subsequent effects on individual fitness (Elgar & Pierce 1988). Furthermore, because the relative importance of adult size and timing of adult emergence may differ between conspecific males and females (Fagerstrom & Wiklund 1982; Gotthard et al. 2000), in some lycaenid species there are sex differences in the effects of ant tending (Fiedler & Hölldobler 1992). Assessing the effects of ant tending on lycaenid larvae and identifying the factors that explain variation in the interaction among and within species are central issues for furthering our understanding of these complex relationships.

Larvae of the imperiled Miami blue butterfly, *Cyclargus thomasi bethunebakeri* (Comstock & Huntington) (Lycaenidae: Polyommatainae), associate with several species of ants (Minno & Emmel 1993; Smith et al. 1994; Saarinen & Daniels 2006). However, we know very little about the costs and benefits of this relationship (Florida Fish and Wildlife Conservation Commission 2003; Saarinen & Daniels 2006). In addition to reporting new observations of ant tending in the wild and under laboratory conditions, our study is the first to quantify experimentally the effects of ant tending on performance of Miami blue larvae. Specifically, we observed the behavioral components of interactions between Miami blue larvae and different ant species in the field and in the laboratory and raised larvae with several ant species to assess the nature of these interactions. We then quantified the effects of ant tending and butterfly sex on larval survival, time until pupation, mass at pupation, and length of pupation with an experiment in which we raised larvae with 2 ant species and a no-ant control. We also noted the location of pupation for larvae in this experiment. The implications of our work for the conservation of wild Miami blue butterfly populations and future reintroduction efforts are discussed.

## MATERIALS AND METHODS

### Study Organism

The Miami blue butterfly is a small, sexually dimorphic lycaenid formerly found in much of coastal peninsular Florida and outlying barrier islands (Smith et al. 1994; Calhoun et al. 2002; Carroll & Loye 2006). Miami blue larvae have an onisciform or sluglike shape typical of ant-tended lycaenids and have prominent ant-associated organs for communicating with and provisioning ant guards beginning in the third instar (Saarinen & Daniels 2006). The larvae elicit tending behavior from ants through chemical communication originating from a pair of eversible tentacles and, perhaps, other specialized epidermal glands (Pierce et al. 2002). Ants typically respond by rapidly antennating around the head, tentacular organs, and dorsal nectary organ of the larvae and accepting sugar-rich secretions from the latter (Saarinen & Daniels 2006). Although ants may protect Miami blue larvae against parasitoids and predators in the wild, we have not recorded parasitism or predation of any larvae despite extensive field observations.

The Miami blue butterfly is currently listed by the state of Florida as an endangered species (Florida Fish and Wildlife Conservation Commission 2003). More information on the history and current status of this species can be found in Calhoun et al. (2002), Carroll & Loye (2006), and Saarinen & Daniels (2006).

## Field and Laboratory Observations of Ant Tending

Over 3 growing seasons (2006-2008), we conducted field observations of ant tending at Bahia Honda State Park (BHSP), where there is a wild population of Miami blue butterflies, as well as at 2 other sites where we introduced captive-bred larvae, Elliott Key in Biscayne National Park (BNP) and Dagny Johnson Key Largo Hammock Botanical State Park (DJSP). The introduced larvae were from a colony maintained at the University of Florida as part of the Miami blue butterfly's state-mandated management and recovery plan (Florida Fish and Wildlife Conservation Commission 2003). These larvae, and all others used in the studies we report here, originated from stock sourced from a wild population at BHSP and were raised on *Caesalpinia bonduc* L. (Roxb.) as the host plant. Observations at all 3 sites were opportunistic because larvae were often difficult to locate once on the host plant, were not always tended, and ants did not always immediately find recently introduced larvae. We were particularly interested in the behaviors of both ants and Miami blue larvae upon first encounter and then subsequent interactions that would indicate whether ants ignored, tended or depredated larvae.

We observed interactions between Miami blue butterfly larvae and several ant species in the laboratory. We raised dozens of larvae with the 2 focal ant species in this study, *Camponotus floridanus* Buckley and *Linepithema humile* Mayr, prior to the experimental assessment described below. For the other ant species, we raised from 2 to 4 Miami blue butterfly larvae simultaneously with a single, queenright ant colony that had no previous interactions with Miami blue butterflies. We introduced larvae to the ant colonies at the second or early third instar, observed the initial interaction to assure that the ants did not attack the larvae and replaced the host plant daily until the larvae pupated. The only 2 exceptions to this protocol were our discovery of *Pheidole moerens* Wheeler tending larvae after entering the laboratory without our knowledge and when we found *Camponotus floridanus* workers tending larvae in an outdoor flight cage.

## Experimental Design and Analysis

We quantified the effects of ant tending on Miami blue larvae with an experiment in which we raised larvae from the second instar to pupation with 2 ant species, *Camponotus floridanus* and *Linepithema humile*, or in a no ant control. *Camponotus floridanus* is a large formicine that is the most common ant found tending Miami blue larvae in the wild, thereby making it relevant species for our study. *Linepithema humile* is a small, non-native dolichoderine that readily tends hon-

eydew-producing Hemiptera and other lycaenid larvae (Agrawal & Fordyce 2000; Tillberg et al. 2007). Although we had no evidence that *L. humile* interacts with Miami blue larvae in the wild, this species is amenable to experimentation and in our study served as a surrogate for smaller, active ant species that we have frequently observed tending Miami blue larvae in the field (e.g., *Paratrechina longicornis*, *P. bourbonica*, *Tapi-noma melanocephalum*).

We conducted 2 trials in which we randomly assigned 3 larvae to each of 4 replicate shoebox-style trays in each of the 3 ant treatments (total of 72 larvae). We placed new groups of ants in each tray between trials. Larvae were from eggs laid by multiple females in the captive colony and were all the same age in each of the 2 trials. We replaced the host plant daily and manually transferred larvae from the old to the new cuttings. Each tray contained an ant harborage comprising a 90-mm diameter Petri dish containing a layer of dental plaster that we wetted occasionally to maintain humidity and covered with dark paper. For the *C. floridanus* treatment, we placed 50 workers in each tray, and for the *L. humile* treatment we placed 100 workers and 1 queen in each tray. These ants had been maintained in captivity for approximately 6 months prior to the experiment and were allowed a week to acclimate to the tray before we introduced the Miami blue larvae. To assure that malnourishment would not affect the ants' interactions with the Miami blue larvae, we provisioned ants with water, 10% sucrose solution, and cut mealworms (larvae of *Tenebrio* sp.); these were also provided to the control trays. We coated the sides of the trays with a fluoropolymer resin slippery barrier (Insect-a-Slip, BioQuip Products, Rancho Dominguez, CA) to contain the ants but, even with this preventative measure, a small number of Miami blue larvae escaped during our experiments.

We monitored the presence of all larvae each day. Upon pupation, we recorded the location of the pupae, measured their mass with a digital analytical balance accurate to 0.01mg (Denver Instruments SI-215D), and kept them in individual vials in the laboratory under fairly constant temperature and humidity until adult emergence (24-28 C with 40-50% relative humidity). We measured the wing chord length (mm) and recorded the sex of each adult. Thus, our response variables for statistical analyses were age at pupation, location of pupation, pupal mass, length of pupation, and adult wing chord. From these data we calculated 2 additional response variables—the proportion of time spent in the pupal and larval stadia (length of pupation divided by age at pupation) and total time as an immature (age at pupation plus length of pupation)—to increase our understanding of the potential effects of ants on larval development of Miami blue butterflies.

We conducted correlation analysis to test for relationships between the measures of larval performance to elucidate patterns in developmental strategies of Miami blue larvae. We analyzed differences among ant treatments in the location of pupation with Fisher's exact test. To assess the influence of ant treatment and sex on larval performance, we conducted linear mixed-effects analyses in which we tested the effects of ant treatment and sex (both fixed effects) on age at pupation, pupal mass, length of pupation, and the 2 compound variables described above. All models contained a nested random effect of tray within trial to account for variance due to differences between the 2 experimental trials or among groups of the same ant species. All analyses were conducted in the R language and environment for statistical computing (R Development Core Team 2009) and followed protocols for model specification and interpretation described by Pinheiro & Bates (2002) and Faraway (2006).

## RESULTS

### Field and Laboratory Observations of Ant Tending

We observed 8 ant species tending Miami blue larvae in the field, including observations at the natural population in Bahia Honda State Park and the reintroduction sites in Biscayne National Park and Dagny Johnson Key Largo Hammock Botanical State Park (Tables 1 and 2). Saarinen & Daniels (2006) reported 1 additional species, *Forelius pruinosus*, tending Miami blue larvae but we have not since observed this interaction. We most

frequently observed 2 *Camponotus* species, *C. floridanus* and *C. planatus*, tending both wild and recently introduced larvae. Additionally, at both reintroduction sites, *Pseudomyrmex gracilis* was common and tended recently released larvae.

### Experimental Assessment of Ant Effects

We collected data on age at pupation, location of pupation, pupal mass, length of pupation, and wing chord from 64 Miami blues. Of the original 72 larvae, 5 escaped from the ant trays and were excluded from further study and 3 individuals did not successfully eclose from the pupal stage so could only be included in some analyses. Pupal mass and wing chord were highly positively correlated ( $r = 0.69$ ,  $t = 7.31$ ,  $df = 59$ ,  $P < 0.0001$ ), and so we used pupal mass as a measure of size for our analyses. There was no correlation between age at pupation and pupal mass ( $r = -0.14$ ,  $t = -1.13$ ,  $df = 62$ ,  $P = 0.26$ ), but pupal mass was positively correlated with length of pupation ( $r = 0.31$ ,  $t = 2.54$ ,  $df = 62$ ,  $P = 0.014$ ) and age at pupation was negatively correlated with length of pupation ( $r = -0.49$ ,  $t = -4.42$ ,  $df = 62$ ,  $P < 0.0001$ ).

Most larvae (45 of 64) pupated under the paper that shaded the Petri dish harborages regardless of ant presence or identity, but the frequency of pupal location was non-random among the 3 ant treatments (Fisher's exact test  $P = 0.029$ ). Five of 22 larvae raised with *C. floridanus* pupated inside the Petri dish with the ants, whereas no larvae from either the *L. humile* or no ant treatments pupated in that location

TABLE 1. ANT SPECIES OBSERVED TENDING MIAMI BLUE LARVAE IN THE FIELD AND THE INTERACTION STATUS SUGGESTED BY SAARINEN & DANIELS (2006), IF AVAILABLE. THE TENDING BEHAVIOR WE OBSERVED FOR ALL ANTS LISTED HERE WAS CONSISTENT WITH A MUTUALISTIC INTERACTION.

Ant taxa	Interaction status	Sites and observers
Subfamily Pseudomyrmecinae <i>Pseudomyrmex gracilis</i>	P	DJSP <sup>2</sup> , BNP <sup>2</sup>
Subfamily Myrmicinae <i>Crematogaster</i> cf. <i>ashmeadi</i> <i>Monomorium floricola</i>	S u	BHSP <sup>1,2</sup> BHSP <sup>1,2</sup>
Subfamily Dolichoderinae <i>Forelius pruinosus</i> <i>Tapinoma melanocephalum</i>	S S	ENP <sup>1</sup> , BHSP <sup>1</sup> BHSP <sup>1,2</sup>
Subfamily Formicinae <i>Camponotus floridanus</i> <i>Camponotus inaequalis</i> <i>Camponotus planatus</i> <i>Paratrechina bourbonica</i> <i>Paratrechina longicornis</i>	S S pS pS	BHSP <sup>1,2</sup> , BNP <sup>2</sup> , ENP <sup>1</sup> KWNWR <sup>3</sup> DJSP <sup>2</sup> , BNP <sup>2</sup> , BHSP <sup>1</sup> BHSP <sup>2</sup> BHSP <sup>2</sup> , KWNWR <sup>3</sup>

Interaction status suggested by Saarinen & Daniels (2006): P = potential predator, pS = potential symbiont (i.e., potential mutualist), S = symbiont (i.e., mutualist), u = unknown. 1 = observations reported by Saarinen & Daniels (2006); 2 = new observations by the authors; 3 = new observations by P. Cannon (pers. comm.).

TABLE 2. ANT SPECIES OBSERVED TENDING MIAMI BLUE LARVAE UNDER EXPERIMENTAL LABORATORY CONDITIONS AND THE INTERACTION STATUS SUGGESTED BY SAARINEN & DANIELS (2006), IF AVAILABLE. WE SUCCESSFULLY RAISED AT LEAST 2 LARVAE FROM APPROXIMATELY THIRD INSTAR TO PUPATION WITH ALL OF THE ANT SPECIES LISTED HERE UNDER CONDITIONS DESCRIBED IN THE MATERIALS AND METHODS EXCEPT *PHEIDOLE MOERENS*, WHICH WE DISCOVERED TENDING LARVAE IN THE LABORATORY.

Ant taxa	Interaction status
Subfamily Pseudomyrmecinae	
<i>Pseudomyrmex ejectus</i>	P
<i>Pseudomyrmex gracilis</i>	
Subfamily Myrmicinae	
<i>Pheidole moerens</i>	P
<i>Solenopsis invicta</i>	
Subfamily Dolichoderinae	
<i>Dorymyrmex bureni</i>	S
<i>Linepithema humile</i>	
<i>Tapinoma melanocephalum</i>	
Subfamily Formicinae	
<i>Brachymyrmex patagonicus</i>	S
<i>Camponotus floridanus</i>	

Interaction status suggested by Saarinen & Daniels (2006): P = potential predator, pS = potential symbiont (i.e., mutualist), S = symbiont (i.e., mutualist), u = unknown.

We present summarized values of the performance parameters measured in Table 3 and the results of all statistical analyses of the effects of larval sex and ant treatment on larval performance in Table 4. There was no significant effect of ant treatment on any of the measures of larval performance, although the analysis suggested that larvae raised with *C. floridanus* may have a

shorter length of pupation relative to the time spent in the larval stadium. Male larvae pupated at a significantly smaller mass than females but females tended to complete pupation faster.

Although we did not quantify the frequency of tending behaviors in this study, both *C. floridanus* and *L. humile* regularly tended Miami blue butterfly larvae throughout the course of the experiment. We rarely found untended larvae, particularly in the later instars, with either ant species. The interactions conformed to the common behavioral pattern of the larvae everting their tentacular organs and secreting from the dorsal nectary organs, followed by the ants antennating the larvae and consuming the nectar. However, upon disturbance associated with maintaining the experiment, *L. humile* usually abandoned the larvae whereas *C. floridanus* showed typical defensive behaviors such as running around larvae, tapping on the host plant or other surfaces with their mandibles, and antennating the air with open mandibles.

#### DISCUSSION

Relationships between lycaenid butterfly larvae and ants range dramatically in the effects on the interacting species. Previous studies have suggested that some ant species may depredate Miami blue butterfly larvae or may opportunistically tend larvae without providing protection against predators or other benefits (Saarinen & Daniels 2006). However, through both field observations and laboratory trials, we recorded a universal tending response among ants consistent with a mutualistic interaction. Indeed, including the observations we report here, a total of 17 ant species have been observed tending Miami blue larvae either in wild populations, in reintroduction sites following releases of captive raised larvae or in the laboratory (Saarinen & Daniels 2006; Carroll & Loyer 2006). We have not observed

TABLE 3. SUMMARY OF DEVELOPMENTAL PARAMETERS (MEANS  $\pm$  SE) FOR MIAMI BLUE BUTTERFLY LARVAE, SEPARATED BY ANT SPECIES TREATMENT, AND SEX. FOR EASE OF INTERPRETATION, MEANS AND SE HERE ARE POOLED ACROSS TRIALS AND TRAYS; TEST STATISTICS FROM THE MIXED-EFFECTS MODEL ANALYSIS ON THE EFFECTS OF ANT SPECIES AND SEX ON DEVELOPMENTAL VARIABLES ARE PRESENTED IN TABLE 4.

	Age at pupation (d)	Pupal mass (mg)	Length of pupation (d)	Time as immature (d)	Length pup./Age at pup.
Ant treatment					
<i>C. floridanus</i> (n = 22)	19.09 $\pm$ 0.31	60.52 $\pm$ 1.56	8.73 $\pm$ 0.13	27.82 $\pm$ 0.27	0.460 $\pm$ 0.013
<i>L. humile</i> (n = 21)	18.81 $\pm$ 0.44	58.11 $\pm$ 2.23	8.76 $\pm$ 0.19	27.57 $\pm$ 0.39	0.471 $\pm$ 0.019
No ants (n = 21)	18.57 $\pm$ 0.44	62.06 $\pm$ 2.23	9.0 $\pm$ 0.19	27.57 $\pm$ 0.39	0.489 $\pm$ 0.019
Sex					
Male (n = 39)	18.51 $\pm$ 0.36	58.92 $\pm$ 1.86	9.10 $\pm$ 0.15	27.51 $\pm$ 0.32	0.490 $\pm$ 0.015
Female (n = 25)	19.32 $\pm$ 0.28	62.27 $\pm$ 1.45	8.56 $\pm$ 0.12	27.88 $\pm$ 0.25	0.446 $\pm$ 0.012
All (n = 64)	18.83 $\pm$ 0.18	60.23 $\pm$ 0.92	8.82 $\pm$ 0.079	27.66 $\pm$ 0.16	0.473 $\pm$ 0.008

TABLE 4. RESULTS OF MIXED-EFFECTS MODEL ANALYSES TESTING THE EFFECTS OF ANT TREATMENT, SEX, AND THEIR INTERACTION ON MEASURES OF LARVAL PERFORMANCE. TRAY IN TRIAL WAS A NESTED RANDOM EFFECT FOR ALL ANALYSES AND VARIANCE COMPONENTS ARE PRESENTED AS STANDARD DEVIATIONS FOR BOTH TERMS FOR EACH MODEL. ALTHOUGH PRESENTED TOGETHER IN THIS TABLE, WE TESTED EACH RESPONSE VARIABLE INDEPENDENTLY.

Response variables	Predictor variables	F-value (df)	P-value
Age at pupation	Ant treatment	2.15 (2, 51)	0.13
	Sex	0.41 (1, 51)	0.53
	Ant treatment * Sex	0.23 (2, 51)	0.80
	Trial $\sigma = 1.16$ ; Tray in Trial $\sigma = 0.10$		
Pupal mass	Ant treatment	1.68 (2, 51)	0.20
	Sex	8.91 (1, 51)	0.0043
	Ant treatment * Sex	0.18 (2, 51)	0.84
	Trial $\sigma = 0.0021$ ; Tray in Trial $\sigma = 3.57 * 10^{-7}$		
Length of pupation	Ant treatment	1.40 (2, 51)	0.26
	Sex	3.60 (1, 51)	0.063
	Ant treatment * Sex	0.76 (2, 51)	0.47
	Trial $\sigma = 0.27$ ; Tray in Trial $\sigma = 4.91 * 10^{-5}$		
Time as immature	Ant treatment	0.57 (2, 51)	0.57
	Sex	0.17 (1, 51)	0.68
	Ant treatment * Sex	0.20	0.82
	Trial $\sigma = 0.87$ ; Tray in Trial $\sigma = 0.089$		
Length of pupation/ Age at pupation	Ant treatment	2.90 (2, 51)	0.064
	Sex	3.14 (1, 51)	0.082
	Ant treatment * Sex	0.81 (2, 51)	0.45
	Trial $\sigma = 0.045$ ; Tray in Trial $\sigma = 1.79 * 10^{-6}$		

any depredation of Miami blue larvae by ants in the field, and laboratory trials suggest that even ant species identified by Saarinen & Daniels (2006) as potential predators in fact tend larvae in ways consistent with a mutualistic interaction. These results are notable for the large number of potential ant partners, the consistency of behaviors toward larvae among distantly related ant taxa and the nearly complete lack of obviously antagonistic interactions.

Despite the high diversity of potential ant partners, our observations suggest that only a small subset of ant species accounts for the vast majority of interactions with Miami blue butterfly larvae in the field. Previous studies have reported that ant species in the genus *Camponotus* most commonly tended larvae (Minno & Emmel 1993; Carroll & Loye 2006; Saarinen & Daniels 2006), and we most commonly found *C. floridanus* and *C. planatus* associated with wild and recently released larvae. Notably, this pattern was consistent across 3 sites (BHSP, BNP, and DJSP) that likely differ dramatically in ant community composition (Deyrup et al. 1988). There are at least 4 likely explanations for the apparent reciprocal affinity between Miami blue larvae and *Camponotus*. First, *Camponotus* commonly forage on nickerbean for plant exudates, honeydew-producing Hemiptera, and insect prey even in sites where

Miami blues do not occur, so frequent tending could be opportunistic association resulting from high rate of encounters compared to other potential ant partners. Second, after discovering Miami blue larvae, even those tended by other ant species, *Camponotus* may competitively exclude other ants from interacting with them through constant tending and defense. Third, following initial contact, the Miami blue larvae may allocate more resources to retaining *Camponotus* compared to other ant species because *Camponotus* are higher quality defenders. Finally, *Camponotus* are quite large and active and therefore researchers may be more likely to find larvae tended by these ants.

Larvae of some facultatively ant-tended lycenid species pupate inside ant nests (e.g., Wagner 1995), but this has not been previously reported for the Miami blue butterfly. We found that a higher than expected number of larvae pupated in the ant harborage in the laboratory when tended by *C. floridanus* and no larvae pupated in that location when kept with *L. humile* or raised without ants, suggesting that this aspect of the relationship may have been overlooked. Clearly, future studies are required to elucidate the precise mechanisms that account for the persistent and geographically widespread association between *Camponotus* species, particularly *C. flori-*

*danus* and *C. planatus* (and possibly *C. inequaelis*), and Miami blue larvae.

In most facultative ant-lycaenid mutualisms, the primary direct cost for the lycaenid larvae is metabolic expense required to produce sugary secretions for ant defenders (Pierce et al. 1987; Daniels et al. 2005). Because the secretions are induced by ant tending (Agrawal & Fordyce 2000), we might expect to find reduced larval growth rate or pupal mass in ant-tended larvae. In our experimental assessment of ant effects on performance of Miami blue butterfly larvae, both *C. floridanus* and *L. humile* almost constantly tended larvae and we frequently observed both species consuming secretions from the dorsal nectary organ. Carroll & Loye (2006) found that *Camponotus* sp. raised with Miami blue larvae lived longer than ants raised without any food source, demonstrating that larval secretions are nutritionally valuable for tending ants. However, contrary to theoretical predictions and the results of studies in similar ant-lycaenid systems, we found no effect of ant presence on any of our measurements of larval performance. It is possible that these secretions are less metabolically expensive than some studies have suggested or that ants tend larvae even when they receive only small nutritional rewards (Fiedler & Saam 1995). Alternatively, larvae may compensate for metabolic expenses of provisioning ants by feeding more efficiently when tended (Fiedler & Hölldobler 1992; Wagner & del Rio 1997). If the latter is true for Miami blue butterfly larvae, we may have found no effect of ant association on larval performance because increased growth of tended larvae offset the costs of the sugary secretions. Regardless of the specific mechanism, it appears that the net costs of ant tending for Miami blue butterflies do not substantially affect their larval development compared to untended conspecific larvae.

The primary benefit of ant association for lycaenid larvae is defense against natural enemies (Atsatt 1981; Pierce & Mead 1981; Weeks 2003). We did not test this important aspect of the relationship for Miami blue butterfly larvae in this study, nor have we observed ants actively protecting larvae against attack by predators or parasitoids in the wild. However, in the laboratory *C. floridanus* displayed strong defensive behaviors (e.g., rapidly circling larvae, recruiting nearby workers and lunging at forceps) when disturbed, particularly when we moved Miami blue butterfly larvae to new host plant. The large size of this ant species and nearly constant tending may serve as a visual deterrent to potential attackers. Although *L. humile* workers assiduously tended Miami blue larvae in our experiment, they are substantially smaller than *C. floridanus* and did not show such a strong defensive response; instead, they usually ran away from the larvae and source

of disturbance. Despite these qualitative observations, it is important to emphasize that we have no definitive evidence from this study that *C. floridanus* are more effective defenders of Miami blue butterfly larvae than small-bodied ant species. Given the apparently small physiological costs for Miami blue larvae of associating with ants, any predator or parasitoid deterrence provided by ant defenders could substantially increase their probability of survival to the adult stage.

Male and female Lepidoptera larvae may have divergent developmental strategies, and may therefore exhibit variation in behaviors, such as foraging or prolonging the larval period that represent trade-offs between growth and mortality (Gotthard 2008). In lycaenid larvae, such differences could result in sex-specific interactions with ants, particularly in species for which timing of adult emergence and adult size have different effects on the relative reproductive success of males and females (Elgar & Pierce 1988). Indeed, Fiedler & Hölldobler (1992) found that *Polyommatus icarus* males benefited from ant association through increased pupal mass, but ant tending appeared to be energetically costly for females. This species has a strongly protandrous mating system with frequent male-male competition for access to females (Lundgren 1977), so it is reasonable that male larvae would interact with ants to optimize adult size as long as the costs to development time were not too great. Similarly, Baylis & Pierce (1992) found that male *Jalmenus evagoras* lost less mass from secretions for ants during the nonfeeding, prepupal instar. By contrast, studies on other lycaenid species have found no sex-related differences in ant association (e.g., Fraser et al. 2001). It is worth noting that most experiments testing lycaenid larval performance with and without ants have not recognized sex as a potentially important predictor of the interaction and its effects. We found that male and female Miami blue larvae differed in some performance parameters, but the sex x ant treatment term did not explain a significant amount of variation in any of the analyses. As such, our results suggest that Miami blue larvae did not have sex-specific costs or benefits of interacting with ants, at least under our experimental conditions.

The state-mandated management plan for the Miami blue butterfly identified examining the relationship between larvae and ants as one of the research goals to inform conservation efforts (Florida Fish and Wildlife Conservation Commission 2003). Saarinen & Daniels (2006) generated further interest in the implications of this relationship for protecting the Miami blue by suggesting that some ant species, particularly the non-native *Solenopsis invicta* and *Pseudomyrmex gracilis*, could be predators of Miami blue larvae. In-

deed, non-native ants can disrupt coevolved mutualistic interactions through competition with native ants or failure to provide the benefits to partner species (Ness & Bronstein 2004). The ant fauna of the Florida Keys contains numerous non-native species, including some that are extremely invasive and have been implicated as major predators of arthropods (Deyrup et al. 1988; Deyrup et al. 2000). However, we found a universal tending response toward Miami blue larvae and very little antagonism across a wide range of ant species, including those previously identified as potential predators. This is a similar result to studies on honeydew-producing Hemiptera that have generally mutualistic relationships with ants, including non-native species (Helms & Vinson 2003; Mondor & Addicott 2007). Different ant species may not have equivalent effects on Miami blue larvae but, based on our observations, we doubt that many ant species regularly depredate larvae. We suggest that ants may be important for protecting larvae from natural enemies, but the identity of the ant attendants may not be particularly important for other measures of larval performance. Furthermore, because the 2 ant species that most commonly tend larvae in the wild and show a strong defensive response, *C. floridanus* and *C. planatus*, are common throughout southern Florida, it is likely that at least 1 of these species would be present at any potential reintroduction site. Our results suggest that the facultative and diffuse interactions between ants and Miami blue larvae are unlikely to be the most important determinant of conservation success for the imperiled butterfly.

#### ACKNOWLEDGMENTS

We thank E. V. Saarinen and 2 anonymous reviewers for helpful comments on a previous version of this paper. Research for this study was conducted under Florida Fish and Wildlife Conservation Commission permit WX02525f. Funding for this work was provided by the Florida Fish and Wildlife Conservation Commission, E. O. Dunn Foundation, U.S. Fish and Wildlife Service and the National Fish and Wildlife Foundation; additionally, M. D. Trager was funded by a National Science Foundation Graduate Research Fellowship. The opinions expressed herein are solely those of the authors and do not represent the position of any of these funding sources.

#### REFERENCES CITED

- AGRAWAL, A. A., AND FORDYCE, J. A. 2000. Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. *Proc. Roy. Soc. B-Biol. Sci.* 267: 1857-1861.
- ATSATT, P. R. 1981. Lycaenid butterflies and ants - selection for enemy-free space. *American Nat.* 118: 638-654.
- AXÉN, A. H. 2000. Variation in behavior of lycaenid larvae when attended by different ant species. *Evol. Ecol.* 14: 611-625.
- AXÉN, A. H., LEIMAR, O., AND HOFFMAN, V. 1996. Signaling in a mutualistic interaction. *Anim. Behav.* 52: 321-333.
- BAYLIS, M., AND PIERCE, N. E. 1992. Lack of compensation by final instar larvae of the myrmecophilous lycaenid butterfly, *Jalmenus evagoras*, for the loss of nutrients to ants. *Physiol. Entomol.* 17: 107-114.
- BRONSTEIN, J. L. 2001. The costs of mutualism. *American Zool.* 41: 825-839.
- BSHARY, R., AND GRUTTER, A. S. 2002. Experimental evidence that partner choice is a driving force in the payoff distribution among cooperators or mutualists: the cleaner fish case. *Ecol. Lett.* 5: 130-136.
- CALHOUN, J. V., SLOTTEN, R. J., AND SALVATO, M. H. 2002. The rise and fall of tropical blues in Florida: *Cyclargus ammon* and *Cyclargus thomasi bethunebakeri*. *Holarctic Lepidoptera* 7: 13-20.
- CARROLL, S. P., AND LOYE, J. 2006. Invasion, colonization, and disturbance; historical ecology of the endangered Miami blue butterfly. *J. Insect Conserv.* 10: 13-27.
- CUSHMAN, J. H., RASHBROOK, V. K., AND BEATTIE, A. J. 1994. Assessing benefits to both participants in a lycaenid-ant association. *Ecology* 75: 1031-1041.
- DANIELS, H., GOTTSBERGER, G., AND FIEDLER, K. 2005. Nutrient composition of larval nectar secretions from three species of myrmecophilous butterflies. *J. Chem. Ecol.* 31: 2805-2821.
- DEJEAN, A., AND BEUGNON, G. 1996. Host-ant trail following by myrmecophilous larvae of Liphyrinae (Lepidoptera, Lycaenidae). *Oecologia* 106: 57-62.
- DEYRUP, M., DAVIS, L., AND COVER, S. 2000. Exotic ants in Florida. *Trans. American Entomol. Soc.* 126: 293-326.
- DEYRUP, M. A., CARLIN, N., TRAGER, J., AND UMPHREY, G. 1988. A review of the ants of the Florida Keys. *Florida Entomol.* 71: 163-176.
- ELGAR, M. A., AND PIERCE, N. E. 1988. Mating success and fecundity in an ant-tended lycaenid butterfly, pp. 59-75 *In* T. H. Clutton-Brock [ed.], *Reproductive Success: Studies of Selection and Adaptation in Contrasting Breeding Systems*. University of Chicago Press, Chicago, IL, USA.
- FAGERSTROM, T., AND WIKLUND, C. 1982. Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. *Oecologia* 52: 164-166.
- FARAWAY, J. J. 2006. *Extending the Linear Model with R: Generalized Linear, Mixed-Effects and Nonparametric Regression Models*. Chapman & Hall CRC Group, Boca Raton, Florida, USA.
- FIEDLER, K., AND SAAM, C. 1995. Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars—Evidence from a survival study. *Oecologia* 104: 316-322.
- FIEDLER, K., AND HÖLLDOBLER, B. 1992. Ants and *Polyommatus icarus* immatures (Lycaenidae) - Sex-related developmental benefits and costs of ant attendance. *Oecologia* 91: 468-473.
- FIEDLER, K., HÖLLDOBLER, B., AND SEUFERT, P. 1996. Butterflies and ants: The communicative domain. *Experientia* 52: 14-24.
- FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION. 2003. Management plan: Miami blue *Cyclargus* (= *Hemiargus*) *thomasi bethunebakeri*. State of Florida, Tallahassee, Florida, USA.
- FRASER, A. M., AXÉN, A. H., AND PIERCE, N. E. 2001. Assessing the quality of different ant species as part-

- ners of a myrmecophilous butterfly. *Oecologia* 129: 452-460.
- GOTTHARD, K., NYLIN, S., AND WIKLUND, C. 2000. Mating opportunity and the evolution of sex-specific mortality rates in a butterfly. *Oecologia* 122: 36-43.
- GOTTHARD, K. 2008. Adaptive growth decisions in butterflies. *Bioscience* 58: 222-230.
- HELMS, K. R., AND VINSON, S. B. 2003. Apparent facilitation of an invasive mealybug by an invasive ant. *Insect. Soc.* 50: 403-404.
- LUNDGREN, L. 1977. The role of intra- and interspecific male:male interactions in *Polyommatus icarus* Rott. and some other species of blues (Lycaenidae). *J. Res. Lepidoptera* 16: 249-264.
- MILLER, T. E. X. 2007. Does having multiple partners weaken the benefits of facultative mutualism? A test with cacti and cactus-tending ants. *Oikos* 116: 500-512.
- MINNO, M. C., AND EMMEL, T. C. 1993. Butterflies of the Florida Keys. Scientific Publishers, Gainesville, Florida.
- MONDOR, E. B., AND ADDICOTT, J. F. 2007. Do exaptations facilitate mutualistic associations between invasive and native species? *Biol. Invasions* 9: 623-628.
- NESS, J. H., AND BRONSTEIN, J. L. 2004. The effects of invasive ants on prospective ant mutualists. *Biol. Invasions* 6: 445-461.
- NESS, J. H., MORRIS, W. F., AND BRONSTEIN, J. L. 2006. Integrating quality and quantity of mutualistic service to contrast ant species protecting *Ferocactus wislizeni*. *Ecol.* 87: 912-921.
- PIERCE, N. E., AND MEAD, P. S. 1981. Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. *Science* 211: 1185-1187.
- PIERCE, N. E., KITCHING, R. L., BUCKLEY, R. C., TAYLOR, M. F. J., AND BENBOW, K. F. 1987. The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behav. Ecol. Sociobiol.* 21: 237-248.
- PIERCE, N. E., BRABY, M. F., HEATH, A., LOHMAN, D. J., MATHEW, J., RAND, D. B., AND TRAVASSOS, M. A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annu. Rev. Entomol.* 47: 733-771.
- PINHEIRO, J. C., AND BATES, D. M. 2002. Mixed Effects Models in S and S-Plus. Springer-Verlag. New York, USA.
- R DEVELOPMENT CORE TEAM. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL <http://www.R-project.org>.
- SAARINEN, E. V., AND DANIELS, J. C. 2006. Miami blue butterfly larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae): New information on the symbionts of an endangered taxon. *Florida Entomol.* 89: 69-74.
- SMITH, D. S., MILLER, L. D., AND MILLER, J. Y. 1994. The Butterflies of the West Indies and South Florida. Oxford University Press. New York, New York, USA.
- TILLBERG, C. V., HOLWAY, D. A., LEBRUN, E. G., AND SUAREZ, A. V. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. *Proc. Natl. Acad. Sci. U.S.A.* 104: 20856-20861.
- WAGNER, D. 1995. Pupation site choice of a North American lycaenid butterfly: The benefits of entering ant nests. *Ecol. Entomol.* 20: 384-392.
- WAGNER, D., AND DEL RIO, C. M. 1997. Experimental tests of the mechanism for ant-enhanced growth in an ant-tended lycaenid butterfly. *Oecologia* 112: 424-429.
- WEEKS, J. A. 2003. Parasitism and ant protection alter the survival of the lycaenid *Hemiargus isola*. *Ecol. Entomol.* 28: 228-232.