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Assessing the quality of different ant species as partners of a myrmecophilous butterfly

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Abstract We assessed the quality of different ant species as partners of the facultatively myrmecophilous lycaenid butterfly *Glaucopsyche lygdamus*. We compared disappearance and parasitism rates of *G. lygdamus* larvae in the field, and development of non-feeding prepupae in the laboratory, when individuals were untended or tended by one of four ant species. *Formica podzolica* was the only ant species to provide a clear benefit to *G. lygdamus*, in the form of reduced larval parasitism relative to untended larvae. *F. 'neogagates'* (*F. neogagates* + *F. lasioides*) and *Tapinoma sessile* were essentially neutral partners, providing no significant cost or benefit for any of the parameters measured. Relative to untended individuals, association with *F. obscuripes* significantly increased larval disappearance and significantly decreased pupal mass. Thus, *F. obscuripes* may act as a parasite of the general association between *G. lygdamus* and ants under certain conditions. Ant species also differed in their persistence as tenders of *G. lygdamus* larvae once an interaction was established. Over the lifetime of a larva, *F. podzolica* and *F. obscuripes* usually remained as the attendant ant species on plants over consecutive census dates, while *F. 'neogagates'* and *T. sessile* were frequently replaced, most commonly by *F. obscuripes*. It remains to be determined if disappearance and developmental outcomes reported here reflect true fitness costs (i.e. reduced survivorship and lower reproductive success) for *G. lygdamus*. The potential and limitations for specialization in association between *G. lygdamus* and high quality ant partners are discussed.

Keywords Conditional mutualism · Cost-benefit analysis · Lycaenid butterfly-ant interactions · Parasitism · Species-specific effects

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Introduction

Mutualisms between species often involve multiple partners on both sides of the interaction (Howe 1984; Jordano 1987; Waser et al. 1996). When participants on one side of the interaction differ in the costs and benefits they confer on a common partner, there is potential for the evolution of specialization in association (Schemske and Horvitz 1984). Specialization (i.e. a reduction in the number of partners with which a species interacts) may be limited, however, by spatial or temporal variation in the availability of high quality partners (Horvitz and Schemske 1984, 1990; Herrera 1988; Peterson 1995; Waser et al. 1996). Knowledge of the costs and benefits conferred by different partners, and of how partner abundances vary over space and time can therefore provide insights into the selective forces influencing the evolution and maintenance of association patterns between species.

Larvae of many species of butterflies from the family Lycaenidae associate with ants. The majority of these associations are facultative, multi-species mutualisms in which lycaenid juveniles secrete nectar-like food rewards to ants via exocrine glands, in exchange for protection against predators and parasitoids (e.g. Pierce and Mead 1981; Pierce and Easteal 1986; DeVries 1991; Wagner 1993; Fiedler and Saam 1994; Savignano 1994). Highly species-specific and obligate associations with ants do occur, however (Pierce 1987; Fiedler 1991; Eastwood and Fraser 1999), suggesting that selection has favored specialization in some species.

Ants may influence lifetime fitness of lycaenids directly, through effects on juvenile survivorship (e.g.

Pierce and Easteal 1986; Pierce et al. 1987; Wagner 1995; Seufert and Fiedler 1996; but see Peterson 1993) and indirectly, through effects on juvenile development and adult reproductive success (Pierce et al. 1987; Elgar and Pierce 1988; Baylis and Pierce 1992; Fiedler and Hölldobler 1992; Wagner 1993; Cushman et al. 1994; Fiedler and Saam 1994). In this study, we assessed the effect of different ant species on fitness-related measures of *Glaucopsyche lygdamus*, a lycaenid that is widely distributed across temperate North America (Scott 1986), and that associates with a broad range of ant species (Pierce and Mead 1981; Pierce and Easteal 1986; Carey 1992; Spomer and Hoback 1998). We quantified disappearance and parasitism of *G. lygdamus* larvae in the field, and developmental performance of *G. lygdamus* prepupae in the laboratory when associated with four ant species. Ant-tended treatments were compared with an untended treatment, from which ants were experimentally excluded, to determine whether each ant species behaved as a mutualist of *G. lygdamus*. The persistence of different ant species as tenders once an interaction was established was also examined in the field.

Materials and methods

Disappearance and parasitism of *G. lygdamus* larvae were assessed in a ca. 150×50 m plot in a subalpine meadow near the Rocky Mountain Biological Laboratory (RMBL), Gothic, Colorado (elevation 2,900 m). The study area has been referred to as the Naked Hills field site by others (e.g. Ehrlich et al. 1972; Pierce and Mead 1981; Pierce and Easteal 1986). At this site, *G. lygdamus* larvae feed predominantly on *Lupinus bakeri*, a common herbaceous perennial, and are tended by at least ten ant species belonging to three different ant subfamilies. These include *Camponotus herculeanus*, *Formica lasioides*, *F. neogagates*, *F. obscuripes*, *F. podzolica* and *F. puberula* (subfamily Formicinae); *Tapinoma sessile* (Dolichoderinae); *Myrmica incompleta*, *M. tahoensis*, and *Leptothorax muscorum* group sp. (Myrmicinae).

Butterflies eclose in spring (May-June) and females lay eggs singly on unopened inflorescences of *L. bakeri*. First and second instar larvae usually feed inside flower buds where they are either untended (Pierce and Easteal 1986), or tended by *T. sessile* ants (A.M. Fraser, personal observation). Third and fourth instar larvae feed in exposed positions and are usually tended by ants. Final instar larvae leave the food plants to pupate and overwinter in the ground.

The ant species used in this study were the most common attendant ant species at the Naked Hills site. They include *F. podzolica* (referred to as *F. fusca* in Pierce and Mead 1981; Pierce and Easteal 1986), *F. obscuripes*, *F. neogagates*, *F. lasioides* and *T. sessile*. *F. neogagates* and *F. lasioides* are indistinguishable in the field and thus will be referred to collectively as *F. 'neogagates'*. Ant species differed in size (range in body length, from anterior edge of the head to posterior tip of the gaster): *F. obscuripes* 4.0–7.5 mm, *F. podzolica* 4.5–6.0 mm, *F. 'neogagates'* 3.0–5.0 mm, *T. sessile* 2.5–3.0 mm.

Five or six nests of each ant species were located in the study plot and three to eight *L. bakeri* plants situated within 1 m of each nest were tagged for use. Vegetation surrounding plants was clipped to ground level and a 10 cm high × 30 cm diameter plastic collar was dug into the ground around the base of each plant. Of these 150 'manipulated' plants, 50 were randomly assigned to an experimental ant exclusion treatment and a sticky barrier of Tanglefoot (The Tanglefoot Company, Grand Rapids, Mich.) was applied to the plastic collar surrounding each plant and to the bases of plant stems. The remaining 100 manipulated plants were accessible to ants (i.e. no Tanglefoot was applied).

G. lygdamus eggs were removed from plant inflorescences and hatched in the laboratory. Resulting larvae were reared on *L. bakeri* leaves for 2–3 days, from which three larvae (first to second instar) were replaced on each manipulated plant (each larva on a separate inflorescence). Plants were censused every 2–3 days to record the number and approximate age (instar) of larvae, the species and number of ants attending each larva, and the species and number of ants per species foraging on the plant. Censuses were conducted between 13 July and 20 August 1995 on a total of 16 census dates. The order, and hence time of day, in which plants were censused varied by date to reduce any effect that time of day might have on ant activity patterns.

Persistence of ant species as tending partners

Using census data, the history of larval attendance on each plant was followed over the course of the season to determine whether ant species differed in their persistence as tending partners of *G. lygdamus* once an interaction was established. Each plant represented an independent data point, and multiple observations were made of each plant (one observation per census). Persistence data were analyzed using the *G* test of independence (Sokal and Rohlf 1981) in a 2×4 contingency table by comparing the number of plants on which each ant species remained as the attendant ant with the number of plants on which each was replaced by another ant species [Contingency table setup: (REMAINED vs REPLACED) × (ANT SPECIES)].

Larval disappearance and parasitism

As larvae reached the late final instar they were collected into vials and brought into the laboratory to complete development. The percentage of larvae that disappeared per plant was calculated from the number of larvae placed on a plant (n=3 larvae) minus the number of larvae collected from a plant, divided by the number placed on the plant (n=3).

Collected larvae either pupated or died when parasitoids emerged. The percentage of larvae parasitized per plant was calculated from the number of individuals parasitized per plant divided by the number collected from the plant. Parasitism was quantified using larvae collected from the 150 manipulated plants, as well as from an additional 103 'unmanipulated' plants on which eggs were left to develop in situ and for which surrounding vegetation was not clipped, nor Tanglefoot applied. Unmanipulated plants were censused every 2–3 days and final instar larvae were collected and reared as described for manipulated plants. Pupae were overwintered in a refrigerator at 4°C for 6 months, then reared under ambient light at 25°C to check for additional parasitoids that emerge from the pupal stage (Pierce and Easteal 1986).

Larval disappearance and parasitism were compared among the untended and four ant-tended treatments on a per plant basis (i.e. each plant represented a single data point) using a generalized linear model with a binomial error structure and a logit link (Crawley 1993). The test statistic, Δdev, was compared to values in a chi-square table under the appropriate degrees of freedom. Corrections for over dispersion were applied based on scaled estimates generated from the maximal model. Required significance levels for pairwise comparisons with the untended treatment were adjusted for non-independence using the sequential Bonferroni method (Rice 1989). Parasitism was analyzed separately for manipulated and unmanipulated plants.

Developmental performance

Developmental performance of *G. lygdamus* prepupae was measured in a laboratory at the RMBL. Prepupae are larvae that have ceased feeding in preparation for pupation. During this time, prepupae continue to secrete nutrients to ants via the dorsal nectary organ, often at rates higher than during the feeding phase (Wagner 1993; Fiedler and Hummel 1995; Burghardt and Fiedler 1996).

One colony of each ant species was collected from the field and housed in test tubes or soil nests. Colonies contained workers and brood, and all except *F. neogagates* contained a fertile queen. Nest chambers were placed at one end of a 50×80×30 cm plastic arena, the walls of which were painted with Fluon (Northern Products, Woonsocket, R.I.) to prevent ants from escaping. Ants were provided with artificial diet (Bhatkar and Whitcomb 1970) and water daily, and frozen crickets twice per week.

Late fourth instar larvae of *G. lygdamus* were collected from the field, weighed and placed individually into 125 ml plastic cups. Ants entered the cups via a hole near the bottom; mesh netting across the top prevented larvae from escaping. Larvae were randomly assigned to one of the five treatment arenas (without ants or containing one of four ant species). Larvae were supplied with fresh *L. bakeri* cuttings daily (leaves and either flowers or seed pods, depending upon availability). There were approximately equal numbers of individuals of the same age in all treatments at any given time. Thus, any variability in development due to the type of plant material provided to larvae was evenly distributed across treatments. Rearing arenas received natural light (from a nearby window) and overhead fluorescent lighting, for an approximate 18:6 h light: dark regime. Ambient room temperatures ranged from 23°C to 28°C. Compared with natural conditions, day length was extended by approximately 2 h, and nightly minimum temperatures were higher than field values by approximately 10°C. Daytime maximum temperatures were similar to natural conditions.

Larvae were weighed at the beginning of the experiment, and at eight hour intervals thereafter. Only larvae that exhibited at least one period of weight gain, and two consecutive periods of weight loss were included in the analysis. The maximum mass attained by an individual was deemed the 'prepupal mass' and the onset of the prepupal phase. Weighing of individuals was discontinued after two consecutive periods of weight loss. Developmental progress was then monitored visually until individuals pupated, at which point they were removed from the experiment and pupal mass was recorded. Pupae were overwintered as described above and individuals were sexed upon eclosion. Developmental parameters used in the analysis and their units of measure were as follows:

a. Initial mass (mg) = larval mass upon introduction to the experiment
b. Prepupal mass (mg) = maximum mass attained by an individual
c. Pupal mass (mg) = mass of an individual within eight hours after pupation
d. $$\text{Relative weight loss}(\%) = \left(\frac{\text{prepupal mass} - \text{pupal mass}}{\text{prepupal mass}}\right) \times 100$$
e. Prepupal duration (h) = time elapsed between onset of prepupal phase and pupal formation

Treatment- and sex-related effects on prepupal mass were tested using two-way ANCOVA with initial mass as the covariate. The effects of treatment and sex on pupal mass and prepupal duration were tested using two-way ANCOVA, with prepupal mass as the covariate. ANCOVA models were simplified from the full model by removing non-significant interaction terms involving the covariate. Only results from the simplified models are reported. Treatment- and sex-related effects on relative weight loss of prepupae were tested using two-way ANOVA. Pairwise comparisons of each ant-tended treatment with the untended treatment were conducted using the Bonferroni-Dunn procedure (Gagnon et al. 1989).

Statistical analyses and associated pairwise comparisons were performed using the software programs GLMStat version 3.0.1 (generalized linear models), Statview version 4.01 (*G*-tests, Friedman test, Wilcoxon Signed Rank test) and SuperANOVA version 1.11 (ANOVA, ANCOVA).

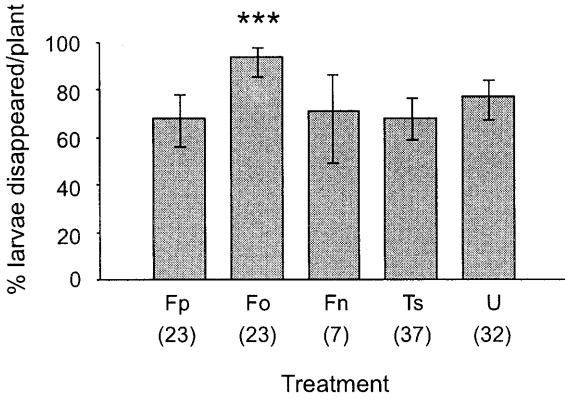

Fig. 1 Mean percentage (± 95% C.I.) of *Glaucopsyche lygdamus* larvae that disappeared from plants when tended by one of four ant species or untended. Treatment: Fp *Formica podzolica*, Fo *F. obscuripes*, Fn *F. 'neogagates'*, Ts *Tapinoma sessile*, U untended. The numbers of plants per treatment are shown in parentheses below treatment name. *** Larvae tended by *F. obscuripes* disappeared at a significantly higher rate than did untended larvae (P=0.001)

Results

Larval disappearance

Larval disappearance differed significantly among the untended and four ant-tended treatments (Δdev=14.68, P=0.005). Larvae associated with *F. obscuripes* disappeared from plants at a significantly higher rate than did untended larvae (pairwise comparison, Δdev=9.74, P=0.002; Fig. 1), while larvae associated with *F. podzolica*, *F. 'neogagates'* and *T. sessile* disappeared at the same rate as untended larvae (untended vs: *F. podzolica* P=0.27; *F. 'neogagates'* P=0.65; *T. sessile* P=0.27; Fig. 1). The majority of *G. lygdamus* larvae (61–90% of larvae lost, depending upon treatment) disappeared from plants during the first to third instar of their development, but all treatments lost fourth instar larvae as well (15–43% of fourth instar larvae, depending upon treatment).

Larval parasitism

Two types of parasitoids, *Aplomya theclarum* (Diptera: Tachinidae) and *Cotesia cyaniridis* (Hymenoptera: Braconidae), emerged from *G. lygdamus* caterpillars during the prepupal phase of the host's development (Table 1). The number of parasitoids per caterpillar ranged from one to four for *A. theclarum*, and from five to nine for *C. cyaniridis*. Only one type of parasitoid emerged from each host caterpillar, but overall, all treatments were attacked by both types of parasitoids (Table 1).

Parasitism on manipulated plants differed significantly among the untended and four ant-tended treatments (Δdev=8.22, df=2, P=0.02; Fig. 2A). Larvae tended by *F. obscuripes* and *F. 'neogagates'* were excluded from this analysis because of small sample sizes (n<6 plants).

Table 1 Summary of parasitism of *Glaucopsyche lygdamus* larvae on manipulated and unmanipulated plants (see Fig. 2 for plant sample size per treatment). (Treatment: Fp *Formica podzolica*, Fo *F. obscuripes*, Fn *F. 'neogagates'*, Ts *Tapinoma sessile*, U untended)

	Treatment				
	Fp	Fo	Fn	Ts	U
Manipulated plants					
Number larvae collected	26	5	6	48	24
Number larvae parasitized	3	2	1	8	8
(% parasitized)	(12%)	(40%)	(17%)	(17%)	(33%)
Parasitism breakdown					
Tachinid fly	3	1	1	6	3
Braconid wasp	0	1	0	2	5
Unmanipulated plants (*n*)					
Number larvae collected	34	18	23	56	0
Number parasitized	3	0	5	7	–
(% parasitized)	(9%)	(0%)	(22%)	(12%)	
Parasitism breakdown					
Tachinid fly	1	0	0	3	–
Braconid wasp	2	0	5	4	–

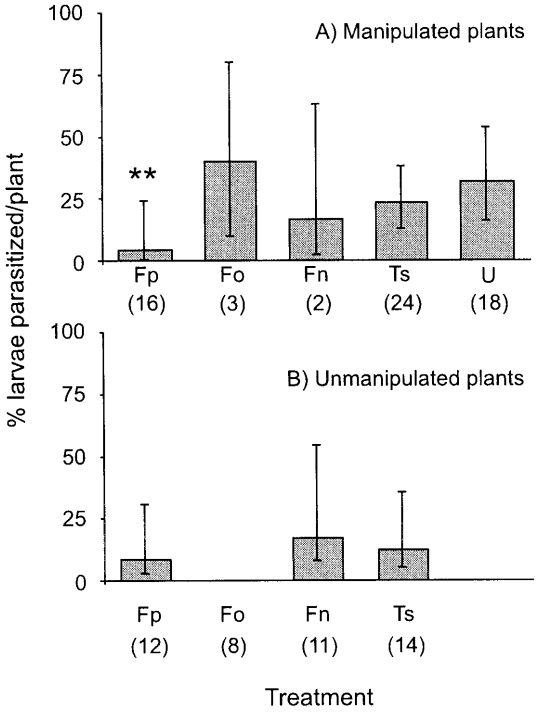

Fig. 2 Mean percentage (± 95% C.I.) of *G. lygdamus* larvae that were parasitized on **A** manipulated plants and **B** unmanipulated plants, when tended by one of four ant species or untended (manipulated plants only). Treatments as in Fig. 1. The numbers of plants per treatment are shown in parentheses below treatment name. ** Significantly fewer larvae associated with *F. podzolica* were parasitized relative to untended individuals (P=0.01; manipulated plants only). *F. obscuripes* and *F. 'neogagates'* were omitted from the analysis of manipulated plants (see text)

A significantly smaller percentage of larvae associated with *F. podzolica* were parasitized compared with untended larvae (Δdev=8.44, P=0.004). Parasitism rates on unmanipulated plants did not differ among treatments (Fig. 2B; Δdev=5.27, *df*=3, P=0.15).

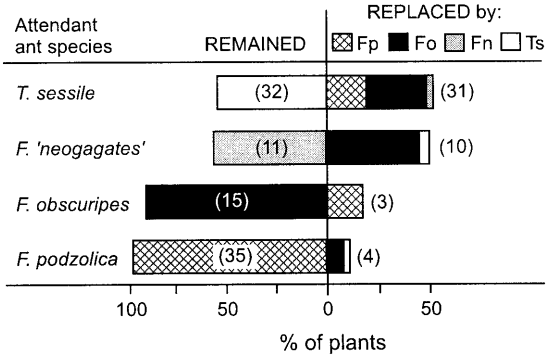

Fig. 3 The relative frequency with which an ant species found tending *G. lygdamus* larvae persisted as the attendant species over consecutive census dates (= REMAINED), or was replaced by another ant species (= REPLACED), differed among ant species (G test of independence, P<0.001). Numbers of plants per category used in the 2×4 contingency table analysis are given in parentheses on the respective sides

No additional parasitoid species were found when *G. lygdamus* pupae were overwintered and reared to eclosion the following year.

Persistence of ant species as tending partners

Two or more ant species were commonly observed on a single plant during a census, but a single ant species usually tended all larvae on the plant. Across census dates, however, the ant species tending larvae on a plant sometimes changed (Fig. 3). The frequency with which one ant species was replaced by another depended on the species in attendance (REMAINED vs REPLACED × ANT SPECIES: G=22.45, *df*=3, P<0.001; Fig. 3). *F. podzolica* and *F. obscuripes* usually remained as attendant ants while *F. 'neogagates'* and *T. sessile* were replaced as attendant species on half of all plants on which

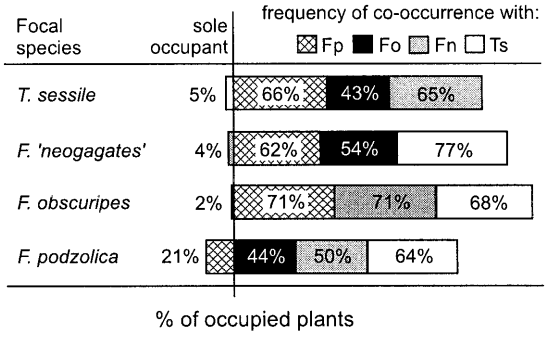

Fig. 4 Percentage of plants occupied by a given ant species for which that ant species was the sole occupant, or co-occurred with other ant species. Percent occupancies sum to greater than 100% since plants were often occupied by more than two ant species

they tended larvae (Fig. 3). When turnovers occurred, *F. obscuripes*, and to a lesser extent *F. podzolica*, was most likely to be the replacing ant species (Fig. 3).

The frequency with which an ant species remained, or was replaced, as the attendant ant was not related to the relative frequency with which ant species encountered one another on plants (compare Figs. 3 and 4). For example, *F. podzolica* was the sole occupant of only 21% of the plants it occupied, and co-occurred with *F. neogagates*, *T. sessile* and *F. obscuripes* on 64%, 50% and 44% of its occupied plants, respectively (Fig. 4). Nonetheless, *F. podzolica* remained as the attendant ant on 91% of plants it occupied as the attendant ant, and was most frequently replaced by *F. obscuripes*, the ant species it encountered least frequently. *F. obscuripes* had the lowest encounter rate with other ant species, yet it was the predominant replacement species when turnovers in larval attendance occurred (compare Figs. 3 and 4).

Developmental performance

Initial masses of field-collected larvae did not differ among treatments or by sex (Tables 2, 3), reflecting an unbiased assignment of larvae to the different treatments.

Females were significantly heavier than males at the onset of the prepupal period, (ANCOVA: P=0.05), but the two sexes pupated at similar masses (P = 0.78; Tables 2 and 3). Masses were similar among treatments at the onset of the prepupal period (P=0.48) but differed significantly at pupation (P=0.02; Tables 2 and 3). Untended pupae were significantly heavier than individuals tended by *F. obscuripes* (P<0.01; Table 2).

There was no significant interaction between treatment and sex for any of the mass parameters measured (Table 3), indicating that the performance of males relative to females was similar among treatments (see Table 2).

Pupal mass was influenced by an interaction between prepupal mass (the covariate) and treatment (ANCOVA: P=0.02; Table 3). Only when prepupae tended by *T. sessile* were excluded from the analysis did the interaction term became non-significant. Plots of prepupal mass against pupal mass for each treatment indicated that, as prepupal mass increased, individuals associated with *T. sessile* pupated at lower weights than did individuals in other treatments.

Ant-tended prepupae lost a significantly greater percentage of their prepupal mass than did untended individuals (ANOVA: P<0.001; Tables 2 and 3; all pairwise comparisons with untended: P<0.05). Neither prepupal sex, nor its interaction with treatment, influenced relative weight loss (Table 3).

Prepupal duration was negatively correlated with prepupal mass (ANCOVA: P=0.03; Table 3), indicating that larger individuals pupated more quickly than smaller counterparts. Neither treatment nor sex influenced prepupal duration, and there was no interactive effect of these two factors on prepupal duration (Table 3).

Table 2 Summary of developmental performance (mean ± 1SE) for *G. lygdamus* juveniles tended by one of four ant species or untended. *n*= number of individuals per treatment. Dependent variables for which a significant treatment- or sex-related effect was found are denoted by an asterisk *. See Table 3 and text for details

Treatment	Initial mass (mg)	Prepupal mass (mg)*	Pupal mass (mg)*	Relative weight loss (%)*	Prepupal duration (h)
Sex (*n*)					
F. podzolica					
Females (9)	89.08±5.73	128.30±3.18	81.66±3.34	36.55±1.28	115±4
Males (8)	95.39±5.80	128.38±4.81	80.14±5.21	37.96±2.67	113±5
F. obscuripes					
Females (7)	97.36±4.77	129.78±4.74	81.30±4.50	37.62±1.56	119±2
Males (11)	86.41±5.35	118.40±3.48	75.11±3.33	36.79±1.37	114±2
F. neogagates					
Females (5)	94.24±7.28	129.66±5.08	85.09±3.64	34.38±0.94	114±2
Males (6)	81.42±6.92	123.82±4.75	79.81±4.42	35.48±2.80	112±4
T. sessile					
Females (11)	91.36±2.71	131.90±4.92	82.93±2.55	36.87±1.30	113±2
Males (6)	90.67±3.43	126.81±4.81	78.93±4.12	37.92±1.12	109±3
Untended					
Females (7)	91.74±5.96	129.32±4.50	90.19±4.58	30.35±2.09	103±4 (*n*=6)
Males (13)	91.87±3.22	115.86±5.12	81.97±4.11	29.39±0.99	113±4 (*n*=12)

Table 3 Two-way ANOVA and ANCOVA results from tests of the effect of tending treatment (untended or tended by one of four ant species) and sex on developmental performance of *G. lygdamus* prepupae

Source	*df*	*MS*	*F*	*P*	
Initial mass (ANOVA)					
Treatment	4	39.34	0.19	0.94	
Sex	1	247.08	1.19	0.28	
Treatment × Sex	4	241.49	1.17	0.33	
Error	73	207.11			
Prepupal mass (ANCOVA)					
Treatment	4	149.84	0.89	0.48	
Sex	1	665.16	3.94	0.05	*
Initial mass	1	1617.81	9.58	0.003	**
Treatment × Sex	4	97.97	0.58	0.68	
Error	72	168.86			
Pupal mass (ANCOVA)					
Treatment	4	85.98	3.11	0.02	*
Sex	1	2.11	0.08	0.78	
Prepupal mass	1	6324.87	228.80	<0.001	***
Treatment × Sex	4	24.46	0.88	0.48	
Treatment × Prepupal mass	4	90.15	3.26	0.02	*
Error	68	27.64			
Relative weight loss (ANOVA)					
Treatment	4	183.27	8.17	<0.001	***
Sex	1	2.39	0.11	0.74	
Treatment × Sex	4	5.45	0.24	0.91	
Error	73	22.44			
Prepupal duration (ANCOVA)					
Treatment	4	157.94	1.66	0.17	
Sex	1	39.00	0.41	0.52	
Prepupal mass	1	454.26	4.76	0.03	*
Treatment × Sex	4	133.34	1.40	0.24	
Error	70	95.41			

ANOVA, ANCOVA:
* P <0.05; ** P <0.01;
*** P <0.001

Discussion

Outcomes of interactions between *G. lygdamus* and ants were influenced by the identity of the attendant ant species. Moreover, relative to untended individuals, only one of the four ant species provided a measurable benefit to *G. lygdamus*. Thus, the relationship between *G. lygdamus* and ants seems to be one of conditional mutualism (Bronstein 1994). The significant reduction in parasitism for larvae associated with *F. podzolica* is consistent with previous studies of *F. podzolica* and its close relative, *F. altipetens* (Pierce and Mead 1981; Pierce and Easteal 1986). *F. 'neogagates'* and *T. sessile* were essentially neutral partners of *G. lygdamus*. Attendance by *T. sessile* influenced prepupal development, but this effect covaried with prepupal mass, resulting in a potential benefit to small prepupae but a cost to heavier individuals. *F. neogagates* and *T. sessile* may generally be ineffective tenders of nectar-secreting insects. Relative to untended individuals, these ants had no effect on disappearance rates of *Icaricia acmon*, another facultatively myrmecophilous lycaenid (Peterson 1993), and did not influence performance of the aphid *Aphis varians* (Addicott 1979) or the membracid *Publilia retuculata* (Bristow 1984). *T. sessile* did provide some protection to *A. vernoniae*, however (Bristow 1984). Under the conditions of our study, *F. obscuripes* appeared to act as a parasite of this generalized association (see Janzen 1975). Relative to untended individuals, association with *F. obscuripes* significantly increased larval disappearance and significantly decreased pupal mass of *G. lygdamus*. *F. obscuripes* has not previously been studied as a partner of lycaenid larvae, but has been shown to protect the sunflower *Helianthella quinquenervis* from seed predation by flies (Inouye and Taylor 1979).

If the species-specific outcomes reported here are consistent across years, and among sites, then selection may favor *G. lygdamus* larvae that preferentially associate with *F. podzolica*, or perhaps avoid *F. obscuripes*. However, a difference in the effect of ant species on lycaenid fitness is a necessary, but not a sufficient, precondition for the evolution of specialization (Schemske and Horvitz 1984). Even if ant species consistently differ in quality as partners of *G. lygdamus*, unpredictability in the availability of a high quality partner, such as *F. podzolica*, may severely limit the potential for specialization (see Howe 1984; Herrera 1988; Waser et al. 1996).

The persistence of ant species as tenders of larvae within a season (and hence over the lifetime of larva) provides some measure of the stability of an association once it is established. Both *F. podzolica* and *F. obscuripes* were persistent partners of *G. lygdamus*, indicating that once they began tending larvae they were unlikely to abandon or lose these larvae to other ant species. *F.*

'neogagates' and *T. sessile*, on the other hand, were frequently replaced as attendant ants, usually by *F. obscuripes* and less frequently by *F. podzolica* (Fig. 3). This pattern of turnover in attendant ant species, from smaller species to larger, and apparently more aggressive ant species, has also been documented in other lycaenid-ant systems (Peterson 1993, 1995). Whether turnovers are due to direct competition among ant species, or to abandonment by one ant species followed by replacement by another is unclear. We did not visit the field site at night, but during the day larvae were rarely found without ants.

Studies of ant protection mutualisms report considerable spatial and temporal variation in ant species abundances (e.g. Horvitz and Schemske 1990; Peterson 1995; Alonso 1998), and similar findings are reported in generalized plant-pollinator systems (e.g. Herrera 1988; Waser et al. 1996). We conducted our study at a single site, and in a single season and therefore do not know the extent to which abundances of the ant species included in our study vary over space and time. If studies of other systems (e.g. Horvitz and Schemske 1990; Peterson 1995; Alonso 1998) are any indication, however, we can expect considerable spatio-temporal variation in ant abundances, and thus limited potential for specialization with *F. podzolica.*

The presence and abundance predators and parasitoids may also vary within and among years, resulting in variation in the selective pressure on *G. lygdamus* to associate with ants. In addition to braconid and tachinid parasitoids, two species of ichneumonid wasps have been reported as parasitoids of *G. lygdamus* at the Naked Hills site (Pierce and Easteal 1986). We overwintered and reared over 175 *G. lygdamus* pupae and did not find additional parasitoids, suggesting that pupal parasitoids were absent in the year our study was conducted. The selective pressure from tachinid and braconid parasitoids appears to be consistent across years, however (compare Pierce and Mead 1981; Pierce and Easteal 1986; this study).

Among the two parasitoids present during our study, we found a slight bias in the number of caterpillars parasitized by each species, depending upon the plant treatment from which the caterpillars were collected (manipulated or unmanipulated plants; Table 1). We suspect that this reflects a difference in the phenology of the two parasitoids rather than an effect of plant treatment. Because female butterflies prefer to lay eggs on unopened floral buds (Carey 1992), and the plants selected for the manipulated treatment were 1–2 weeks more advanced in their flowering phenology than were unmanipulated plants, the two treatments differed in timing of egg deposition and larval development. Based on emergence patterns of parasitoids, it appears that *A. theclarum* may be the predominant parasitoid early in the season, while *C. cyaniridis* predominates later in the season. Parasitism of *G. lygdamus* larvae collected from a late-flowering lupine species, *L. argenteus*, at the study site supports this hypothesis. Caterpillars collected from these plants were parasitized exclusively by *C. cyaniridis* (A.M. Fraser, unpublished data).

The two parasitoid species that attacked *G. lygdamus* are reported to be specialists on the Lycaenidae, although they attack a range of lycaenid genera (Arnaud 1978; Marsh 1979). It is not known at what stage they attack *G. lygdamus* larvae. *C. cyaniridis* is a Microgastinae braconid whose members typically attack early instar larvae, while *A. theclarum* is a member of the Eryciini, which typically deposit their eggs externally on the host and tend to attack later instar larvae (J. Stireman, personal communication).

Measuring true fitness costs and benefits

Parasitism of *G. lygdamus* larvae had a direct negative effect on lycaenid fitness because it resulted in larval death. Further work is needed, however, to determine if the elevated disappearance of larvae associated with *F. obscuripes* represents a true fitness cost. Causes underlying larval disappearance were not investigated but could include predation or drop-off from plants, perhaps in response to predator or parasitoid attack (see Pierce and Easteal 1986). Predaceous insects (large wasps, coccinellid beetle larvae, spiders, and an unidentified hemipteran) were seen on plants, but we did not witness a predation event. Ant species may also act as predators. *Formica* and other nectar-harvesting ant species have been observed preying on lycaenid larvae and aphids in certain situations, while tending in others (Way 1963; Malicky 1970; Andersen 1991; Sakata 1994; Savignano 1994). By choosing manipulated plants close to ant nests, we may have inadvertently selected for heightened ant aggression toward lycaenid larvae (Malicky 1970), especially with *F. obscuripes*. We also observed that inflorescence stalks near *F. obscuripes* nests were often stripped of blossoms, and many did not set fruit. *F. obscuripes* may, therefore, be indirectly responsible for increased larval disappearance through the removal of blossoms on which larvae were feeding. Whatever the causes of disappearance, this measure undoubtedly overestimates larval mortality in the field because fallen larvae could wander to adjacent plants.

While all ant species increased relative weight loss of *G. lygdamus* prepupae relative to untended individuals, only *F. obscuripes*, and potentially *T. sessile*, caused a significant reduction in pupal mass. It remains to be determined if this negative effect on pupal mass would persist if *G. lygdamus* larvae were reared in the presence versus the absence of ants throughout the latter two instars. Several studies have demonstrated that ant attendance stimulates larval development (Baylis and Pierce 1992; Fiedler and Saam 1994; Wagner and Martinez del Rio 1997), such that ant-tended larvae pupate at similar, or heavier wet weights than their untended counterparts (Fiedler and Hölldobler 1992; Wagner 1993; Cushman et al. 1994; Fiedler and Saam 1994; Wagner and Martinez del Rio 1997), despite the significantly greater weight loss by tended individuals during the non-feeding period (Wagner 1993; this study). We attempted to rear *G.*

lygdamus on cuttings in the laboratory to address this question, but all laboratory-reared larvae died before, or shortly after, reaching the fourth instar. None of the studies on lycaenid development to date, including our study, have examined whether the effect of ant-attendance on pupal or adult mass is due to a difference in tissue biomass, or simply a difference in water retention among treatments. Future studies should attempt to address this.

Relative to the untended treatment, tended *G. lygdamus* females experienced a larger reduction in pupal mass than did tended males, suggesting a sex-related developmental cost similar to that reported for other lycaenid species (Pierce et al. 1987; Fiedler and Hölldobler 1992).

In summary, under the conditions which this study was conducted, it appears that the relationship between *G. lygdamus* and ants differs with the species of ant under consideration. Furthermore, associating with a broad range of ants may be costly. Larvae tended by *F. obscuripes* disappeared from plants at a higher rate than did untended larvae, and pupated at smaller masses than did untended individuals. Even if *G. lygdamus* females could avoid ovipositing on plants bearing *F. obscuripes*, there would be a high probability that *F. obscuripes* would eventually become the attendant ant species since it frequently replaced *F. 'neogagates'* and *T. sessile* as the attendant species. Future work should examine whether the apparent cost of association with *F. obscuripes* represents a true fitness cost and, if so, whether this negative outcome is a general phenomenon or peculiar to the conditions under which this study was conducted.

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