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ASSESSING BENEFITS TO BOTH PARTICIPANTS IN A LYCAENID–ANT ASSOCIATION¹

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Abstract. We examined interactions between the ant *Iridomyrmex nitidiceps* and the lycaenid butterfly *Paralucia aurifera* in southeastern Australia, and present data supporting the hypothesis that both participants benefit from their association. In the field, lycaenids persisted only on those host plants that ants subsequently colonized. In the laboratory, lycaenid larvae reared with ants were 31–76% heavier, developed 37% faster, and commonly completed one or two fewer instars than larvae reared without ants. Ant tending also resulted in 20% heavier pupae, 69% shorter pupal duration, and 5% larger adults as measured by forewing length; adults were not significantly different as measured by body length. We hypothesize that these positive effects occurred largely because ant-tended lycaenid larvae spent more time feeding than did untended larvae. Field data documented that ants colonized host plants only after lycaenid larvae were present, indicating that ants actively maintained the association. In laboratory experiments, 40% more ant workers survived when lycaenid larvae were present than when they were absent, although ant mass was not significantly affected. We hypothesize that the survivorship effects occurred because ants consumed the lycaenid's nectary gland secretions, which contained considerable amounts of glucose and amino acids. Our results show that lycaenids can benefit from ants in ways other than, or in addition to, protection from natural enemies and that they incur minimal developmental costs from associating with ants.

Key words: ant worker survival; lycaenid–ant interactions; lycaenid growth and development; mutualism; southeastern Australia.

INTRODUCTION

The participants in most nonsymbiotic mutualisms can be classified as either hosts or visitors. Thompson (1982) was the first to propose this distinction, defining hosts as plants or animals that provide food and/or domicile and visitors as animals that provide beneficial services (e.g., protection, dispersal, or pollination). Previous studies of putative mutualisms have concentrated primarily on the effects of visitors on the survival and reproduction of their hosts (see Boucher et al. 1982, Addicott 1984). Considerably less attention has been directed toward potential host benefits related to development time, which is especially pertinent in animal–animal mutualisms (see Bristow 1984, Pierce et al. 1987, Fiedler and Hölldobler 1992). This is surprising given that development time can have substantial effects on reproductive success and population dynamics. For example, faster development can shorten generation time and thereby increase the intrinsic

rate of natural increase (Cole 1954, May 1976, Charlesworth 1980). Faster development can also reduce exposure to natural enemies and increase the probability of survival (Feeny 1976, Price et al. 1980).

Although the definition of mutualism requires that both participants benefit from their association (Boucher et al. 1982, Boucher 1985), most studies have neglected to consider the perspective of visitors or have simply assumed that visitors benefit from their interactions with hosts (Cushman and Beattie 1991). There are at least two reasons why the perspective of visitors is so commonly overlooked. First, they are often difficult to study, due to their greater mobility, and in some cases, their complex social structure (e.g., social Hymenoptera). Second, the benefits to visitors, food and/or domicile, may often seem obvious and therefore unnecessary to document. However, despite logistical difficulties and the appearance of benefits to visitors, studying only half of an interaction may result in a one-sided, and perhaps inaccurate, view of how mutualisms work. In addition, focusing exclusively on hosts ignores the possibility that hosts deceive their visitors and thus leaves unresolved the question of whether or not associations are actually mutualistic (Cushman and Beattie 1991).

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Ants are particularly conspicuous visitors, as they form close associations with a diverse range of plants, animals, and fungi (Thompson 1982, Beattie 1985, Hölldobler and Wilson 1990, Cushman and Beattie 1991). Many of these associations involve larvae in the families Lycaenidae (the blue, copper, and hairstreak butterflies) and Riodinidae (the metalmark butterflies), which together comprise 30–40% of all butterfly species (Vane-Wright 1978, Shields 1989). Roughly half of the species in these two families are tended by ants: their larvae possess numerous structures that facilitate the association, including dorsal nectary glands on the abdomen that produce secretions containing sugars and amino acids (Atsatt 1981, Kitching 1983, Pierce 1984, 1987, 1989).

Although a substantial literature on the natural history of lepidopteran–ant associations has developed over the past century (reviews by Atsatt 1981a, Cottrell 1984, Pierce 1987), the larvae of only three species have been shown experimentally to benefit from being tended by ants (*Glaucopsyche lygdamus*: Pierce and Mead 1981, Pierce and Easta 1986; *Jalmenus evagoras*: Pierce et al. 1987; *Thisbe irenea*: DeVries and Baker 1989, DeVries 1991). In each case, ants were found to protect lepidopteran larvae from their natural enemies. Only one study has shown experimentally that ants can benefit from associating with Lepidoptera (Nash 1989), although two studies provide indirect evidence of ant benefits (Pierce et al. 1987, Fiedler and Maschwitz 1988).

Here, we address the perspective of both participants in a putative mutualism, focusing on the interactions between larvae of the lycaenid butterfly *Paralucia aurifera* and the ant *Iridomyrmex nitidiceps*. First, we assess the lycaenid's perspective by examining (a) the influence of ants on the persistence of lycaenids in the field and (b) the effect of ants on the growth and development of lycaenids in laboratory experiments. Second, we consider the ant's perspective by documenting the influence of lycaenids on (a) the colonization behavior of ants in the field and (b) the mass and survival of ant workers in laboratory experiments.

NATURAL HISTORY OF THE SYSTEM

The butterfly *Paralucia aurifera* (the bright copper) occurs in southeastern Australia, from southern Queensland to Tasmania. Its primary food plant is a perennial shrub, *Bursaria spinosa* (Blackthorn; Pittosperaceae). The lycaenid has 1–4 generations per year, depending on the local climate. Adults fly from August to April, with peaks in abundance during November and February (R. L. Kitching, *personal communication*). They remain close to patches of their host plant and do not exhibit hill-topping behavior like many other butterfly species (see Alcock 1987). Thus, juvenile stages (eggs, larvae, pupae) and adults are found in close proximity (Common and Waterhouse 1981).

Adult females deposit eggs primarily on the under-

side of leaves of the host plant, either singly or occasionally in groups of up to four. The eggs usually hatch in 6–9 d, whereupon the larvae go through 5–6 instars and pupate in the soil at the base of their host plant. The lycaenid overwinters as pupae (J. H. Cushman, *personal observation*) and perhaps late-instar larvae, as found for *Paralucia pyrodiscus lucida* (Braby 1990).

The larvae and pupae of *P. aurifera* always associate with ants and are found exclusively with *Iridomyrmex nitidiceps* (Common and Waterhouse 1981; the *I. nitidiceps* group is being reclassified as *Anonychomyrma nitidiceps* group, species A; S. Shaddack, *personal communication*). Ants recruit to early-instar *P. aurifera* larvae, found only on the foliage, but are prevented from actively tending them by the lycaenid's long dorsal setae. Beyond the third instar, larvae are found on the foliage only at night and spend the day in subterranean chambers constructed by *I. nitidiceps* at the base of their host plant. The larvae emerge from these shelters shortly after sunset, ascend the food plant to feed, and descend to the shelters just before sunrise. Both in the field and under laboratory conditions, each nocturnal-feeding *P. aurifera* larva is tended continuously by up to 25 ants, with the number of ants increasing with larval size. In the laboratory, individual pupae were tended by 3–12 ants. The egg stage is untended (J. H. Cushman and V. K. Rashbrook, *personal observations*).

The subterranean shelters that *I. nitidiceps* workers construct at the base of *B. spinosa* plants consist of an elaborate network of chambers that can contain up to 20 lycaenid larvae and 10 pupae. *Iridomyrmex nitidiceps* appears to use these chambers as "outpost" or satellite nests, with well-maintained connections in terms of exchange of workers and presumably resources between these outposts and the main colony (J. H. Cushman and V. K. Rashbrook, *personal observations*).

Both *P. aurifera* larvae and pupae produce secretions that *I. nitidiceps* workers may harvest. In another study (J. H. Cushman et al., *unpublished data*), we used high-pressure liquid chromatography (HPLC) to analyze the amino acid and monosaccharide content of hydrolyzed larval secretions. We detected 13–15 amino acids (primarily proline, valine, serine, glutamine, and asparagine) at average individual concentrations ranging from 0.5 to 14.8 mmol/L; total amino acid concentration averaged 97 mmol/L. The monosaccharide analyses detected only glucose in concentrations averaging 34%.

Paralucia aurifera eggs suffer varying degrees of parasitism by a scelionid wasp (*Telenomus* sp.), ranging from 10 to 25% depending on the site and time of year. By contrast, our extensive rearings of field-collected larvae and pupae ($n > 350$) have produced only 12 parasitized individuals (<3.4%). Ten larvae were attacked by an ichneumonid wasp (*Habronyx* sp.) and two by a trigonalid wasp (*Taeniogonalos* sp.); in all cases, parasitoids emerged after larvae pupated. There

are a variety of nocturnal spiders that may be important predators of *P. aurifera* larvae.

METHODS

We conducted this study in the laboratory at Macquarie University and at two field sites near Sydney, New South Wales, Australia. Both field sites occur at low elevation and are located in Dural (30 km northwest of Sydney) and Wyong (90 km north of Sydney).

Host-plant colonization

From September to December 1991 (spring–summer), we surveyed *B. spinosa* at the two field sites to determine the pattern and sequence of plant colonization by *I. nitidiceps* and *P. aurifera* larvae. At each site, we randomly selected and labeled 20 uncolonized plants and monitored them every 2–3 wk. After 10 wk, we classified each of the 40 plants as (1) uncolonized, (2) colonized by ants only, (3) colonized by lycaenid larvae only, (4) colonized by ants first and lycaenid larvae second, or (5) colonized by lycaenid larvae first and ants second. We considered a plant as having been colonized by ants when *I. nitidiceps* workers had excavated the soil at its base and constructed a satellite nest. We classified plants as having been colonized by lycaenids after we observed the distinctive feeding furrows on the underside of leaves made by first- to third-instar larvae. This damage was easily distinguished from that inflicted by older larvae which chewed entire leaves. In all cases, larvae present on a given host plant arose from eggs deposited on that plant (J. H. Cushman and V. K. Rashbrook, *personal observations*).

Effect of ants on lycaenid mass

We performed three laboratory experiments to assess the effect of ants on the mass of lycaenid larvae. We conducted these experiments under conditions of natural light and ambient temperature ($21^{\circ} \pm 3^{\circ}\text{C}$). Each of five captive *I. nitidiceps* colonies contained at least one queen and large numbers of workers, eggs, larvae, and pupae. We housed each colony in a fluon-coated box ($70 \times 40 \times 20$ cm) and provided them with moistened cotton wool, ample amounts of artificial diet (Bhatkar and Whitcomb 1970), and occasional *Drosophila*.

In the first experiment (March 1991), we paired 10 potted *B. spinosa* plants according to size, density of foliage, and overall condition. Plants ranged from 30 to 40 cm in height. We then transferred 34 field-collected lycaenid larvae of known mass onto the plants; these larvae ranged between the third and sixth instar and varied widely in mass. We placed three larvae on each of six plants, while the remaining four plants each received four larvae. We randomly assigned half of the lycaenid-occupied potted plants to the ant treatment while the other half served as a control. We placed the five plants assigned to the ant treatment into two ant boxes, while placing the control plants immediately

adjacent to these boxes. After 7 d, we removed all remaining larvae on plants from both treatments (often requiring excavation of the soil around the base of ant-treatment plants) and weighed each of them. To assess the hypothesis that the effect of ants on larvae was mass-specific, we categorized the initial mass of all larvae as above (large, 20.3–33.7 mg) or below (small, 7.3–20.0 mg) the median.

In the second experiment (April 1991), we transferred 12 laboratory-reared third-instar larvae, each 20 d old and weighing from 0.5 to 1.3 mg, onto two potted plants matched for overall quality. We placed one plant into an ant box while the other was placed immediately outside the box. After 14 d, we collected and weighed all remaining larvae.

In the third experiment (November–December 1991), we used 36 field-collected larvae ranging from the third to sixth instar and varying widely in mass. We grouped them by mass into the lowest, middle, and highest third (small, 0.9–3.8 mg; medium, 4.8–10.8 mg; large, 11.1–17.8 mg). We paired 12 plants as before, transferred three larvae onto each plant, and assigned the plants in each pair to opposing treatments. We placed six plants into the ant boxes (two plants per box) and positioned the other six immediately outside the boxes as before. After 21 d, we collected and weighed all remaining larvae.

In all three experiments, we weighed each larva twice (at the beginning and end of each experiment), but were unable to keep track of the identity of individuals. In the first and third experiment, we performed two-way ANOVAs on the final larval masses, with ants (present/absent) and larval size (small/large or small/medium/large) as the grouping factors. In the second experiment, we performed a one-way ANOVA on the final larval masses. We did not control for lycaenid sex in these mass experiments.

Effect of ants on lycaenid development time

We reared *P. aurifera* from egg to adult stage to determine the effect of ants on the number of larval instars, duration of each instar, total larval development time, pupal mass and duration, and adult eclosion and size. Between October 1991 and February 1992, we collected 42 eggs from the field and reared them in the laboratory. We placed each egg into a mesh-covered vial with moistened filter paper and, after hatching, added freshly cut *B. spinosa*. We transferred a total of 19 vials into an ant box and a total of 23 vials immediately outside the box. The mesh covering prevented the larvae from escaping but allowed ants to enter. Every 2nd d until the larvae pupated, we replaced *B. spinosa* with fresh material and cleaned out the frass from vials. We noted the number of days that larvae spent in each instar and determined the number of instars by collecting all discarded head capsules and, secondarily, by noting the cessation of feeding that occurred prior to molting and instar-related differences

TABLE 1. Colonization of 40 *Bursaria spinosa* plants by *Paralucia aurifera* and *Iridomyrmex nitidiceps* at two field sites. Three of the 40 plants were uncolonized by either ants or lycaenids.

A) Colonization sequence for plants colonized by both species		
Colonizer	Arrival sequence	
	Colonized first	Colonized second
	No. plants	
Lycaenids	32	0
Ants	0	32
B) Lycaenid persistence on colonized plants		
Fate	Colonizer	
	Lycaenids only	Lycaenids + (later) ants
	No. plants	
Persist	0	32
Die	5	0

in the number of dorsal setae. We also recorded the mass of newly pupated individuals, duration of the pupal stage, adult size as measured by forewing length and body length, and sex of eclosed adults. We analyzed the data on days/instar through time using a one-way repeated-measures ANOVA, with ants (presence/absence) as the grouping factor and time (instars 1–5) as the repeated measure (because some larvae began pupating in the fifth instar, we omitted the data for sixth and seventh instars in this analysis). We analyzed all other data with two-way ANOVAs, using ant treatment and sex as the grouping factors.

Effect of lycaenids on ant mass and survival

We performed two laboratory experiments to assess the short-term effect of *P. aurifera* larvae on the mass and survival of *I. nitidiceps* workers. We focused on the worker caste because they are the portion of the colony that require energy from sugars for foraging (see Beattie 1985). In both experiments, our aim was first to test the common assumption that ant workers benefit from consuming lycaenid secretions (the benefit hypothesis). This test was especially important in our system, given that we rarely observed *I. nitidiceps* actually collecting the secretions of *P. aurifera* (although they actively tend the lycaenid larvae at all times). Our second aim was to evaluate the quality of lycaenid secretions relative to a known high-quality artificial diet (the resource-quality hypothesis).

In the first experiment, we placed a total of 225 randomly chosen ants (from one laboratory colony) of known weight into 15 vials (15 ants/vial), the upper halves of which had been coated with fluon (liquid teflon) to prevent ants from escaping. Into each vial we placed moistened filter paper, a piece of the host plant *B. spinosa*, and either (1) nothing, (2) a late-instar

P. aurifera larva, or (3) ≈ 300 mg of artificial ant diet (5 vials/treatment). We used 15 ants/vial, because this was the average number of ants we observed tending late-instar larvae in the field. After 24 h, we re-weighed all living ants to generate mean mass per ant per vial and calculated the percent change in mean mass per vial.

In the second experiment, we assessed the survival of 450 randomly chosen ants (from the same colony as before) after they were subjected to the three treatments for 36 h (plant only, plant plus lycaenid, and plant plus artificial diet). We used the same procedure as before, but did not weigh the ants, and determined the percent ant survival per vial (15 ants/vial, 10 vials/treatment). In both experiments, we first performed one-way ANOVAs on the mass change and survival data, and then compared the three treatments with Scheffé multiple-comparison tests. Comparison between the plant only and plant plus lycaenid treatments evaluated the benefit hypothesis, and comparison between the plant plus lycaenid and plant plus artificial diet treatments evaluated the resource-quality hypothesis.

RESULTS

Host-plant colonization

Our field surveys of 40 plants at two sites documented the colonization behavior and persistence of the ants and lycaenids (Table 1). First, the data showed that *I. nitidiceps* actively maintained its association with *P. aurifera* larvae. After 10 wk, 80% of the plants had been colonized by ants. In all cases, this happened only after lycaenid larvae had colonized the plants; ants neither colonized plants without lycaenids nor preceded the arrival of lycaenids (Table 1A). Second, given that *P. aurifera* colonized plants prior to ants, the data suggest that females did not exhibit ant-dependent oviposition, as has been shown for two other lycaenid species (Atsatt 1981b, Pierce and Elgar 1985). Third, lycaenid larvae persisted only on those host plants that were colonized subsequently by ants (Table 1B).

Effect of ants on lycaenid mass

In the absence of natural enemies, the mass of developing *P. aurifera* larvae was greatly influenced by ants. In the first experiment (7-d duration), larvae reared with ants were 32% heavier than larvae reared without ants (Fig. 1A). A two-way ANOVA revealed that the main effect of ants on larval mass was significant, while the ant \times larval size interaction was not (Table 2A). In the second experiment (14-d duration), larvae reared with ants were 76% heavier than larvae reared without ants (Fig. 1B; Table 2B). In the third experiment (21-d duration), larvae reared with ants were 72% heavier than those without ants (Fig. 1C). We also detected a significant ant \times larval size interaction, as the mass of larvae in the medium size class was not significantly influenced by the ant treatment (Table 2C).

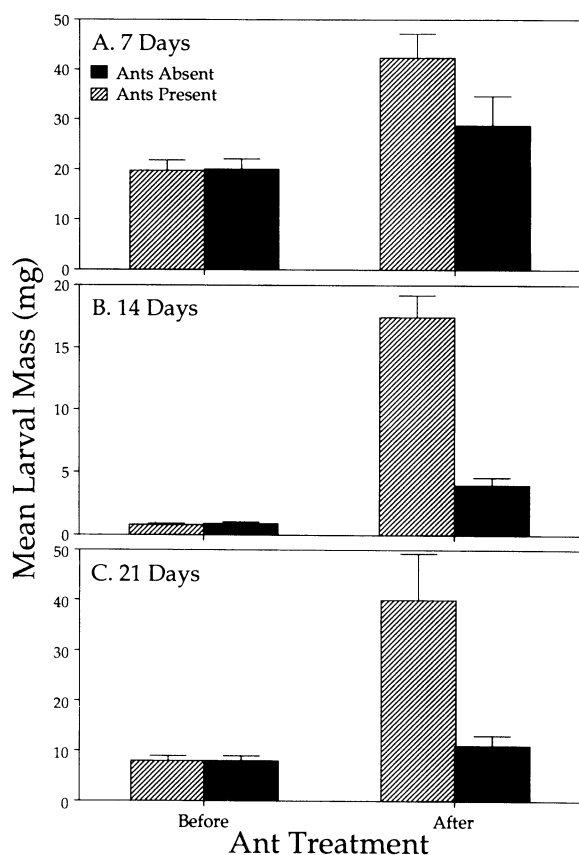


FIG. 1. Effect of the presence or absence of *Iridomyrmex nitidiceps* workers on the mass of *Paralucia aurifera* larvae after 7, 14, and 21 d. Narrow vertical bars represent 1 SE.

Effect of ants on lycaenid development

In the absence of natural enemies, *I. nitidiceps* had a positive effect on the development of *P. aurifera*. Lycaenids reared with ants spent significantly less time in each larval instar through time than those reared without ants (Fig. 2; Table 3A). In addition, larvae went through significantly fewer instars in the presence of ants; tended larvae pupated in the fifth or sixth instar, while untended larvae pupated in the sixth or seventh instar (Fig. 3; $\chi^2 = 15.55$, $df = 2$, $P < .0001$). The combination of these two effects resulted in reduced development times, with ant-reared individuals developing from hatched larvae to pupae in 37% fewer days on average than untended individuals (Tables 3B and 4).

Ants also had a positive effect on the development of lycaenid pupae (Table 4). Tended individuals were 20% heavier at pupation than untended individuals (Table 3C). Even when larval instar at pupation was held constant, by considering only those lycaenids pupating in the sixth instar, ant tending still resulted in significantly heavier pupae (Table 4; $F_{1,27} = 23.32$, $P < .0001$). In addition, duration of the pupal stage was 69% shorter for ant-tended lycaenids than their un-

TABLE 2. ANOVA tables for laboratory experiments evaluating the effect of *Iridomyrmex nitidiceps* workers on the body mass of *Paralucia aurifera* larvae.

Source	df	MS	F	P
A) After 7 d				
Ant	1	922.2	4.30	.0482
Size	1	6383.0	29.75	.0001
Ant \times size	1	34.9	0.16	.6899
Error	26	214.5		
B) After 14 d				
Ant	1	488.7	58.23	.0001
Error	9	8.4		
C) After 21 d				
Ant	1	3223.2	22.30	.0003
Size	2	1098.2	7.60	.0053
Ant \times size	2	691.2	4.78	.0247
Error	15	144.5		

tended counterparts (Table 3D). While there was a trend for fewer tended individuals to eclose compared to untended individuals (14/19 for ant treatment vs. 20/23 for the no-ant treatment), this trend was not significant (Yates' corrected $\chi^2 = 1.65$, $df = 1$, $P = .28$).

One of two measured characteristics of newly eclosed adults was also influenced by the presence or absence of ants (Table 4). Forewing length was significantly longer for ant-tended individuals compared to untended individuals (Table 3E). However, while there was a trend for ant-tended adults to be longer in body length, this pattern was not significant (Table 3F). Finally, neither the sex main effects nor the sex \times ant interaction terms were significant for any of the lycaenid variables considered in this experiment (Table 3B–F).

Effect of lycaenids on ant mass and survival

In the first experiment, there was a significant overall effect of the diet treatments on the mass of ant workers (Fig. 4A; Table 5A). After 24 h, ants with access to the

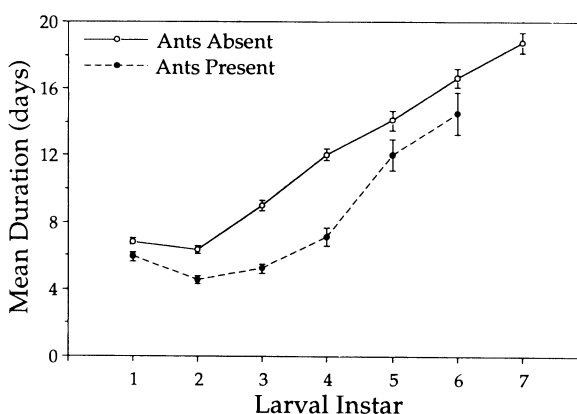


FIG. 2. Duration of each instar for *Paralucia aurifera* larvae reared with and without *Iridomyrmex nitidiceps* workers. Vertical bars represent ± 1 SE.

TABLE 3. ANOVA tables for a laboratory experiment evaluating the effect of *Iridomyrmex nitidiceps* workers and lycaenid sex on *Paralucia aurifera* larval duration, pupal mass and duration, and adult size.

Source	df	MS	F	P
A) Larval development by instar (instars 1–5)				
Ant	1	379.1	77.01	.0001
Error (ant)	40	4.9
Time	4	403.9	104.65	.0001
Ant \times time	4	27.9	7.23	.0001
Error (ant \times time)	160	3.9
B) Total larval duration				
Ant	1	6899.3	98.76	.0001
Sex	1	12.3	0.77	.6594
Ant \times sex	1	45.7	0.74	.3979
Error	29	62.1
C) Pupal mass				
Ant	1	2049.4	11.34	.0022
Sex	1	335.7	1.86	.1833
Ant \times sex	1	244.3	1.35	.2544
Error	29	180.7
D) Pupal duration				
Ant	1	43 125.5	17.93	.0002
Sex	1	1103.4	0.46	.5036
Ant \times sex	1	5860.8	2.44	.1294
Error	29	2405.8
E) Forewing length				
Ant	1	2.1	4.65	.0412
Sex	1	0.6	1.43	.2429
Ant \times sex	1	0.4	0.98	.3329
Error	24	0.5
F) Body length				
Ant	1	0.6	1.48	.2346
Sex	1	0.2	0.59	.4479
Ant \times sex	1	0.7	1.72	.2012
Error	26	0.4

host plant plus moisture decreased in mass by an average of 5.2%, compared to average mass increases of 6.7 and 22.2% for ants that also had access to a lycaenid larva and artificial diet, respectively. However, multiple-comparison tests failed to detect significant dif-

ferences between the plant-only and plant plus lycaenid treatments (the benefit hypothesis) and between the plant plus lycaenid and plant plus artificial diet treatments (the resource-quality hypothesis).

In the second experiment, there was also a significant overall effect of the three dietary treatments on the survival of ant workers (Fig. 4B; Table 5B). After 36 h, fewer ants had survived with the host plant and moisture alone (54.8%) than with a lycaenid larva or artificial diet (95.1 and 96.3%, respectively). Multiple-comparison tests detected significant differences in worker survival between the plant-only and plant plus lycaenid treatments (the benefit hypothesis), but not between the plant plus lycaenid and the plant plus artificial diet treatments (the resource-quality hypothesis).

DISCUSSION

We have presented experimental and observational data consistent with the hypothesis that interactions between *P. aurifera* and *I. nitidiceps* are mutualistic. Our data are important for three reasons. First, they attempt to address the perspectives of both participants. Second, they show that lycaenid larvae can benefit from ants in ways other than, or in addition to,

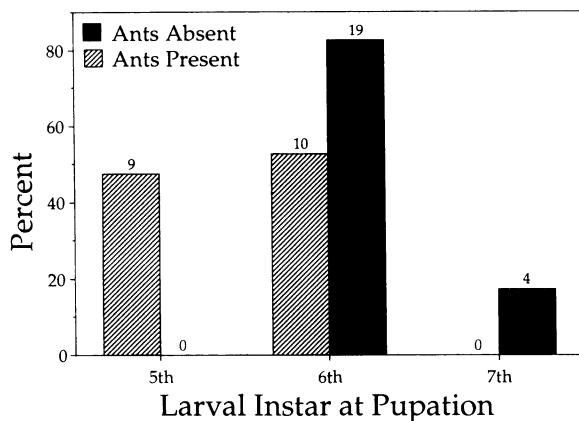


FIG. 3. Effect of the presence or absence of *Iridomyrmex nitidiceps* workers on the instar at which *Paralucia aurifera* larvae pupate. Numbers above bars correspond to the number of lycaenid individuals in each category.

TABLE 4. Effect of the presence or absence of *Iridomyrmex nitidiceps* workers on *Paralucia aurifera* development. Values given are means \pm 1 SE.

Variable	Ants	No ants
Larval duration (d)	43.18 \pm 1.92	68.93 \pm 1.74
Pupal duration (d)	31.79 \pm 11.13	102.55 \pm 13.1
Mass at pupation (mg)		
All individuals	90.92 \pm 4.76	70.59 \pm 1.73
Sixth-instar pupation only	96.96 \pm 7.13	69.12 \pm 1.79
Adult size (mm)		
Forewing length	12.0 \pm 0.21	11.35 \pm 0.16
Body length	10.49 \pm 0.19	10.11 \pm 0.15

protection from natural enemies. Third, we document that lycaenid larvae incur minimal developmental costs from associating with ants.

Benefits to the lycaenid

We found that the presence of *I. nitidiceps* increased the mass of *P. aurifera* larvae by 31–76% and reduced the development time of larvae by 37% and that of pupae by 69%. As a result of these effects, we estimate that ants reduced generation time by 50%, from 197.5 d for untended individuals (8.0 d as egg, 68.9 d as larva, 102.6 d as pupa, and 14.0 d as adult) to 97.0 d for ant-tended individuals (8.0 d as egg, 43.2 d as larva, 31.8 d as pupa, and 14.0 d as adult). In environments at higher elevations and/or latitudes (which have shorter potential breeding seasons), the main significance of shortened generation time is that *P. aurifera* populations will be more likely to successfully complete a generation. In environments at lower elevations and/or latitudes, ant tending may allow *P. aurifera* populations to undergo additional generations. In both cases, this would lead to a substantial increase in the intrinsic rate of natural increase.

Ant-tended *P. aurifera* larvae also underwent one or two fewer instars than their untended counterparts. To our knowledge, no study has previously shown that ants affect the number of developmental stages that lycaenids go through. Indeed, we do not know of any studies reporting that one participant in a mutualism influences the number of developmental stages of another participant. However, studies have shown that reduced humidity, temperature, and/or nutritional quality of host plants can lead to additional larval in-

stars in a number of Lepidoptera (see Taylor 1984 and references therein).

There are at least two mechanisms that may have generated the lycaenid growth and development results. One possibility is that the ant-constructed shelters provided improved physiological conditions, such as increased humidity, that promoted larval growth. A second possibility is that untended lycaenid larvae behaved abnormally in the absence of ants, becoming disoriented and spending less time feeding than ant-tended larvae. Both mechanisms could be operating in this system, and our observations strongly suggest that the second mechanism is particularly important.

TABLE 5. ANOVA tables for laboratory experiments evaluating the effect of *Paralucia aurifera* larvae on the mass and survival of *Iridomyrmex nitidiceps* workers.

Source	df	MS	F	P
A) Mass				
Diet	2	939.7	6.88	.0102
Error	12	136.5
B) Survival				
Diet	2	5594.9	27.13	.0001
Error	27	206.2

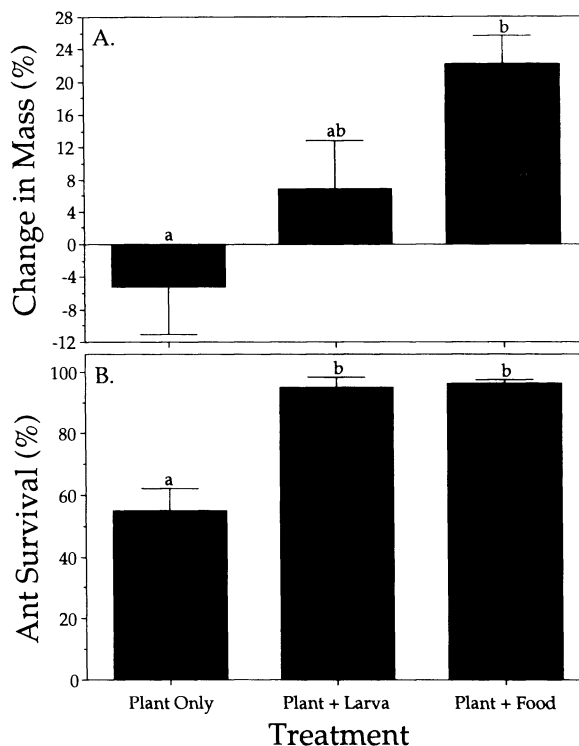


FIG. 4. The effect of artificial diet, *Paralucia aurifera* larvae, and control treatments on (A) percent change in the body mass of *Iridomyrmex nitidiceps* workers after 24 h and (B) percent ant survival after 36 h. In both panels, we present means and 1 SE. Lowercase letters above each bar correspond to the results of Scheffé multiple-comparisons tests.

Although increased feeding rates may explain the positive effects of ants on the growth and development of lycaenid larvae, the mechanism does not fully explain the results for lycaenid pupae. It clearly applies to the finding that ant-tended pupae were heavier than untended pupae (heavier larvae simply become heavier pupae), but it is unclear how increased larval feeding rates could influence pupal development time. One possibility is that individuals that developed quickly as larvae also developed quickly as pupae. However, such "carryover" effects would not explain why ant tending decreased larval development by 37% but decreased pupal development by 69%. Further experiments are needed to untangle this relationship, such as rearing larvae with and without ants and then rearing the resulting pupae in each group with and without ants.

The increased mass of ant-tended larvae and pupae that we detected in our laboratory experiments may translate into increased reproductive success of adult butterflies. Although we do not have such data for *P. aurifera*, numerous studies of other Lepidoptera have shown positive correlations between pupal and/or adult mass and lifetime female fecundity (Hayes 1981, Jones et al. 1982, Karlsson 1987, Elgar and Pierce 1988, Karlsson and Wickman 1990, Honek 1993). Thus, ant tending may increase female fecundity for *P. aurifera*.

Although the net effect of ant tending on lycaenids may be positive, the prevailing view is that lycaenids incur a cost to maintain the association, because the sugary larval secretions that attract ants are thought to be metabolically expensive to produce (Pierce 1987). Pierce and co-workers demonstrated the existence of such costs for the lycaenid *J. evagoras* (Pierce et al. 1987, Elgar and Pierce 1988, Pierce 1989, Baylis and Pierce 1992). Although they detected a 19% reduction in larval development time, ant-tended individuals weighed 25% less as pupae than their untended counterparts, thus decreasing their reproductive success. Robbins (1991) may also have evidence of costs, as he observed that ant-tended larvae of the lycaenid *Arawacus lincoides* took longer to develop than untended larvae.

In our lycaenid-ant system, we failed to detect any costs for *P. aurifera*, either because associating with ants did not result in costs or, more likely, they were minimal and larvae could rapidly compensate for them. Costs also appear to be minimal for the lycaenid *Hemiarctus isola*, where three ant species did not affect development time, and one of the species enhanced larval growth and produced significantly heavier adults (Wagner 1993). DeVries and Baker (1989) also presented data suggesting that costs were minimal or absent for *T. irenea*, as ant-tended larvae were 30% heavier than untended larvae. Fiedler and Hölldobler (1992) reported more complex results for the lycaenid, *Polyommatus icarus*. While ant-tended larvae and pupae were not different from untended individuals in terms of

development time, sex-dependent costs and benefits may occur: tended females lost significantly more mass during the pupal stage than untended females and tended males were significantly heavier as pupae than untended males.

The finding that lycaenid larvae were heavier and developed faster when tended by ants in the absence of natural enemies does not detract from the hypothesis that protection from enemies was a major factor driving the evolution of this mutualism. Rather, enhanced growth and development in the presence of ants (and the associated reductions in generation time) may have been an additional selective advantage for lycaenids. Further, enhancement of larval feeding by ants may have made the supply of ant rewards more certain, thereby creating strong positive feedback within the interaction. Such benefits would have been important during the evolution of the interaction if there was temporal and spatial variation in the need for protection from enemies. In addition, evolution of the lycaenid-ant interaction may have been intensified not only by the coincidence of multiple selective advantages, but by the apparent minimal cost of the association to the lycaenid.

Benefits to the ant

Several lines of evidence suggest that *I. nitidiceps* benefits from associating with *P. aurifera*. Field data showed that ants exhibited behaviors that promoted their association with lycaenids, as they constructed nests and elaborate shelters only at the base of plants previously colonized by the lycaenid. Given that the intensity of ant foraging is commonly related to resource quality, quantity, and/or stability (Sudd and Frank 1987, Hölldobler and Wilson 1990), it seems unlikely that colonies (or portions of them) would distribute themselves in such a way if they were not receiving substantial benefits from their lycaenid associates. Presumably, such behavior allows ants to harvest more effectively the glucose- and amino acid-rich secretions of lycaenids. In addition, if recruitment, foraging trails, and shelter building represent a hierarchy of increasing investment in resource acquisition for ants in general (see Hölldobler and Wilson 1990), then the results of this study may indicate that lycaenid larvae are not only beneficial to ant colonies, but are among the highest quality resources available to them.

While we feel that the ant colonization data are compelling, they do not rule out the possibility that lycaenids are deceptive hosts (sensu Cushman and Beattie 1991). For example, lycaenid larvae may attract ants through the release of volatile chemicals, but fail to provide them with food rewards, or do so only occasionally. However, our laboratory experiments offer support for the commonly held, but rarely tested, assumption that ants benefit from associating with lycaenids. Although there were no significant effects of lycaenids on the mass of ant workers, 40% more work-

ers survived when associating with lycaenid larvae compared to those subjected to starvation in the plant-only treatment. The most probable explanation is that ants consumed lycaenid secretions that provided sufficient nutrients to meet their metabolic requirements. Moreover, we failed to detect significant differences in the survival of ant workers feeding on an artificial diet compared with those tending lycaenid larvae, suggesting that lycaenid secretions are a high-quality food resource for ants.

Our data on the ant's perspective have a number of potential limitations. First, our laboratory experiments were of short duration, and thus we can only speculate that the positive effects of lycaenids on ants persist for longer periods. Second, our experiments assessed benefits to ants at the level of individual workers rather than colonies, the latter being most appropriate for eusocial insects. Whether or not benefits to workers translate into significant colony gains is an open question and requires further consideration. Under most conditions, we suspect that workers would be the only direct beneficiaries of sugar-rich lycaenid secretions, given that previous studies indicate that workers are usually the primary metabolizers of sugars (Beattie 1985: Chapter 8). However, ant colonies as a whole may benefit indirectly from lycaenid secretions if these rewards fuel the foraging activities of workers and increase the intake of protein-rich food that the colony brood and queen require. Third, our data assess primarily the existence of benefits, and only begin to address the value of lycaenid rewards relative to other resources available in the environment (i.e., we do not consider the substitutability of these benefits). We previously discussed the value of lycaenid secretions to ants when proposing a hierarchy of increasing investment. In our laboratory experiments, we also assessed the value of lycaenid secretions relative to a high-quality artificial diet, and found that the two resources were of equal value with respect to worker survival. However, because *I. nitidiceps* is omnivorous and commonly found in areas that lack *P. aurifera* (J. H. Cushman, *personal observation*), much more attention needs to be directed toward assessing the value of lycaenid secretions relative to other food resources available to ants.

Three other studies have considered the effects of lycaenids on their ant associates. Pierce et al. (1987) showed that ant workers weighed significantly more after tending lycaenid larvae compared to those that were about to begin tending. Both Pierce et al. (1987) and Fiedler and Maschwitz (1988) made detailed estimations indicating that energy intake from lycaenid secretions exceeded the energy expenditure of ant colonies in acquiring them. In laboratory experiments, Nash (1989) showed that, while *Iridomyrmex vicinus* colonies attained higher growth rates when *Jalmenus evagoras* larvae were present, growth rates were higher for colonies that associated with a single larva than for

those with five larvae. Nash also showed that another ant species (*I. anceps*) acquired greater net energy from tending homopterans than from tending lycaenids, even though more ants tended the lycaenids. Such counter-intuitive results suggest that lycaenid larvae may at times manipulate their ant associates to behave in ways that reduce benefits and underscore the need for future studies that consider the ant's perspective.

Conclusions

In this study, we have assessed the possibility that a pair of species benefit from their association with each other. While our work supports the mutualism hypothesis, we recognize that most putative mutualisms are not species specific: in nature, one or both participants usually interact with an array of partners that can vary greatly in their ability to provide beneficial rewards and services and therefore in their contributions to fitness (Addicott 1979, Bristow 1984, Schemske and Horvitz 1984, Thompson and Pellmyr 1992). To reflect the multispecies nature of mutualistic systems, a major objective of ecological studies is to document not only the effects that a particular pair of species have on each other, but also to assess the degree to which both participants interact with other species and the relative importance of these additional partners. In our lycaenid-ant system, only *P. aurifera* is species specific for the association, while *I. nitidiceps* interacts with other mutualists and acquires additional foods from nonmutualistic sources. Thus, lycaenid secretions are only part of a suite of resources that ants use. Such findings, where one or both participants rely on the benefits received from multiple mutualists, are not unique to this system and the field would benefit from future studies that attempt to estimate the relative ranking of mutualists in terms of their value to focal species and to identify those mutualists that provide unique benefits vs. those that provide benefits that can be attained from other sources.

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