The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants

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Summary. The larvae and pupae of the Australian lycaenid butterfly, *Jalmenus evagoras* associate mutualistically with ants in the genus *Iridomyrmex*. Four ant exclusion experiments in three field sites demonstrated that predation and parasitism of *J. evagoras* are so intense that individuals deprived of their attendant ants are unlikely to survive. Larvae and pupae of *J. evagoras* aggregate, and the mean number of attendant ants per individual increases with larval age and decreases with group size. Field observations showed that young larvae could gain more attendant ants per individual by joining the average size group of about 4 larvae than by foraging alone. Aggregation behaviour is influenced by ant attendance: young larvae and pupating fifth instars aggregated significantly more often on plants with ants than on plants where ants had been excluded. In return for tending and protecting the larvae, ants were rewarded by food secretions that can amount to as much as 409 mg dry biomass from a single host plant containing 62 larvae and pupae of *J. evagoras* over a 24 h period. Larval development in the laboratory lasted approximately a month, and larvae that were tended by ants developed almost 5 days faster than larvae that were not tended. However, tended individuals, particularly females, pupated at a significantly lower weight than their untended counterparts, and the adults that eclosed from these pupae were also lighter and smaller. On average, pupae that were tended by ants lost 25% more weight than untended pupae, and in contrast with larvae, they took longer to eclose than pupae that were not tended. These experimental results are discussed in terms of costs and benefits of association for both partners, and of aggregation for the lycaenids.

Introduction

The family Lycaenidae comprises nearly 40% of all butterfly species (Vane Wright 1978), and the larvae of about half of these species associate with ants (Pierce 1987; Downey 1962). Associations with ants can be mutualistic, commensal, or parasitic in nature (see Hinton 1951; Atsatt 1981; Cottrell 1984 for review). In mutualistic interactions, larvae produce substances to appease ants that could otherwise be threatening predators (Malicky 1969, 1970; Henning 1983; cf Hölldobler 1970, 1971). Several species have been shown to secrete food in the form of carbohydrates and amino acids that attendant ants harvest from specialized glands (Maschwitz et al. 1975; Kitching 1983; Kitching and Luke 1985; Pierce 1983; Pierce et al. in preparation). In return for these rewards, attendant ants protect larvae against parasitoids and predators (Ross 1966; Pierce and Mead 1981; Pierce and Easteal 1986). In clearly parasitic interactions, larvae are carried by ants into the brood chamber of the ant nest where they become carnivorous and feed on the ant brood. In others, lycaenid larvae may mimic ant recognition signals and chemically fool attendant ants into providing care and defense

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in the absence of significant return rewards (Cottrell 1984).

The interaction between lycaenid butterflies and ants offers an excellent opportunity to identify the selective forces that have shaped the evolution of mutualism, and to examine the behavioral, ecological, and biochemical mechanisms that maintain it. By "mutualism", we simply mean an interaction in which the genetic fitness of each mutualist is increased by the action of its partner. Interspecific mutualisms have attracted considerable theoretical interest in the past decade (see Boucher 1985). This interest has stimulated the development of models of population dynamics (reviewed in Boucher et al. 1982; see also Wolin 1985; Addicott 1984, 1986a; Pierce and Young 1987) and of evolution (Trivers 1971; Roughgarden 1975; Keeler 1981, 1985; Wilson 1980, 1983; Axelrod and Hamilton 1981; Maynard-Smith 1982; Axelrod 1984; Vandermeer 1984; Templeton and Gilbert 1985).

Despite this current wealth of theory, relatively few empirical studies have attempted to measure quantitatively the costs and benefits of mutualism for both species involved (see Janzen 1979; Schemske 1983; Addicott 1984; Morse 1985). In part this is because it is difficult to determine all of the sources of costs and benefits, particularly if the mutualism is diffuse in its effects (Janzen 1985). In addition, there are often practical difficulties in assessing the effects on fitness of benefits that are meted out in different biological currencies, such as food vs. defense (Addicott 1986b). Although these difficulties apply to lycaenid/ant interactions as well, the lycaenids nevertheless offer exceptional study material because depending upon the species, their relationships range from facultative to obligate mutualism, and this allows the technique of interspecies comparison to be employed effectively. Moreover, certain species lend themselves easily to experimental analysis using simple manipulations of the system under natural field conditions and in the laboratory.

In this study we examine the costs and benefits of ant association for the Australian lycaenid, *Jalmenus evagoras*. We determine whether ants protect the eggs, larvae, and pupae against predators and parasitoids, and evaluate some of the possible advantages of larval aggregation to *J. evagoras*. We assess whether ants receive substantial amounts of food from the larvae and pupae that they tend, and investigate the effect that ant attendance has on the development of *J. evagoras*.

Natural history and study site. Jalmenus evagoras is a multivoltine, Australian lycaenid that occurs from Melbourne, Victoria in the south to Gladstone, Queensland in the north, and is found both inland and near the coast (Common and Waterhouse 1981). The eggs of *J. evagoras* are laid in clusters, and the larvae and pupae aggregate. Larvae are known to feed on at least 17 different species of *Acacia* (Hawkeswood 1981; Dunn 1984; Pierce and Elgar 1985). Pupation occurs on the food plant, and clusters of both larvae and pupae are heavily tended by several species of ants in the genus *Iridomyrmex*. Both the late instar larvae (N. Pierce, unpublished observations) and the pupae stridulate when disturbed (see Downey 1962), and the vibrations they produce may serve to alert their attendant ants of danger.

Our study site at Mount Nebo, Queensland (152 47 E/27 23 S), is a subtropical region where *J. evagoras* has at least three broods a year. The most common food-plant in this area is *Acacia irrorata*, and the larvae usually feed on the newly produced foliage of young trees that form secondary growth in cleared areas.

The primary ant that tends *J. evagoras* at Mt. Nebo is in the *Iridomyrmex anceps* species group (sp. 25, Australian National Insect Collection), and we will refer to it as *I. anceps* for convenience. Colonies of *I. anceps* are usually large, containing multiple queens, many workers, and numerous nest entrances. Workers are extremely sensitive to vibrations of the substrate and possess a mass recruitment form of defence; they stream out by the hundreds if larvae or pupae of *J. evagoras* are disturbed. So many workers tend the juveniles of *J. evagoras* that they are virtually concealed by the dense coating of ants. The eggs are not tended. *J. evagoras* infrequently associates with another ant at Mt. Nebo, *I. rufoniger*, a species that also forms extensive polygynous and polydomous colonies.

Methods

Ant exclusion experiments

Plants infested with larvae at our field site in Mt. Nebo were selected and roughly paired on the basis of plant size and number of larvae on the plant. Ants were excluded from one of each pair of plants by wrapping a band of removable surveyor's tape around the stem at the base of the plant, and covering it with a viscous band of Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan). Controls were treated in the same manner except that the stem was coated with Tanglefoot on one side only so that ants could still have access to the larvae and pupae. Clear plastic drop cloths smeared with Tanglefoot were placed beneath each tree to ensure that the only way larvae and pupae could disappear from the trees was by being transported away by aerial predators (see Pierce and Easteal 1986).

Ant exclusion experiments at Mt. Nebo were repeated three times. The first experiment ran from January 20–27, 1981 (inclusive); the second from February 8–21; and the third from February 12–21. In the third experiment, five of the control trees from the first experiment were redesignated as "experimentals" (without ants), and four out of the five new "control" trees (with ants) were originally experimental trees from the first experiment. Every day during the first experiment, and every other day during the second and third experiment, the following data were collected:

Predation. The number of eggs, larvae, pupae, and exuviae were itemized for each tree during each census. Drop cloths were checked for larvae and pupae. Any evidence of predation, such as desiccated carcasses or larvae in spider webs was recorded, and a tally was made of other arthropods present on the plant.

Parasitism. At the end of the first experiment, all larvae and pupae were collected, placed in individual petri dishes, and reared in a growth chamber until they either emerged as adults or died upon the emergence of parasitoids. Egg masses were tagged with identification numbers and left on the trees until they had hatched or parasitoids had emerged. Parasitised eggs were identifiable by the small exit holes left by the parasitoids.

For comparison, an identical ant exclusion experiment was performed from March 12–20, 1981, using trees occupied by *J. evagoras* at Black Mountain and at Mount Stromlo in Canberra, A.C.T. Larvae at these sites were feeding on the foliage of young trees of *A. decurrens* and were also tended by ants in the *Iridomyrmex anceps* species group. Although eggs were not followed during the course of this experiment, all larvae and pupae were collected and checked for parasitism as before.

Larval and pupal aggregations

During each census of control and experimental plants at Mt. Nebo, we noted whether a larva was solitary or in group, and for control plants, the number of attendant ants. For our analysis, we included only plants that contained four or more larvae at the time of census. A larva was considered to be in a group if it was within 5 mm of another individual.

Ant rewards

We used two methods to estimate whether *J. evagoras* produces rewards for its attendant ants, and these were both measured in terms of weight:

Differences in weights of tended and untended pupae. Initial weights were taken of a sample of pupae that had just undergone metamorphosis (July 15, 1984). Half the pupae were then positioned on poles where they could be tended by foraging workers from a queenright, laboratory colony of *I. anceps* (see below), and half were placed adjacent to the first set, but on poles where ants were not allowed access. After five days, all pupae were removed and weighed again.

Field estimate of food secreted by the larvae of J. evagoras on a single plant of A. irrorata over a 24 h period. On March 13, 1981 (toward the end of the summer at Mt. Nebo), we chose a plant of *A. irrorata* approximately 1 m high containing 62 juveniles of *J. evagoras*, and made the following observations.

Foraging rates. We measured the rate of ants going up the tree and ants going down the tree by wrapping a narrow band of white tape around the bottom of the tree trunk and counting the number of ants passing over it. One observer counted the number going up, and another counted the ants going down. Counts were made for 10 successive 50 s intervals at each sampling time.

Forager weights. After counting the ants foraging up and down the tree, we collected samples of about 30 ascending and 30 descending ants. These were immediately frozen on dry ice, and remained frozen until a few minutes before they were weighed. In the laboratory, individual ants were weighed on a Kahn electrobalance. After its wet weight had been recorded, each ant was dried in an oven at 80 C for 15 min, and weighed again.

Laboratory cultures

J. evagoras and its attendant ants were cultured in a greenhouse at Griffith University in Brisbane using a modified version of the technique described by Kitching and Taylor (1981). Larvae were reared on potted plants of *A. irrorata* that were approximately three months old and less than 1 m high. Ant colonies composed of at least one queen, workers and brood were housed in aquaria where they could nest inside test tubes wrapped in foil. Some of these tubes were half-filled with water and tightly plugged with cotton wool, allowing enough seepage for the ants to drink. In addition to being able to forage on the potted plants of *A. irrorata*, ants were provided with daily Bhatkar diet (Bhatkar and Whitcomb 1970) and an occasional chopped cockroach. Foraging ants were allowed free access to larvae by means of bridges made from sticks. Each system was sealed to prevent ant escape by means of sticky, Tanglefoot barriers smeared around the trunk of the food plants and the rim of the ant aquaria.

Development

Eggs of *J. evagoras* were collected from *A. irrorata* at Mt. Nebo. On hatching, larvae were reared at 28 C in a greenhouse at Griffith University in the manner described above. They were assigned to one of three treatments: plants without ants, plants with foragers of *I. anceps,* and plants with foragers of *I. rufoniger*. Two large, queenright colonies of each ant species were used in the experiment. Each colony tended larvae of *J. evagoras* on two separate plants, and these plants were replaced every few days by fresh plants to maintain an unlimited supply of young foliage for the developing larvae.

Each larva was observed once daily, and its behaviour and number of attendant ants noted. We recorded the following variables for each individual: number of days spent as larva and as pupa, pupal weight, forewing length, body length, amount of silk produced by the final instar larva to fasten the pupa (scored on an increasing subjective scale from 1 to 5), adult weight, and sex. The experiment was initiated on July 10, 1984 and ended when all individuals had eclosed approximately six weeks later.

Results

Ant exclusion experiment

Predation. Eggs of *J. evagoras* that were laid on plants without ants during the first experiment were preyed upon more heavily than eggs laid on plants with ants. Females of *J. evagoras* use ants

Table 1. Predation of ant tended and untended larvae and pupae (juveniles) of *J. evagoras*. Data are combined for all experimental and control trees in each experiment, but were compared on a tree by tree basis using the Mann-Whitney U test. Standard deviations are in parentheses. Mean number of juveniles/tree were compared using the t-test

	Treatment		
	With ants	Without ants	Test statistic
Mt Nebo, Queensland			
Experiment I			
Mean number of juveniles per tree	33.5	24.7	$t_s = 0.84$
(13 paired trees)	(9.18)	(5.19)	
Overall predation (%)	13	41	$U = 217^{***}$
Experiment II			
Mean number of juveniles per tree	21.8	35.5	$t_s = 0.92$
(9 paired trees)	(4.00)	(14.43)	
Overall predation (%)	28	54	$U = 116^{***}$
Experiment III			
Mean number of juveniles per tree	61.4	60.6	$t_s = 0.40$
(5 paired trees)	(19.86)	(25.62)	
Overall predation (%)	14	100	$U = 33^{***}$
Canberra, ACT			
Experiment IV			
Mean number of juveniles per tree[a]	11.0	10.17	$t_s = 0.24$
	(4.96)	(5.21)	
Overall predation (%)	3	85	$U = 41.5^*$

[a] 8 trees with ants; 6 trees without ants

* $P < 0.05$; *** $P < 0.005$

as cues in oviposition, and during Experiment I they generally did not lay eggs on plants without ants (139 egg masses on plants with ants versus 20 egg masses on plants without ants; see Pierce and Elgar 1985). However the mean number of eggs per mass was the same in both treatments (with ants: 13.87 ± 1.34 eggs; without ants: 15.45 ± 2.03 eggs; $t_{s, 157} = 0.44$; ns). Of these, two solitary eggs disappeared from plants with ants whereas 38 eggs from 6 separate egg masses disappeared from plants without ants (Mann-Whitney U test comparing percent lost per egg mass from trees with and without ants: $U = 1783$, $P < 0.05$).

Workers of *I. anceps* guard larvae and pupae of *J. evagoras* against predators (Table 1). In all three experiments, larvae and pupae disappeared significantly more often from plants without ants than from plants with ants. Only two fourth instar larvae and two partially consumed pupae were found on drop cloths. All other disappearances could only be ascribed to predation by an airborne predator (see Pierce and Easteal 1986).

Because of the relatively gradual and incremental loss of juveniles from trees without ants, we believe that the dominant predators of *J. evagoras* were arthropods. We never observed birds settling on the food plants, nor did we find bird droppings on the drop cloths. During our daily observations, we were able to keep a complete account of predation resulting from both spiders (which we observed either feeding on larvae or with larvae tangled in their webs) and sucking predatory bugs such as reduviids or pentatomids (that left desiccated carcasses). Ninety-seven percent ($n = 95$) of the mortality caused by these predators occurred on trees without ants. Spider attack accounted for 7.44% ($n = 605$) of the overall mortality of juveniles of *J. evagoras*, and sucking predators of 8.26% ($n = 605$). Of these, 75% ($n = 92$) were first, second or third instars. The spiders included: *Theridion pyramidale* (Theridiidae), *Olios punctatus* (Sparassidae), *Thomisus spectabilis* (Thomisidae), *Chiracanthium* sp. (Clubionidae), and *Araneus* sp. (Araneidae).

The most effective arthropod predators appeared to be social insects, including the primitive jumper ant, *Myrmecia nigrocincta* and the vespid wasp, *Polistes (Polistella) variabilis*. We found workers of *M. nigrocincta* attacking larvae on three separate occasions. As their common name implies, these ants are able to leap over a drop cloth, seize a larva, and leap back across the drop cloth

Table 2. Parasitism of ant tended versus untended larvae and pupae of *J. evagoras*. Data are combined for all experimental and control trees in Experiment I, but were compared on a tree by basis using the Mann-Whitney *U* test

	Treatment		
	With ants	Without ants	
Egg masses (N)	59	7	
Number of trees from which eggs were collected	8	5	
Parasitised (%)	62	66	$U=25$
Dead (%)	11	3	$U=30$
Larvae (N)	179	48	
Number of trees from which larvae were collected	11	7	
Parasitised (%):			
braconid wasp	22	23	$U=39.5$
Dead (%)	8	17	$U=44$
Pupae (N)	65	56	
Number of trees	8	10	
Parasitised (%):			
chalcid wasp	–	88	
tachinid fly	–	3	
overall	0	91	$U=68**$
Dead (%)	9	4	$U=40$

** $P<0.01$

to carry it back to the nest. One reason why social insects might be ocnsidered particularly threatening predators is because they are able to remember the location of trees containing larvae without ants. For example, we observed an individual vespid wasp return repeatedly to a plant without ants to attack larvae.

Parasitism. Fifty-nine of the 139 egg masses laid on trees with ants and 7 of the 20 laid on trees without ants during Experiment I were observed again several weeks after the experiment had finished and after all larvae and parasitoids had hatched out. Eggs of *J. evagoras* are not tended by ants and are heavily parasitised by a wasp in the genus *Trichogramma* that leaves a characteristically small exit hole when it emerges from the egg. There was no difference in either parasitism or death (possibly caused by a fungus, as dead eggs turned black) of eggs laid on plants with or without ants (Table 2).

Similarly, ants did not appear to be effective against a braconid wasp, *Apanteles* sp., that attacks the larvae (Table 2). Indeed, Common and Waterhouse (1981, p. 497) mention that populations of *J. evagoras* often suffer heavy parasitism by braconid wasps despite their attendant ants. This wasp attacks primarily second instar larvae, and usually emerges during the fourth instar. It is possible that some of the larvae we reared from the first experiment were parasitised before the ex-

periment began, and that this is why we detected little difference in parasitism between treatments. If we include only those larvae that were first or seond instars at the start of the experiment, we find that braconid wasps parasitised 31% ($n=61$) of the ant tended larvae as opposed to none ($n=7$) of the untended larvae. These data suggest that the *Apanteles* wasps may use ants as cues in finding larvae. Alternatively, larvae that are parasitised may be more vulnerable to predators than their unparasitised counterparts, and we may not be detecting high levels of parasitism in the untended group because the parasitised larvae have been preferentially eaten by predators.

In contrast, ants were completely effective against a chalcid wasp, *Brachymeria* (*Brachymeria*) *regina* that attacks the prepupa of *J. evagoras* and emerges from the pupa. This wasp is known to attack a number of different kinds of lepidopteran pupae and appeared to be common in our field site (I. Naumann, pers. comm.). Parasitism of the pupae of *J. evagoras* by chalcid wasps was so intense that, without ants, survival was negligible (Table 2). In addition to the wasps, two pupae were parasitised by a tachinid fly (subfamily Goniinae).

Canberra exclusion experiment. Results of the ant exclusion experiments performed in Canberra were similar to those from Mt. Nebo (Table 1). Again, significantly more larvae disappeared from plants
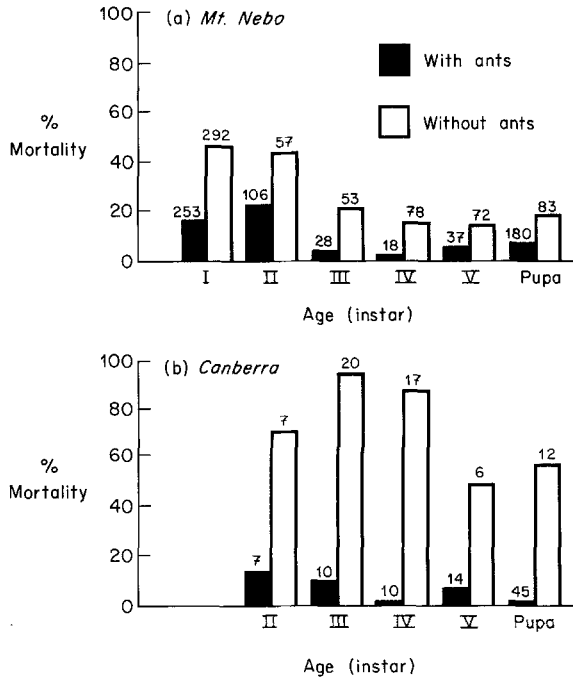
Fig. 1a, b. Age-specific mortality due to predation of larvae and pupae of *Jalmenus evagoras* with and without ants in two field sites: Mt Nebo, Queensland and Canberra, ACT. Sample sizes are given above each bar
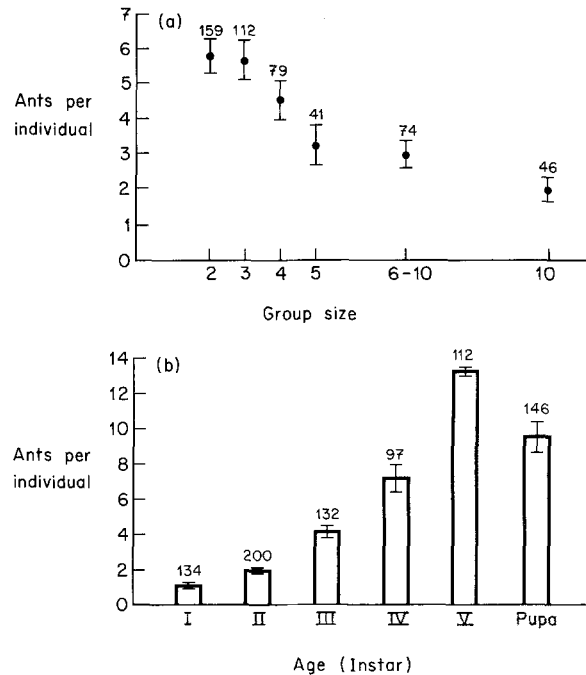

Fig. 2a, b. Mean number of attendant ants per individual of *Jalmenus evagoras* as a function of a group size and b age at Mt Nebo, Queensland. All individuals in b were solitary. Bars represent standard errors and sample sizes are given above

without ants than from plants with ants. However, none of the larvae or pupae that survived the experiment were parasitised.

Age specific mortality due to predation: differences between sites. Data from Experiments I, II, and IV were broken down by comparing the numbers of each age class at the start of the experiment with the number that died before reaching the next age class. The percent mortality of each age class was then compared between experiments. A pairwise comparison of the age-specific mortalities of larvae and pupae on trees with ants in Experiments I and II showed no significant difference (Wilcoxon signed-ranks test: Wilcoxon $T = 5$, $Z = 0.54$, $P > 0.6$). Similarly, the pattern of age-specific mortality of larvae and pupae without ants was the same between the two experiments (Wilcoxon $T = 10$, $Z = 0$, $P > 0.6$). These two experiments were initiated 20 days apart at sites approximately 1 km distant from each other.

We compared the pooled results of Experiments I and II at Mt. Nebo with the results of Experiment IV at Canberra, and again found no significant difference in the age-specific mortality of individuals that were tended by ants (Fig. 1; Wilcoxon $T = 3$, $Z = 0.55$, $P > 0.6$). However the mortalities of larvae and pupae without ants was

different between the Mt. Nebo and Canberra experiments: mortalities due to predation were greater in Canberra for all age classes (Fig. 1; Wilcoxon $T = 0$, $Z = 1.89$, $P < 0.6$).

Ant attendance and larval and pupal aggregation

The mean number of ants per solitary larva increased as a function of larval age, but the mean number of ants per larva decreased with larger group sizes (Fig. 2). The mean group size for tended larvae at Mt. Nebo was 3.86 (SE = 0.88, $n = 1218$), with a range of 1 to 31 individuals. There was no correlation between the proportion of aggregated larvae on a plant and the total number of larvae on the plant for either tended larvae (Spearman rank correlation: $r_s = 0.22$; $n = 19$; $P > 0.4$) or untended larvae ($r_s = 0.20$; $n = 23$; $P > 0.3$); thus larvae do not aggregate randomly, but actively join groups whatever the densities of conspecifics on each plant.

Aggregation behaviour is affected by both ant attendance and larval age. Young larvae (first, second and third instars) aggregated significantly more on plants with ants than on plants without ants (Mann-Whitney U test: $Z_{21,15} = 2.28$; $P < 0.03$), whereas the behaviour of old instars (fourths and fifths) remained unchanged ($Z_{14,15} = 0.611$;

Table 3. Weight loss over five days by pupae of *J. evagoras* reared with and without attendant ants. Mean weights were compared using the *t*-test

	With ants $n=16$		Without ants $n=12$		
	Mean	SE	Mean	SE	
Mean starting weight (mg)	158.91	9.33	161.12	8.88	$t_s=0.17$
Mean weight loss (mg)	25.08	1.31	19.24	1.63	$t_s=2.79**$
Mean % weight loss	16.12	0.74	11.91	0.71	

** $P<0.01$

$P>0.5$, ns). Fifth instars that were about to pupate, however, were also influenced by the presence or absence of ants: pupae were more aggregated on plants with ants than on plants without $(Z_{11,11}=2.69; P<0.01)$.

Ant rewards

Pupal weight loss. Pupae of both sexes lost significantly more weight when tended by ants than when not tended (Table 3). On average, pupae that were tended by ants lost 25% more weight than their untended counterparts over the five days of the experiment.

Field estimates of net biomass/day produced by a tree containing J. evagoras. The tree that we chose for our observations contained a total of 62 larvae of *J. evagoras* having the following age distribution: 1 first, 5 second, 5 third, 22 fourth, 18 fifth instars, and 11 pupae. A two-way analysis of variance for wet weights of ants showed that both time of observation and differences in weights between ants travelling up and down the tree had significant effects (for time: $F_{8,8}=5.18; P<0.02$; for differences in up-down weights: $F_{1,8}=9.77; P<0.01$). Foragers returning to the nest were heavier than foragers going up the tree in all time intervals but one (just after a brief shower). Foraging loads were also heavier at some time intervals than at others (Fig. 3a).

The dry weight of foragers returning to the nest was also significantly greater than that of their counterparts going up the tree, but this difference did not vary with time as the wet weight differences did (for up-down differences: $F_{1,8}=19.27; P<0.002$; for time: $F_{8,8}=1.55; P<0.28$) (Fig. 3b). We conclude from this that while the overall foraging loads varied at different times of day, the total amount of dry food contained in these loads remained approximately the same. The only quantitative feature of the food that varied was the amount of water it contained.
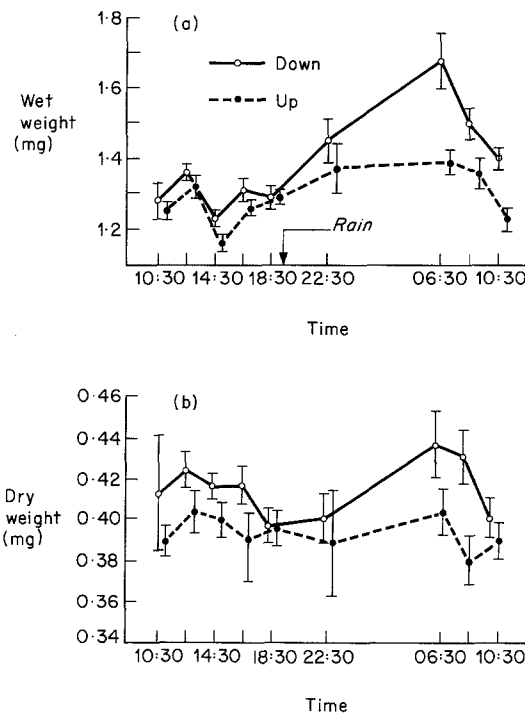

Fig. 3a, b. Differences in (a) wet weights and (b) dry weights of ants foraging on a tree containing 62 juveniles of *Jalmenus evagoras* over a 24 h period. "Up" weights are means for ants travelling up the tree and "down" weights are means for ants travelling back down again. *Bars* represent standard errors; sample sizes are over 20 in all time zones, except 22:30, when they are both 7

Depending on the time of day and the ambient temperature, the foraging rate ranged from a maximum ($\pm$SE) of 2228 ± 106 ants/h at 10:30 h to 58 ± 18 ants/h at 6:30 h, with a mean of 765 ± 149 ants/h. To estimate the net biomass removed from the tree over the course of the day, we pooled the dry weight data over time to render the mean (down – up) dry weight difference per ant. This foraging load was approximately 0.0223 mg ($n_D=258; n_U=285$). We multiplied this by the mean rate of foraging ants per hour, and summed this figure over 24 h. This amounted to

Table 4. Developmental time (days) of *J. evagoras* reared with and without attendant ants. The effects of sex have been removed from the ANOVA

	With ants			Without ants			
	Mean	SE	n	Mean	SE	n	
Larval duration	23.29	0.48	80	28.83	0.66	59	$F_{1,11} = 9.52^*$
Pupal duration	7.79	0.08	80	7.54	0.10	59	$F_{1,136} = 4.08^*$
Total development	31.08	0.47	80	36.37	0.66	59	$F_{1,11} = 7.82^*$

* $P < 0.05$

Table 5. Pupal and adult weight and size of *J. evagoras* reared with and without attendant ants. The effects of sex have been removed from the ANOVA

	With ants			Without ants			
	Mean	SE	n	Mean	SE	n	
Pupal weight (mg)[a]							
males	123.94	4.64	40	132.60	5.78	37	$F_{1,136} = 9.32^{***}$
females	133.05	4.31	40	164.87	10.93	22	
Adult weight (mg)[b]							
males	35.88	2.92	12	48.95	2.77	35	$F_{1,82} = 10.54^{***}$
females	59.84	4.56	16	80.69	7.43	22	
Forewing length (mm)	18.91	0.25	79	19.48	0.24	59	$F_{1,135} = 3.69^*$
Body length (mm)	14.44	0.16	79	15.19	0.18	59	$F_{1,135} = 8.94^{***}$
Silk produced to attach pupa	2.40	0.13	77	2.12	0.17	59	$F_{1,133} = 1.57$

* $P < 0.07$; *** $P < 0.005$
[a] Interaction with sex; $F_{1,135} = 3.64$, $P < 0.07$
[b] Interaction with sex; $F_{1,81} = 0.55$, ns

approximately 409 mg dry biomass removed from the tree over the 24 h period.

Effect of ants on larval development

Larvae that were tended by ants developed more quickly than larvae that were not tended by ants. However, they pupated at a significantly lower weight than their untended counterparts, and the adults that emerged from these pupae were also lighter and smaller (Tables 4 and 5). No differences in development were detected between larvae tended by *Iridomyrmex anceps* and *I. rufoniger* (e.g. for total developmental period, $F_{1,135} = 0.29$, ns; for pupal weight, $F_{1,135} = 0.33$, ns). Regression analysis revealed a significant effect of individual host plant assignment on larval developmental time, and data for this character were analysed using tree means that were weighted by the number of larvae in each tree. In all other cases, individuals were treated as statistically independent (and all analyses cited as significant remain so even when

the effects of host plants are removed). Ant colony did not have a significant effect in any of the analyses.

While larvae with ants developed more than five days faster than larvae without ants, tended pupae developed slightly more slowly than untended pupae (Table 4). Pupal weight was not correlated with pupal duration either for individuals with ants ($r_s = -0.052$; $n = 80$; $P = 0.33$; ns), or without ants ($r_s = -0.028$; $n = 59$; $P = 0.42$; ns). The difference in adult weight between tended and untended individuals were reflected by a significant decrease in body length. However, the presence or absence of ants had no statistically significant effect on either forewing length, or the amount of silk that fifth instars produced when they attached themselves to the plant to pupate (Table 5).

Males that were tended by ants pupated at a weight that was only slightly smaller than that of untended males, whereas females that were tended by ants underwent metamorphosis at a substantially lower weight than their untended counter-

parts (Table 5). This interaction between ant attendance and sex of pupa is significant at only the 7% level and needs further investigation.

The mean number ($\pm$ SE) of attendant workers of *I. anceps* per solitary instar in our laboratory culture was as follows: for seconds: 0.74 ± 0.17 ($n = 19$); for thirds: 1.30 ± 0.15 ($n = 47$); for fourths: 2.04 ± 0.22 ($n = 70$); for fifths: 3.44 ± 0.23 ($n = 150$); and for pupae: 4.11 ± 0.66 ($n = 9$). These represent considerably fewer attendant ants than are found in natural populations (compare with Fig. 2), and thus the results of this experiment are probably quantitatively conservative estimates of the effect that ants have on the development of *J. evagoras*.

Discussion

Costs and benefits of ant association

The degree of mortality caused by predation and parasitism in the Mt. Nebo and Canberra field sites indicates that while predators and parasitoids of *J. evagoras* are patchily distributed, the selective force they exert in both locations is strong enough to result in an obligate dependency on the part of the lycaenids: larvae and pupae deprived of attendant ants cannot survive. In addition to providing protection, attendant ants shorten larval development, thereby reducing the time that larvae are exposed to the threat of predators and parasitoids. Ants may also decrease the probability of mould or fungal attack by removing larval secretions and faecal pellets (e.g. Hinton 1951). As would be predicted from the nature of the relationship, females of *J. evagoras* use ants as cues in oviposition (Pierce and Elgar 1985).

The energetic cost of associating with ants results in a reduction in adult weight and size, and these are important to mating success in males and fecundity in females of *J. evagoras* (Elgar and Pierce 1987). An understanding of the proximate mechanism underlying the reduction in weight and size of tended individuals requires further investigation. Certain Lepidoptera, such as *Manduca sexta*, are known to pupate after they have achieved a specific weight (Nijhout and Williams 1974 a, b; Nijhout 1975, 1981); however the situation with *J. evagoras* appears to be more complex. Perhaps a proximate cue underlying pupation in *J. evagoras* is associated with the amount of food that has been ingested by an individual rather than its actual weight. If ant tended individuals spend more time feeding than their untended counterparts and have to secrete a significant proportion

of that food for attendant ants, this could explain why they pupate earlier and at a lower weight.

The possible difference in response to ant attendance between males and females of *J. evagoras* is interesting in light of the separate factors influencing reproductive success of the two sexes (Elgar and Pierce 1987). Females of *J. evagoras* are about 60% heavier than males, and appear to have a much shorter lifespan in the field (about 3 days vs. 7 days in males). Males of *J. evagoras* are protandrous, and individuals that eclose early in the season have a higher encounter rate with unmated females than those that eclose later. Relatively larger males are more successful than smaller males in competing for mates. Females of *J. evagoras* are mated upon eclosion, and egg production is highly correlated with body size. Given these observations, it is difficult to explain why males and females appear to differ in their developmental response to the absence of ants, and why females in particular are affected by ants.

There are likely to be additional, indirect costs to ant attendance that would influence the population dynamics and evolution of this butterfly (see discussion in Pierce 1984, 1987). The most obvious of these is that an obligate dependency on ants restricts the range of *J. evagoras* to only those areas where appropriate ants and host plants occur together. Similarly, ant dependent lycaenids may be restricted to feeding on only those species of plants that can provide a diet adequate to support both lycaenids and ants: the secretions of *J. evagoras* and other congeneric species are rich in amino acids (Pierce et al. in preparation), and there is a strong correlation between ant association in the Lycaenidae and consumption of protein rich food plants (Pierce 1985).

This study only begins to assess the costs and benefits to the attendant ants of associating with lycaenid butterflies. The main cost to the ants is a metabolic one meted out by the time energy required both to forage from the larvae and to protect them. In addition, ants tending larvae may be more conspicuous to their own predators and parasitoids.

Several lines of evidence indicate that colonies of *I. anceps* receive substantial rewards for their efforts. First, colonies of *I. anceps* have multiple nest openings, and satellite nests containing ant brood are contructed at the base of virtually every plant containing larvae and pupae of *J. evagoras*. It seems unlikely that colonies would distribute themselves in this manner if they were not also receiving significant food rewards from their lycaenid associates. Second, pupae that are tended

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by ants for only 5 days lose 25% more weight than their untended counterparts, and develop significantly more slowly. This suggests that pupae are supplying rewards for ants by diverting metabolic resources from metamorphosis. Finally, the mean dry weight of an individual worker of *I. anceps* is about 0.4 mg (from data in Fig. 3), and our estimate of biomass removal from a tree containing 62 juveniles was about 409 mg. If there is a 10% rate of biomass conversion from one trophic level to the next, then foraging on a single tree containing about 60 juveniles of *J. evagoras* can result in the equivalent production of about 100 new workers of *I. anceps* in one day. This observation provides only a crude estimate of the contribution of *J. evagoras* to its attendant ants; for example, it was not possible to occlude the extra-floral nectaries of the food plant during our observations (although their contribution could have amounted to only a small fraction of the total biomass removal). Analyses of the secretions of *J. evagoras* have shown that they contain simple sugars (about 10%) and high concentrations of amino acids, particularly serine, which ranges from 20–50 mM (Pierce 1983; Pierce et al. in preparation).

Costs and benefits of aggregation

There are several possible benefits to aggregating for larvae and pupae of *J. evagoras*. If a threshold number of ants is necessary to protect the larvae and pupae, then aggregating is one mechanism by which *J. evagoras* could simultaneously increase its collective defence and decrease the amount of food that each individual would need to produce to attract that defence. For example, first instars can gain more ants by joining a group of any size than by remaining alone, and solitary second or third instars can have a higher number of attendant ants by joining the mean sized group of about 4 larvae (Fig. 2). Moreover, aggregation is not automatic, but occurs in response to ants: young instars that are not tended by ants are less likely to form groups than their tended counterparts. It is interesting and probably significant that most lycaenid species that lay eggs in clusters and whose larvae aggregate have complex and apparently obligate associations with ants (Kitching 1981; Pierce and Elgar 1985).

Myrmecophilous lycaenids that aggregate may receive secondary benefits from feeding in a group. Larvae may alter the physiology of their host plants to create a nutrient sink that supplies more soluble protein (e.g. Way and Cammell 1970). Furthermore, the formation of large and visible clus-

ters of pupae may facilitate mate finding by *J. evagoras*. Males are able to investigate regularly every plant in an area bearing conspecific pupae, and engage in active tournaments for eclosing females (Elgar and Pierce 1987).

Two main costs to aggregating may be competition between individuals for food, and an increased risk of contracting diseases such as moulds and fungi. We have never observed the latter under field or laboratory conditions, but it is not uncommon to find larvae of *J. evagoras* that have starved on their host plants after consuming all the available foliage. Finally, larvae that occur in groups are likely to be more conspicuous to their predators and parasitoids: it is possible, for example, that the species of *Apanteles* that attacks the larvae of *J. evagoras* in our field site actually uses ants and larval clusters as cues in oviposition.

Ants benefit energetically from aggregation in *J. evagoras*: larvae and pupae that occur in clusters are easier to collect secretions from and to defend. However, if larvae and pupae can regulate the amount of secretion they produce, then individuals in groups may be able to provide less food for ants than they would on their own while still receiving the same degree of defence. Furthermore, aggregations may become so attractive to predators (such as other ant species) that the ants themselves are endangered. On several occasions we observed *Iridomyrmex nitidus* attack and take over trees of *A. irrorata* containing homopterans and juveniles of *J. evagoras*, inflicting heavy mortality on attendant workers of *I. anceps*.

References

Addicott JF (1984) Mutualistic interactions in population and community processes. In: Price PW, Slobodchikoff CN, Gaud BS (eds) A new ecology: novel approaches to interactive systems. Wiley, New York, pp 437–455
Addicott JF (1986a) On the population consequences of mutualism. In: Diamond J, Case TJ (eds) Community ecology. Harper and Row, New York
Addicott JF (1986b) Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. Oecologia (Berl) 70:486–494

Atsatt PR (1981) Lycaenid butterflies and ants: selection for enemy-free space. Am Nat 118:638–654

Axelrod R (1984) The evolution of cooperation. Basic Books, New York

Axelrod R, Hamilton WD (1981) The evolution of cooperation. Science 211:1390–1396

Bhatkar A, Whitcomb WH (1970) Artificial diet for rearing various species of ants. The Fla Entomol 53:230–232

Boucher DH (1985) The idea of mutualism, Past and Future. In: Boucher DH (ed) The biology of mutualisms. Croom Helm, Kent

Boucher DH, James S, Keeler KH (1982) The ecology of mutualism. Ann Rev Ecol Syst 13:315–347

Common IFB, Waterhouse DF (1981) Butterflies of Australia, 2nd edn. Angus and Robertson, Sydney

Cottrell CB (1984) Aphytophagy in butterflies: its relationship to myrmecophily. Zool J Linn Soc 79:1–57

Downey JC (1962) Myrmecophily in Plebejus (Icaricia) icarioides (Lepidoptera: Lycaenidae). Entomol News 73:57–66

Dunn KL (1984) Acacia diffusa Lind.: a new larval foodplant for Jalmenus evagoras evagoras (Donovan) (Lepidoptera: Lycaenidae). Vict Entomol 14:8

Elgar MA, Pierce NE (1987) Mating success and fecundity in an ant-tended lycaenid butterfly. In: Clutton-Brock TH (ed) Reproductive success: studies of selection and adaptation in contrasting breeding systems. Chicago University Press, Chicago (in press)

Hawkeswood TJ (1981) The food plants of Jalmenus evagoras (Donovan) (Lepidoptera: Lycaenidae). Aust Entomol Mag 8:1–2

Henning SF (1983) Chemical communication between lycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae). J Entomol Soc South Afr 46:341–366

Hinton HE (1951) Myrmecophilous Lycaenidae and other Lepidoptera – a summary. Proc Soc Lond Entomol Nat Hist Soc 1951:111–175

Hölldobler B (1970) Zur Physiologie der Gast-Wirt-Beziehungen (Myrmecophilie) bei Ameisen. II. Das Gastverhältnis zu Myrmica und Formica. Z Vergl Physiol 66:215–250

Hölldobler B (1971) Communication between ants and their guests. Sci Amer 224:86–93

Janzen DH (1979) How many babies do figs pay for babies? Biotrop 11:48–50

Janzen DH (1985) The natural history of mutualisms. In: Boucher DH (ed) The biology of mutualism. Croom Helm, London, pp 40–99

Keeler KH (1981) A model for a facultative, non-symbiotic mutualism. Am Nat 118:488–498

Keeler KH (1985) Cost: benefit models of mutualism. In: Boucher DH (ed) The biology of mutualism. Croom Helm, London, pp 100–127

Kitching RL (1981) Egg clustering and the Southern Hemisphere lycaenids: comments on a paper by N.E. Stamp. Am Nat 118:423–425

Kitching RL (1983) Myrmecophilous organs of the larvae and pupae of the lycaenid butterfly Jalmenus evagoras (Donovan). J Nat Hist 17:471–481

Kitching RL, Luke B (1985) The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. J Nat Hist 19:259–276

Kitching RL, Taylor MFJ (1981) The culturing of Jalmenus evagoras (Donovan) and its attendant ant, Iridomyrmex anceps (Roger). Aust Ent Mag 7:71–75

Malicky H (1969) Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Entomol 112:213–298

Malicky H (1970) New aspects on the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). J Lepid Soc 24:190–202

Maschwitz U, Wust M, Schurian K (1975) Blaulingsraupen als Zuckerlieferanten für Ameisen. Oecologia (Berl) 18:17–21

Maynard Smith J (1982) Evolution and the theory of games. Cambridge University Press, Cambridge

Morse D (1985) Costs in a milkweed, Asclepias syriaca and bumblebee, Bombus spp. mutualism. Am Nat 125:903–905

Nijhout HF (1975) A threshold size for metamorphosis in the tobacco hornworm, Manduca sexta (L.). Biol Bull 149:214–225

Nijhout HF (1981) Physiological control of molting in insects. Am Zool 21:631–640

Nijhout HF, Williams CM (1974a) Control of moulting and metamorphosis in the tobacco hornworm, Manduca sexta (L.): Growth of the last-instar larva and the decision to pupate. J Exp Biol 61:481–491

Nijhout HF, Williams CM (1974b) Control of moulting and metamorphosis in the tobacco hornworm, Manduca sexta (l.): cessation of juvenile hormone secretion as a trigger for pupation. J Exp Biol 61:493–501

Pierce NE (1983) The ecology and evolution of symbioses between lycaenid butterflies and ants. PhD thesis, Harvard University, Cambridge

Pierce NE (1984) Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants. In: Vane Wright RI, Ackery PR (eds) Biology of butterflies. XI Symp R Entomol Soc (Lond). Academic Press, London, pp 197–200

Pierce NE (1985) Lycaenid butterflies and ants: selection for nitrogen fixing and other protein rich food plants. Am Nat 125:888–895

Pierce NE (1987) The evolution and biogeography of associations between lycaenid butterflies and ants. Oxford Surv Evol Biol (in press)

Pierce NE, Easteal S (1986) The selective advantage of attendant ants for the larvae of a lycaenid butterfly, Glaucopsyche lygdamus. J Anim Ecol 55:451–462

Pierce NE, Elgar MA (1985) The influence of ants on host plant selection by Jalmenus evagoras, a myrmecophilous lycaenid butterfly. Behav Ecol Sociobiol 16:209–222

Pierce NE, Mead PS (1981) Parasitoids as selective agents in the symbiosis between lycaenid butterfly caterpillars and ants. Science 211:1185–1187

Pierce NE, Young WR (1987) Lycaenid butterflies and ants: two-species stable equilibria in mutualistic, commensal, and parasitic interactions. Am Nat 128 (in press)

Ross GN (1966) Life history studies on Mexican butterflies. IV. The ecology and ethology of Anatole rossi, a myrmecophilous metalmark (Lepidoptera: Riodinidae). Ann Entomol Soc Am 59:985–1004

Roughgarden J (1975) Evolution of marine symbiosis – a simple cost-benefit model. Ecol 56:1201–1208

Schemske DW (1983) Limits to specialization and coevolution in plant-animal mutualisms. In: Nitecki MH (ed) Coevolution. Univ of Chicago Press, Chicago

Templeton AR, Gilbert LE (1985) Population genetics and the coevolution of mutualism. In: Boucher DH (ed) The biology of mutualism. Croom Helm, London, pp 128–144

Trivers RL (1971) The evolution of reciprocal altruism. Q Rev Biol 46:35–57

Vandermeer J (1984) The evolution of mutualism. In: Shorrocks B (ed) Evolutionary ecology. Symp Brit Ecol Soc, 23. Blackwell, London, pp 221–232

Vane Wright RI (1978) Ecological and behavioural origins of diversity in butterflies. In: Mound LA, Waloff N (eds) Diversity of insect faunas. IX Symp R Entomol Soc (Lond). Blackwell, London, pp 56–59

Way MJ, Cammell M (1970) Aggregation behaviour in relation to food utilisation by aphids. In: Watson A (ed) Animal populations in relation to their food resources. Blackwell, Oxford, pp 229–247

Wilson DS (1980) The natural selection of populations and communities. Benjamin/Cummings, Menlo Park

Wilson DS (1983) The effects of population structure on the evolution of mutualism: a field test involving burying beetles and their phoretic mites. Am Nat 121:851–870

Wolin CL (1985) The population dynamics of mutualistic systems. In: Boucher DH (ed) The biology of mutualism. Croom Helm, London, pp 248–269