Oviposition tests of ant preference in a myrmecophilous butterfly

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Keywords:

diversification;
Formicidae;
geographical specialization;
host selection;
host shift;
local adaptation;
Lycaenidae;
myrmecophily;
oviposition preference.

Abstract

Butterflies in the family Lycaenidae that have obligate associations with ants frequently exhibit ant-dependent egg laying behaviour. In a series of field and laboratory choice tests, we assessed oviposition preference of the Australian lycaenid *Jalmenus evagoras* in response to different species and populations of ants. Females discriminated between attendant and nonattendant ant species, between attendant ant species, and to some extent, between populations of a single ant species. When preferences were found, ovipositing butterflies preferred their locally predominant attendant ant species and geographically proximate attendant ant populations. A reciprocal choice test using adults from a generation of butterflies reared in the absence of ants indicated a genetic component to oviposition preference. Individual females were flexible with respect to oviposition site choice, often ovipositing on more than one treatment during a trial. Preferences arose from a hierarchical ranking of ant treatments. These results are discussed in terms of local adaptation and its possible significance in the diversification of ant-associated lycaenids.

Introduction

Diversification within the butterfly family Lycaenidae is thought to have been enhanced by the tendency for the majority of its members to associate with ants (Pierce, 1984). Associations range from mutualistic to parasitic interactions, and vary in their specificity for, and dependence on, ant partners (Cottrell, 1984; Pierce, 1987; Fiedler, 1991, 1997; Pierce *et al.*, 2002). If ant association has influenced lycaenid diversification, it should be most evident among the obligately myrmecophilous species, which account for 15–20% of lycaenid species diversity (Fiedler, 1991, 1997). Their associations with ants tend to be species-specific (Cottrell, 1984; Pierce, 1989; Thomas *et al.*, 1989; Fiedler, 1991; Eastwood & Fraser, 1999) and adult females use their ant partners as cues during oviposition (e.g. Atsatt, 1981a; Pierce & Elgar, 1985; Seufert & Fiedler, 1996; van Dyck *et al.*, 2000).

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The evolution of oviposition preference is seen as a driving force in the divergence of phytophagous insect populations (Futuyma, 1986; Diehl & Bush, 1989; Thompson & Pellmyr, 1991). Preference, in this context, refers to both the order in which females rank different hosts and the specificity or degree to which they prefer one host over another (Wiklund, 1981; Singer, 1986). Thus, shifts in ant preference by ovipositing lycaenid butterflies may contribute to divergence in ant-associated lycaenid populations. Ultimately, this may lead to speciation events if, for example, genetic divergence in host preference has pleiotropic consequences for reproductive isolation (Funk, 1998).

A phylogenetic study of the lycaenid genus *Ogyris* suggests that shifts in ant partners have been accompanied by speciation events (N.E. Pierce, unpublished data). Association patterns between ants and at least two other obligately ant-associated lycaenid genera, *Maculinea* (Thomas *et al.*, 1989; Elmes *et al.*, 1994) and *Jalmenus* (Pierce, 1989; Eastwood & Fraser, 1999; Braby, 2000) further suggest that shifts in ant partners may contribute to population divergence and diversification.

The degree to which ovipositing butterflies discriminate between different ant species, as well as the extent to which oviposition behaviour has diverged among butterfly populations, has not been explored. We conducted a series of field and laboratory experiments that address these questions in the Australian lycaenid, *Jalmenus evagoras*. In choice tests, we assessed oviposition preference at three levels: in response to attendant and nonattendant ant species, in response to different species of attendant ants, and in response to different populations of a single attendant ant species. A reciprocal choice test was also conducted to determine whether females exhibited local adaptation in oviposition preference, and whether oviposition preference had a genetic component.

Methods and results

Natural history

Jalmenus comprises at least 10 species, distributed across the Australian continent (Braby, 2000). All species feed on the plant genus *Acacia (*some feed on additional plant genera), and all have specialized associations with one or more species of *Iridomyrmex* or *Froggattella* ants (subfamily Dolichoderinae). There is little overlap in the ant species that *Jalmenus* species associate with (Eastwood & Fraser, 1999), and where *Jalmenus* species overlap geographically, they are separated ecologically by their ant partners (Pierce, 1989; Costa *et al*., 1996; Braby, 2000).

Jalmenus evagoras inhabits coastal and inland areas of eastern Australia (Fig. 1). Immature stages are tended by different ant species in different geographical regions (Costa *et al*., 1996; Pierce & Nash, 1999), but are most commonly associated with *Iridomyrmex* species in the *anceps* and *rufoniger* groups (Eastwood & Fraser, 1999; Pierce & Nash, 1999). The geographical distributions of these ants are not fully known, but in areas where several attendant ant species overlap, a given 'colony' of *J. evagoras* is predominantly associated with only a single ant species (Costa *et al*., 1996; Pierce & Nash, 1999). Adult butterflies tend to remain in their natal area (Elgar & Pierce, 1988), creating potential for the evolution of specialization in ant preference.

Adult females lay eggs on *Acacia* host plants in response to the presence of attendant ants (Pierce & Elgar, 1985). Typically, gravid females that land on host plants follow a sequence of behaviours leading to oviposition. These include: (a) *dragging*, in which a female walks up and down the branches of the plant dragging the tip of her abdomen along the substrate – a behaviour that is typical among Lepidoptera searching for suitable oviposition sites (Renwick & Chew, 1994); (b) *probing*, in which a *dragging* female stops, extrudes her ovipositor and probes a crevice and (c) *oviposition*, in which she remains stationary with the tip of her abdomen inserted into a crack or crevice and during which she pumps her abdomen. For the behavioural analysis in this study, we grouped dragging, probing and oviposition into a single category of oviposition-related behaviour.

Collection and identification of material

Butterflies and ants were collected from various localities in eastern Australia (Fig. 1, Table 1). Ant identifications were assigned in consultation with Dr Steve Shattuck, Australian National Insect Collection (ANIC), Canberra and voucher specimens have been deposited at the ANIC

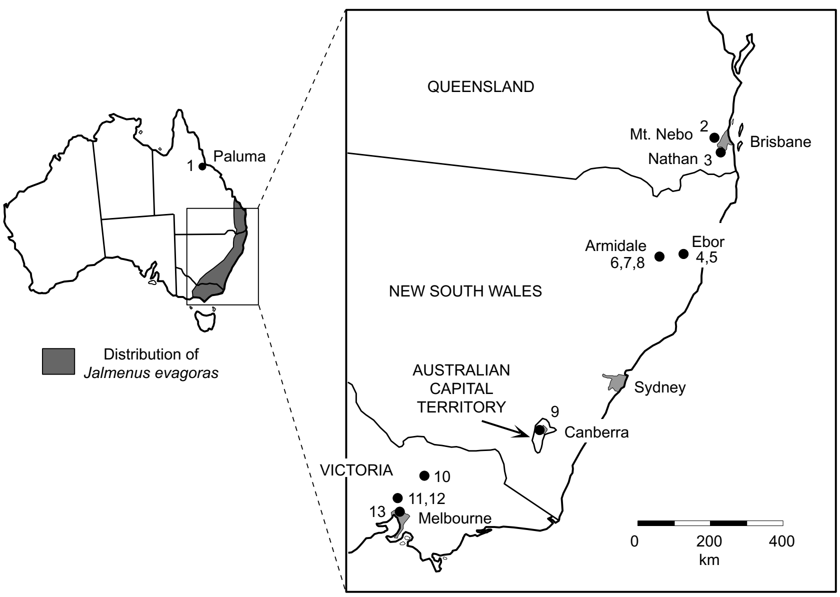

Fig. 1 Distribution of *Jalmenus evagoras* and localities from which ant species were collected.

Table 1 Summary of material collected to assess levels of oviposition discrimination exhibited by *Jalmenus evagoras* butterflies in response to ants. The outcome of each experiment is summarized in the far righthand column. Geographical distance refers to the approximate geographical distance separating each test ant collection locality from the butterfly population used in the experiment. All ant species except *I. purpureus* and *F. kirbii* naturally attend *J. evagoras.*

Experiment	Butterfly locality (site no.)*	Treatment offered	Ant locality (site no.)*	Geographical distance (km)	Outcome
Experiment I:	Mt. Nebo	*I. anceps*†	Mt. Nebo (2)	0	Butterflies discriminate
Choice of	(2)	*I. rufoniger*†	Mt. Nebo (2)	1	between ant species; prefer
attendant and		*I. purpureus*†	Mt. Nebo (2)	1	attendant *I. anceps* over
nonattendant		*F. kirbii*†	Paluma (1)	1200	*I. rufoniger* and nonattendant
ant species					ant species (Table 2)
Experiment II:	Nathan	*I. anceps*	Nathan (3)	0	Butterflies discriminate
Choice of local	(3)	*I. rufoniger*	Armidale (7)	350	between treatments; prefer
and foreign	(two trials)	*I. anceps*	Canberra (9)	950	local *I. anceps* over
attendant ant		No ant	–	–	*I. rufoniger* and foreign
species					*I. anceps* (Fig. 2)
Experiment III:	Nathan	*I. anceps*	Nathan (3)	0	Butterflies do not show
Choice of	(3)	*I. anceps*	Mt. Nebo (2)	30	clear or consistent
local and	(three trials)	*I. anceps*	Ebor (4)	350	preference; when
foreign		No ant	–	–	preference shown, local
attendant ant					ant always among
populations	Ebor	*I. anceps*	Ebor (4)	0	preferred populations
	(4)	*I. anceps*	Nathan (3)	350	(Fig. 3)
	(two trials)	*I. anceps*	Mt. Nebo (2)	350	
		No ant	–	–	
Experiment IV:	Armidale/Ebor	*I. rufoniger*	Armidale/Ebor (5–8)	0	Naïve butterflies prefer
Reciprocal	(5–8)	*I. anceps*	Melbourne (10–13)	1000	local attendant ant species
choice of					(Table 4); suggests local
attendant ant	Melbourne	*I. anceps*	Melbourne (10–13)	0	adaptation in oviposition
species	(10–13)	*I. rufoniger*	Armidale/Ebor (5–8)	1000	preference

*See Fig. 1 for collection localities and corresponding site numbers.
†Each test ant species treatment was also paired with a local, ground-foraging *I. anceps* treatment.

and at the Museum of Comparative Zoology, Harvard University. Species-level taxonomy within the *anceps* and *rufoniger* groups of *Iridomyrmex* is poor. We noted differences in body colour and colony structure between *I. anceps* populations from Ebor, Nathan and Mt. Nebo compared with those from Canberra and Melbourne. It is possible that these two variants represent separate species within the *anceps* groups. Pending a systematic review of the *anceps* group, however, we adopt a conservative approach and refer to all *I. anceps* populations as a single species.

Choice tests experiments were conducted from January to March over several years.

Experiment I: Choice of attendant and nonattendant ant species

Methods

An arena of four pairs of potted host plants (*Acacia irrorata*) was arranged in a 5 m × 5 m square in an open field at Mt. Nebo, Queensland (Fig. 1). Paired plants were placed within 1 m of each another. One plant in each pair was connected to a captive colony of one of four 'test' ant species (Table 1). Of these, *I. purpureus* and *F. kirbii* do not normally attend *J. evagoras* under natural conditions; *I. purpureus* tends *J. ictinus* while *F. kirbii* tends *J. pseudictinus* and *J. aridus*. The second plant in each pair served as a control, on which the locally occurring attendant ant (*I. anceps* sp. 25, Pierce *et al*., 1987) foraged. This design permitted us to assess whether oviposition preferences were influenced by test ant treatment or by treatment position alone. Twelve juveniles of a locally common ant-associated membracid, *Sextius virescens* (Homoptera), were maintained on each plant to attract ants.

Oviposition on plants by freely flying *J. evagoras* butterflies was monitored over 15 days. Plants were exchanged with fresh trees every 5 days. Egg masses were counted and removed at the end of each day and summed across days for each treatment before analysis. Female preference was assessed in a Chi-square (χ^2) test of a 2×4 contingency table. The mean number of ants per plant was obtained by calculating a mean for each of the three trees used in each treatment over the 15-day period, and then finding the mean across these three trees. The numbers of ants foraging on the different treatments were compared using a two-way repeated measures analysis of variance.

Results

Egg mass distribution was not independent of the test ant species ($P = 0.006$, Table 2). Females laid more egg masses on plants bearing *I. anceps* as the test ant species than they did on plants bearing other test ant species. The distribution of egg masses on plants with control *I. anceps* compared with that on plants with test ant species indicates that preference was not a result of treatment position (Table 2). *I. rufoniger* was as unattractive to ovipositing butterflies as the two nonattendant test ant species (Table 2).

Oviposition differences cannot be attributed to the total number of ants in each paired setup (control *I. anceps* + test species). There were significantly more local (control) ants than test ants on plants (repeated measures ANOVA, $F_{1,8} = 9.82$; $P = 0.014$), but the total number of ants (control + test) did not differ among pairs ($F_{3,8} = 0.74$, $P = 0.5$) and the two factors did not interact (within pairs × among pairs: $F_{3,8} = 0.17$, $P > 0.9$) (Table 2). Therefore, ant species and not the total number of ants within a pair explains the observed difference in egg mass distribution.

Although it is possible that only one or two butterflies generated the overall egg mass distribution pattern, this is unlikely given the large numbers of eggs and egg masses that were laid over the course of the experiment (Table 2). To address this uncertainty, however, subsequent experiments followed marked individuals in an enclosed setting.

Experiment II: Choice within and between attendant ant species

Methods

This experiment assessed oviposition preference of females in response to different species of attendant ants (Table 1). When it was conducted, it was assumed that *I. anceps* from Nathan and Canberra were members of different species within the *anceps* group (see above). Until a systematic review of the *anceps* group is undertaken, however, we will treat them conservatively as a single species. Thus, this experiment assesses oviposition preference both within and between attendant ant species. An ant-free control treatment was also included (Table 1).

Oviposition trials were conducted in an outdoor screened enclosure (5 × 5 × 2.3 m) at Griffith University, Nathan, Queensland (Fig. 1, Table 1). Four groups of three potted host plants (*Acacia melanoxylon*) were arranged in a *c.* 3 m × 3 m square. Within each group, plants were in contact with one another and one plant was connected to a captive ant colony or to an empty ant nest container (Table 1). *Jalmenus evagoras* juveniles were placed on plants to attract ants, and as a control in the ant-free treatment. Additional oviposition substrate, in the form of a rough-barked branch, was attached to the main stem of each plant.

Butterflies were derived from juveniles collected from the field at Nathan (Table 1, Fig. 1). Juveniles were tended by their local ant (*I. anceps*) until eclosion. Butterflies eclosed and mated in cages, and were marked with numbers on the underside of the forewings for identification during behavioural observations. An oviposition trial began by releasing up to 12 males and 12 females into the enclosure. Butterflies that died over the experimental period were replaced with new individuals of the same sex. Ants foraging on treatments were counted daily and numbers equalized among treatments by adjusting the number and/or age class of *J. evagoras* juveniles on plants or the number of ants in a nest container.

After 8 days, egg masses were removed from plants and totalled for each treatment. Egg mass distribution among treatments was compared with a random distribution using the *G*-test for goodness of fit. Pairwise comparisons were conducted using the *G*-test, with required significance values adjusted for nonindependence using the sequential Bonferroni procedure (Rice, 1989). Between oviposition trials, plants were left in place but ants and nest containers were moved to the corner diagonally opposite their previous location to control for effects of treatment position and plant quality on oviposition. New butterflies were used for each trial.

During the second trial, the behaviour of female butterflies was recorded during 5-min scans of the enclosure, conducted daily at hourly intervals between 07:30 and 17:30 h. The *G*-test was used to analyse data on visitation and activity patterns of individuals at the various treatments.

Results

Females laid significantly more egg masses on plants with their local *I. anceps* population than on plants with *I. anceps* from Canberra or with *I. rufoniger* (Fig. 2, $P < 0.001$, pairwise comparisons $P < 0.05$). In the first

Table 2 Numbers of egg masses laid by *J. evagoras* butterflies over 15 days on potted host plants containing juveniles of the membracid *Sextius virescens* tended by workers of different test ant species, or a paired control of the predominant local attendant ant species, *I. anceps*. The experiment was conducted in an open field. Host plants were replaced with fresh plants every 5 days. Mean ants per tree (±1 SE) was calculated from the three 5-day averages.

	Test species			
	I. anceps	*F. kirbii*	*I. rufoniger*	*I. purpureus*
Egg masses per treatment*				
Test species	16	4	5	4
Control *I. anceps*	15	11	19	27
Ants per tree ($n = 3$)				
Test species	10.9 ± 3.5	10.0 ± 1.6	6.5 ± 1.0	5.5 ± 2.1
Control *I. anceps*	16.7 ± 0.7	17.0 ± 4.2	16.3 ± 5.9	15.7 ± 2.9

*$\chi^2 = 12.49$, d.f. = 3, $P = 0.006$.

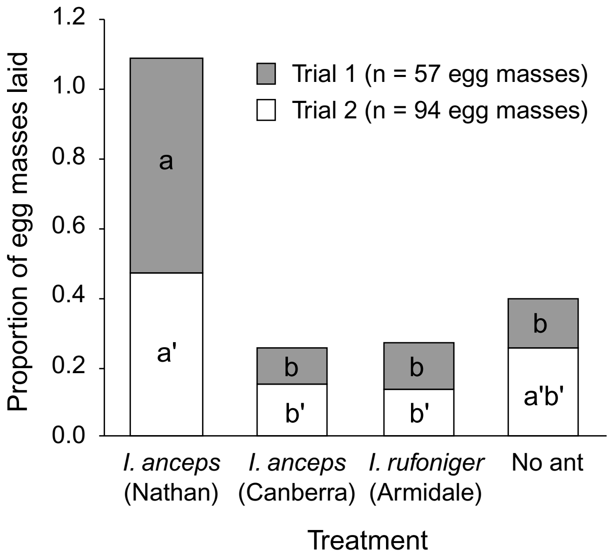

Fig. 2 Oviposition response of *J. evagoras* females from Nathan on host plants bearing conspecific juveniles tended by their local attendant ant (*I. anceps*, Nathan), foreign attendant ants (*I. anceps*, Canberra or *I. rufoniger*, Armidale) or untended. Total numbers of egg masses laid per trial are given in the figure legend. Oviposition was nonrandom with respect to treatment (*G*-test, Trial 1: $G = 34.05$, $P < 0.001$; Trial 2: $G = 25.57$, $P < 0.001$; d.f. = 3). Bars sharing the same letter do not differ significantly (pairwise comparisons adjusted for nonindependence, $P > 0.05$).

trial, females preferred plants with the local *I. anceps* to ant-free plants ($P < 0.05$). Females did not discriminate among treatments with foreign ants or ant-free plants (Fig. 2). Overall, ovipositing females ranked treatments in the following order: *I. anceps* Nathan (local ant) > no ants = *I. anceps* Canberra = *I. rufoniger* Armidale.

The nonrandom distribution of egg masses was related to differences in female behaviour on plants, but not to visitation patterns (Table 3). Visitation to the different treatments was random, both in terms of the number of different females visiting ($P = 0.63$) and the total number of visits received ($P = 0.36$). However, females were most likely to engage in oviposition-related behaviours on plants containing their local ant, secondly on ant-free plants and least likely on plants with foreign attendant ant species ($P = 0.01$). A majority of females (8/14) visited and exhibited oviposition-related behaviours on more than one treatment and five of these females exhibited oviposition-related behaviours on at least three treatments.

Egg mass size ranged from 1 to 86 eggs. Mean clutch size (±1 SE) per treatment ranged from 17.7 ± 4.0 to 28.2 ± 6.2 eggs; median clutch size range per treatment ranged from 10 to 28 eggs. No significant differences in egg mass sizes were detected (Kruskal–Wallis test: $P > 0.05$).

Experiment III: Choice among populations of *I. anceps*

Methods

This experiment assessed oviposition preference of *J. evagoras* females in response to different populations of *I. anceps* (Table 1, Fig. 1). An ant-free control treatment was also included. Experimental conditions were similar to Experiment II except that the three host plants in each treatment were placed within 50 cm of one another, but were not interconnected, and three ant colonies from each locality were used per ant treatment, with each colony connected to a single host plant. Five oviposition trials were conducted (Table 1), each lasting 4–5 days. Fresh host plants were used for each trial to control for any residual ant or butterfly odours deposited on plants during the previous trial. All plants were visually matched for size and condition. Ant colonies were moved to different corners of the enclosure between trials to control for effects of treatment position on oviposition. Butterflies from two localities were used (Table 1) and were derived from field collected, ant-tended juveniles.

Egg mass distribution among treatments was compared with a random distribution using the *G*-test for goodness of fit. Pairwise comparisons were conducted using the

Table 3 Summary of *J. evagoras* female butterfly visitation and behavioural patterns on host plants bearing conspecific juveniles tended by their local attendant ant (*I. anceps*, Nathan), foreign attendant ants (*I. anceps*, Canberra or *I. rufoniger*, Armidale) or untended. Data were compiled from hourly scans conducted during trial 2 of Experiment II using butterflies from Nathan. Fourteen females were used over the course of the trial. Oviposition-related behaviours (ORB) include dragging, probing and oviposition.

	Treatment				
Nathan butterfly behaviour	Local *I. anceps* (Nathan)	Foreign *I. anceps* (Canberra)	Foreign *I. rufoniger* (Armidale)	No ants	*G*-test results
No. of female visitors	10	6	8	11	$G = 1.74$, $P = 0.63$
Total no. of visits	23	13	15	16	$G = 3.22$, $P = 0.36$
No. (%) of visits involving ORB	21 (91)	6 (46)	8 (53)	14 (88)	$G = 10.99$, $P = 0.01$

G-test, with required significance values adjusted for nonindependence using the sequential Bonferroni procedure (Rice, 1989).

Results

Nathan females discriminated among ant populations in the latter two of three trials, preferring the local Nathan and the neighbouring Mt. Nebo ant populations over the more distant Ebor population (Fig. 3a, Trial 2 $P < 0.001$; Trial 3 $P = 0.02$; pairwise comparisons $P < 0.05$). Ebor butterflies did not discriminate among ant populations, but preferred ant-tended treatments over the ant-free treatment in Trial 2 (Fig. 3b, $P < 0.001$; pairwise comparisons $P < 0.05$).

Treatment did not influence the number of eggs per mass in any of the trials (Kruskal–Wallis test: $P > 0.05$). Egg mass size ranged from 1 to 215 eggs. Mean clutch size (±1 SE) per treatment ranged from 16.2 ± 4.2 to 45.0 ± 6.2 eggs; median clutch size per treatment ranged from 13 to 40 eggs.

Experiment IV: Reciprocal choice of two attendant ant species using naïve butterflies

Methods

A reciprocal choice test involving naïve butterflies was conducted to determine whether female discrimination among *I. anceps* and *I. rufoniger* had a genetic component and whether females preferred their local attendant ant species. *Jalmenus evagoras* pupae and a fragment of the attendant ant colony were collected from four field sites in each of two regions (Fig. 1, Tables 1 and 4) and brought to the University of Melbourne, Victoria for rearing and experimentation. Adults that eclosed from field-collected pupae were mated in the laboratory and progeny from these adults were reared without ants on *A. melanoxylon* cuttings, ensuring that F_1 adults were naïve with respect to ant cues.

Oviposition trials using F_1 adults were conducted in the laboratory in cylindrical cages, 50 cm high × 24 cm diameter with clear plastic sides and a net roof. Two plastic foraging arenas were placed in each cage. One arena contained ants from the same population as the butterfly being tested, whereas the other contained ants of the foreign attendant species. A 40-cm long wooden dowel with numerous pits drilled into the surface was mounted vertically in each arena and served as oviposition substrate for butterflies. Thirty ants were placed on each dowel at the start of an oviposition trial. A plastic sleeve containing cotton wool soaked in 10% sucrose solution was placed at the top of each dowel to attract ants onto the dowel.

A single, newly eclosed *J. evagoras* female was placed in a cage with two or three males as mates. Cages were checked every morning. When eggs were detected the trial was terminated and eggs were counted. Only one female from each population was used. Thus, each population represented an independent data point. Similarly, each ant population was used only twice: once as the local attendant ant species, and once as the foreign attendant ant species. Oviposition preference was assessed using a one-tailed binomial probability test.

Results

Naïve female butterflies exhibited a preference for their local ant species over the foreign species (Table 4, $P = 0.035$, $n = 8$). This experiment rules out the possibility that oviposition preference was because of imprinting on attendant ants, as individuals were reared from egg to eclosion in the absence of ants. Moreover, each female butterfly was tested in isolation from other females and over a relatively short time period, which ensured that the presence of conspecific females or eggs on a treatment did not influence behaviour.

Discussion

Jalmenus evagoras females discriminated between attendant and nonattendant ant species, between attendant ant species, and to some extent, between populations of a

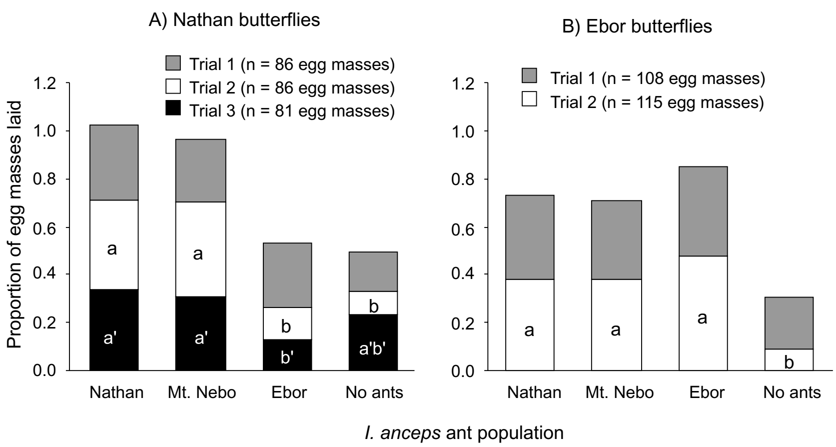

Fig. 3 Oviposition response of *J. evagoras* females on host plants bearing conspecific juveniles tended by three populations of *I. anceps* ants or untended. Butterflies were from (a) Nathan and (b) Ebor. Total egg masses per trial are given in the figure legend. *G*-tests of oviposition preference: Nathan females, Trial 1: $G = 4.40$, $P = 0.22$; Trial 2: $G = 26.80$, $P < 0.001$; Trial 3: $G = 9.54$, $P = 0.02$; d.f. = 3; Ebor females, Trial 1: $G = 4.64$, $P = 0.20$; Trial 2: $G = 26.84$, $P < 0.001$; d.f. = 3. Bars sharing the same letter do not differ significantly (pairwise comparisons adjusted for nonindependence, $P > 0.05$).

Table 4 Number of eggs laid by individual *J. evagoras* females from Armidale / Ebor and Melbourne offered a simultaneous choice of oviposition substrate patrolled by their local attendant ant species or by the reciprocal foreign attendant ant species (see Table 1, Fig. 1).

	Number of eggs laid on treatment		
Butterfly origin site (No.)	Armidale / Ebor ants (*I. rufoniger*)	Melbourne ants (*I. anceps*)	Local ant preferred*
Armidale / Ebor			
Panton's Gully† (5)	9	27	–
Eastwood SF (6)	19	9	+
Scary Road (7)	7	0	+
Kalinda Road (8)	5	0	+
Melbourne			
Violet Town (10)	0	6	+
Wallan 1 (11)	16	109	+
Wallan 2 (12)	0	11	+
LaTrobe (13)	30	54	+

*One-tailed binomial probability, $P = 0.035$; + local ant preferred; – local ant not preferred.
†*I. anceps* is the predominant attendant ant species at this site (Costa *et al.*, 1996) but *I. rufoniger* controls a minority of host plants bearing *J. evagoras*.

single ant species in oviposition choice tests. Our limited testing also suggests that butterflies exhibit local adaptation in oviposition preference and that oviposition preference has a genetic component. A genetic basis for oviposition preference has previously been reported in Lepidoptera and other insect groups and is a necessary prerequisite for the evolution of oviposition preference (Futuyma & Peterson, 1985; Jaenike & Holt, 1991; Thompson & Pellmyr, 1991).

Oviposition preference can evolve rapidly in response to the introduction of a novel host, the loss of a preferred host, or colonization of a new area where the preferred host is absent or rare (Feder & Bush, 1989; Singer *et al.*, 1993). The degree of evolutionary change varies, however, with the organism under study, the amount of gene flow among populations and the scale of the analysis (Thompson & Pellmyr, 1991; Thompson, 1993; Thomas & Singer, 1998). Aspects of the biology of *J. evagoras* and other obligately ant-associated lycaenids that would likely promote and maintain local specialization in oviposition preference include the low dispersal rates of adults from their natal habitat and the use of host plants and attendant ants as rendezvous sites for mating (Atsatt, 1981a; Pierce & Elgar, 1985; Elgar & Pierce, 1988; Seufert & Fiedler, 1996; Fiedler, 1997).

Although this is the first study, to our knowledge, that addresses geographical variation in oviposition preference among myrmecophilous butterflies, at least two studies have examined variation with respect to the attraction of lycaenid larvae to ants. Elmes *et al.* (1994) presented correlative evidence for local specialization in association between populations of *Maculinea alcon* and different *Myrmica* ant species in Europe. Ant-related oviposition behaviour was recently documented in *M. alcon* (van Dyck *et al.*, 2000) and it would be interesting to determine if this contributes to specificity in association patterns in this system. In two populations of *Plebejus argus,* in which larvae are associated with different *Lasius* ant species, Jordano & Thomas (1992) found that larvae were more attractive to their natural host ant species and that larval differences in attractiveness had a genetic basis.

We cannot rule out the possibility that a behavioural imprinting process, whereby individuals learn diagnostic characteristics such as smell, shape and behaviour of their natal ant associate during development or upon eclosion, influences oviposition behaviour. This may have influenced female preference in Experiments I–III, but not in Experiment IV in which an entire generation of *J. evagoras* was reared in the absence of ants. Reciprocal choice tests conducted with these adults still revealed an oviposition preference for the local ant partner.

Our report of local adaptation in oviposition preference by *J. evagoras* is based on widely separated butterfly populations and their respective ant associates. However, specialization within more limited geographical regions may not be evident. Costa *et al.* (1996) did not find a pattern of allozyme variation related to ant associate in the Armidale / Ebor area, where *J. evagoras* associates with *I. rufoniger* and *I. anceps*, respectively, although there was a trend in isolation by distance. Costa *et al.* (1996) suggest that gene flow among butterfly subpopulations and frequent extinction-recolonization events account for the observed genetic variation. Our experiments indicate that *anceps*-associated butterflies always prefer *I. anceps* when discriminating between *I. anceps* and *I. rufoniger*, regardless of the geographical distance that separates butterfly and ant populations. Whether *rufoniger*-associated butterflies exhibit similar behaviour in regions where the two ant species overlap is unclear. Interestingly, the one *rufoniger*-associated butterfly that preferred the foreign *I. anceps* treatment to the local *I. rufoniger* treatment in Experiment IV was collected from a site (Panton's Gully, Table 4) where *I. anceps* is the predominant attendant ant species (Costa *et al.*, 1996).

It would be interesting to know if oviposition preference in *J. evagoras* is locally adaptive. Do females preferentially associate with ant species that confer the greatest benefits or smallest cost to *J. evagoras* juveniles in a given region? Juveniles depend on ants for protection from natural enemies (Pierce *et al*., 1987) and ant species can differ in the level of protection they provide to their trophobiotic partners (Addicott, 1979; Buckley & Gullan, 1991; Savignano, 1994; Fraser *et al*., 2001). At Mt. Nebo, survivorship of *J. evagoras* juveniles was higher when *I. anceps* was in attendance compared with *I. rufoniger* (Pierce, 1989), suggesting that natural selection may favour discrimination by ovipositing butterflies and preference for the most effective attendant ant. Adults of *J. evagoras* juveniles that associate with ants incur a fitness cost, in the form of reduced body size (Pierce *et al*., 1987; Baylis & Pierce, 1992) and thus may experience lower reproductive success relative to untended individuals (Elgar & Pierce, 1988) but *I. anceps* and *I. rufoniger* confer similar costs to developing larvae (Pierce *et al*., 1987). Therefore, it appears that selection acts similarly on juveniles and adults by promoting preferential association with ant species that offer juveniles the most effective protection from natural enemies.

Females may remain flexible in their choice of oviposition sites, despite selection favouring association with a particular ant species. Females in Experiment I oviposited on host plants containing attendant as well as nonattendant ant species, indicating that oviposition 'mistakes' with respect to ant associate may occur in nature. Behavioural observations from Experiment II further suggest that individuals are flexible with respect to specificity in oviposition site selection, but that overall patterns of oviposition preference result from a hierarchical ranking of treatments by individuals. This flexible strategy may be adaptive, because females can continue to oviposit on plants with 'inferior' ant species if the most effective, and presumably preferred, attendant ant species is absent or relatively rare (see also Wiklund, 1981). On the other hand, a flexible strategy may be costly if females deposit eggs on plants visited by ant species that do not recognize developing juveniles favourably.

From local adaptation to speciation?

A sympatric shift in ant associate, followed by speciation, seems unlikely for *J. evagoras* or other lycaenids, owing to the concomitant shift in chemical signalling required between lycaenid larvae and ants (Atsatt, 1981b; Pierce, 1984; Elmes *et al*., 1994; Fiedler, 1997). An allopatric model involving colonization of a new area, in which a butterfly's usual attendant ant species is absent, together with reduced gene flow with the parental species, seems a more plausible scenario (see also Elmes *et al*., 1994). We speculate that a shift in ant associate is probably initiated by a change in adult behaviour rather than a change in larval characteristics because of the greater mobility of adults and the fact that ovipositing females ultimately determine the conditions in which newly hatched larvae will emerge.

We have demonstrated flexibility in oviposition site choice by *J. evagoras* and assume that this flexibility would provide opportunities for interactions with novel ant species in nature. For associations to become established, larvae must be able to appease their new ant partner. Observations of *J. evagoras* larvae being tended by *I. purpureus* and several other novel ant partners in the wild and under laboratory conditions (Pierce, 1989; Eastwood & Fraser, 1999), indicate that appeasement of novel ant partners is possible. Nonetheless, the intensity with which ants participate in these novel associations may vary within and among ant colonies and over time (Pierce *et al*., 1991). As a result, many novel associations may be transient. If they persist and the newly founded lycaenid population remains genetically isolated, selection may act on traits such as oviposition preference and larval performance. This may lead to a situation where ovipositing butterflies no longer recognize their former attendant ant species favourably when reunited and larvae are no longer recognized favourably by their former attendant ant species. Speciation may be promoted if genes involved in traits related to oviposition preference and larval performance have pleiotropic effects on traits involved in sexual reproduction (Funk, 1998).

Acknowledgments

We thank J.D. Fraser and K.L. Nutt for field assistance, and R. Eastwood for comments on the manuscript. R.L. Kitching and the Faculty of Environmental Science graciously provided facilities and hospitality at Griffith University. S.O. Shattuck kindly provided identifications of ants. A.M. Fraser was supported by the Natural Sciences and Engineering Research Council of Canada, a Harvard University Frank Knox Traveling Fellowship, the Putnam Expedition Fund of the Museum of Comparative Zoology and the Explorer's Fund. T. Tregenza was supported by a Royal Society Traveling fellowship. N. Wedell was supported by the Swedish Natural Science Research Council.

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Received 8 January 2001; revised 18 March 2002; accepted 21 March 2002