THE ROYAL SOCIETY

Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies

T. Akino[1]†, J. J. Knapp[1*], J. A. Thomas[2] and G. W. Elmes[2]

[1]*Division of Biodiversity and Ecology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK*
[2]*Institute of Terrestrial Ecology, Furzebrook Research Station, Wareham, Dorset BH20 5AS, UK*

Although it has always been assumed that chemical mimicry and camouflage play a major role in the penetration of ant societies by social parasites, this paper provides the first direct evidence for such a mechanism between the larvae of the parasitic butterfly *Maculinea rebeli* and its ant host *Myrmica schencki*. In the wild, freshly moulted fourth-instar caterpillars, which have no previous contact with ants, appear to be recognized as ant larvae by foraging *Myrmica* workers, which return them to their nest brood chambers. Three hypotheses concerning the mechanism controlling this behaviour were tested: (i) the caterpillars produce surface chemicals that allow them to be treated as ant larvae; (ii) mimetic compounds would include hydrocarbons similar to those employed by *Myrmica* to recognize conspecifics and brood; and (iii) the caterpillars' secretions would more closely mimic the profile of their main host in the wild, *M. schencki*, than that of other species of *Myrmica*. Results of behavioural bioassays and chemical analyses confirmed all three hypotheses, and explained the high degree of host specificity found in this type of highly specialized myrmecophile. Furthermore, although caterpillars biosynthesized many of the recognition pheromones of their host species (chemical mimicry), they later acquired additional hydrocarbons within the ant nest (chemical camouflage), making them near-perfect mimics of their individual host colony's odour.

Keywords: chemical mimicry; *Maculinea*; myrmecophily; social parasitism

1. INTRODUCTION

Larvae of the lycaenid butterfly *Maculinea rebeli* Hir. have a complex parasitic relationship with *Myrmica* ants, which includes penetrating their host's nests and eating the resources in the brood chambers. This exploitation of the richest, but best protected, ecological niche inside an ant colony represents the most evolutionarily advanced and rarest lifestyle known among the social parasites of ants (Hölldobler & Wilson 1990). Most social parasites that inhabit brood chambers are predators of juvenile ants, but *M. rebeli* and a few other species achieve such close integration with their host's society that they are fed directly by the workers. Trophallactic feeding is an efficient way of exploiting a colony's resources, but carries the ecological cost of high host specificity, perhaps because the degree of integration required can be attained only through very close mimicry of one host (Thomas & Elmes 1998).

Hölldobler & Wilson (1990) suggest that all social parasites penetrate ant societies by using mechanical and chemical cues to break their hosts' communication and recognition codes. However, evidence of genuine chemical mimicry (*sensu* Howard *et al.* 1990*a*) involving the biosynthesis of ant recognition pheromones—as opposed to the passive adsorption of colony odours (Vander Meer & Wojcik 1982; Vander Meer *et al.* 1989; Akino *et al.* 1996) or the secretion of agonistic semiochemicals—has been elusive. It has been demonstrated through behavioural studies in *Atemeles* beetles and in a few other species (Hölldobler & Wilson 1990), but perhaps only one (unpublished) description exists of the chemistry of a biosynthesized mimetic pseudopheromone that is apparently uncontaminated by its host (referred to by Henning 1983). In addition, Howard *et al.* (1990*b*) strongly suggest that *Microdon* (syrphid) larvae biosynthesize mimetic cuticular hydrocarbons (Dettner & Liepert 1994).

We attempted to obtain clear evidence of these mechanisms by making behavioural bioassays and chemical analyses of *M. rebeli* and its hosts, to test three hypotheses (Thomas *et al.* 1989; Elmes *et al.* 1991; De Vries *et al.* 1993), as follows. (i) The final-instar caterpillar of *M. rebeli* produce surface chemicals that induce *Myrmica* workers to treat them like ant larvae, giving them access to the brood chambers of these ants. (ii) Mimetic chemicals, if found, would include a cocktail of chemicals resembling the hydrocarbons employed by *Myrmica* to recognize conspecific adults and, probably, their brood (Brian 1975; Cammaerts *et al.* 1978; Winterbottom 1980). (iii) *M. rebeli*'s secretions would most closely mimic the profile of *Myrmica schencki* Emery, explaining its high survival in

* Author for correspondence (jjk@soton.ac.uk).
†Present address: National Institute of Sericulture and Entomological Science, Ohwashi 1–2, Tsukuba, 305–8634, Japan.

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colonies of this ant and low survival with other *Myrmica* species.

2. MATERIAL AND METHODS

(a) *Lifestyle and myrmecophily of* Maculinea rebeli

Adult *M. rebeli* fly for four weeks in summer and oviposit on the flower-buds of an initial larval food plant, *Gentiana cruciata* L., regardless of whether these grow in the territory of any, or a particular, species of *Myrmica* ant (Thomas *et al.* 1989). The larvae (henceforth called caterpillars to avoid confusion with ant larvae) develop quickly inside the seed capsules. In the early evening after moulting to the fourth and final instar, they drop to the ground. This timing coincides with the peak foraging activity of all *Myrmica* species, and caterpillars are soon found by *Myrmica* workers. After brief antennal contact, they are transported to the nest, placed among the ant brood, and are tended and fed with prey, trophic eggs and by trophallaxis as if they were ant larvae. After ten days, many caterpillars are so closely integrated with their host's society that they are transported or fed in preference to the ant's larvae. Caterpillars remain in the brood chambers for 11 or 23 months, growing from 1–2 mg to 80–140 mg (Thomas *et al.* 1998).

The period of entry and integration with a host society is one of the most dangerous in the life of a brood parasite (Hölldobler & Wilson 1990). The adoption behaviour of *M. rebeli* is so effective that over 90% of individuals leaving their gentians are transported into *Myrmica* colonies. However, mortality is often high inside the brood chamber, especially during the early days of integration (Elmes *et al.* 1991). A key variable is which species of *Myrmica* adopts caterpillars. Caterpillar survival is about 30 times greater in *M. schencki* colonies than in those of other *Myrmica* species (Thomas & Elmes 1998), yet caterpillars in the field are usually adopted by the first *Myrmica* workers to encounter them beneath gentians, resulting in 30% of individuals, on average, being adopted by *M. schencki* on known sites ($n = 9$), and the rest by *M. sabuleti* (37%), *M. scabrinodis* (19%), and either *M. ruginodis* or *M. rubra* ($< 5\%$) (Elmes *et al.* 1991, 1996).

Although *Maculinea* caterpillars emit sounds that resemble the stridulations of worker ants, we considered acoustical mimicry an unlikely cue for adoption and integration because *Myrmica* larvae are mute and because the caterpillars' sounds are specific to the genus *Myrmica* rather than to its host species (DeVries *et al.* 1993). Instead, the observed interactions suggested that chemical mimicry was involved (Elmes *et al.* 1991). With many social parasites it is almost impossible to extract secretions that are not contaminated by host pheromones. With *M. rebeli* it is slightly easier because caterpillars can remain healthy for 24 h without ants after entering their final myrmecophilous phase. However, the rarity of *M. rebeli* constrained the amount of extract that could safely be obtained. Experiments were carried out between 1996 and 1998.

(b) *Extracts and bioassays*

M. rebeli caterpillars and three *M. schencki* colonies were collected in the Pyrenees; we used tested procedures to ensure that the next year's butterfly population would be unaffected. Caterpillars were reared on *G. cruciata* in the absence of ants, and were used within 24 h of their final moult to make bioassays or obtain initial extracts, again without ever having been in contact with ants. From each *M. schencki* colony, we established a laboratory culture of 37 workers, brood but no queens (which have litle influence on *Myrmica* recognition odours (Winterbottom 1980)) in a Perspex box (foraging arena) 15 cm × 27 cm × 10 cm, containing a plant-pot saucer as the nest site (Wardlaw *et al.* 1998).

Three solvents were used to extract surface chemicals from five workers and third-instar larvae from each laboratory colony of *M. schencki*, from five workers of *M. sabuleti*, *M. scabrinodis*, and *M. ruginodis*, and from five *M. rebeli* caterpillars both before exposure to ants and after living for seven days with *M. schencki*. The insects were immersed successively in 100 µl hexane for 5 min, in 100 µl ethyl acetate for 1 h, and in 100 µl methanol for 1 h. Each solvent was decanted into a clean vial, sealed with an aluminium lid with nitrogen, and stored at −60 °C until ready for analysis.

Glass dummies, onto which extracts could be placed, were used to assess the role of chemicals in ant–butterfly interactions. A glass rod 1 mm in diameter was modified into 2–3 mm lengths with clubbed ends to mimic the approximate size and shape of butterfly and ant larvae. Dummies were washed in methanol immediately after being made, and each was later treated with 20 µl of the same extract (0.2 larval equivalents per dummy). This was done by placing five clean dummies into a clean small glass tube containing one larval equivalent of extract and allowing the solvent to evaporate for 20 s. Dummies were then put into experimental ant nests, by means of clean forceps. Controls consisted of dummies treated with pure solvent. To eliminate effects of learning or habituation by worker ants, five blank glass dummies were tested after every fourth bioassay. If workers transported these, the colony would be rested until these dummies were again ignored.

In experiments 1 and 2, a single test specimen (caterpillar, larva, dummy) was placed 1 cm from the nest entrance of a *M. schencki* laboratory colony. Interactions between workers and the test specimen were recorded for 60 min or until the test specimen was taken into the nest. The arena was checked again 2 h and 24 h after introduction. This was repeated with a minimum of ten test specimens.

An initial bioassay (experiment 1) was made to confirm that worker behaviour towards ant larvae and *M. rebeli* caterpillars was as described by Elmes *et al.* (1991). This was restricted to *M. schencki* colony 1 owing to the limited material and time when live caterpillars were available. The responses of workers to 29 live *M. rebeli* fourth-instar caterpillars, to ten *M. schencki* kin and ten non-kin larvae, and to ten controls were compared. Having established that colony 1 behaved normally towards larvae and caterpillars, the same nest was used to investigate worker response to cuticular extracts (experiment 2). Dummies washed with solvent extracts from *M. schencki* kin and non-kin larvae, and from *M. rebeli* fourth-instar caterpillars that had never been exposed to ants, plus controls, were introduced singly to the arena. Ten dummies were used for each treatment.

We finally tested how *M. schencki* colonies 2 and 3 responded to a choice of caterpillars and *Myrmica* larvae (experiment 3). Each colony was offered two of each of the following items: live larvae of *M. schencki* (kin), *M. scabrinodis*, *M. ruginodis* and *M. sabuleti*, and *M. rebeli* caterpillars. Unfortunately, by this stage, only frozen fourth-instar *M. rebeli* caterpillars that had never been exposed to ants were available. Five replicates were made with each colony.

(c) *Analysis of extracts*

All extracts were analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The gas chromatograph was a Hewlett Packard HP5890-II equipped with a flame ionization detector (FID) and on-column injection.

Table 1. *The behavioural response of* Myrmica schencki *to live conspecific larvae, live* Maculinea rebeli *caterpillars, and extracts*

(All *M. rebeli* caterpillars and their extracts had never previously been exposed to ants. Values are means (±s.e.); means in each row that share the same superscripts are not significantly different from each other (Mann–Whitney *U*-test, $p<0.01$); *t*, time; N, nest; A, arena.)

	live larvae				extracts			
behavioural response	kin *M. schencki*	non-kin *M. schencki*	*M. rebeli*	control (blank)	kin *M. schencki*	non-kin *M. schencki*	*M. rebeli*	control (solvent)
n	10	29	10	10	10	10		10
t to discovery s^{-1}	15.5 ± 1.9^{a}	14.5 ± 1.9^{a}	49.7 ± 8.1^{b}	322.0 ± 22.6^{c}	12.5 ± 1.7^{e}	16.7 ± 1.7^{e}	54.0 ± 6.9^{f}	270.0 ± 27.2^{g}
t to pick up s^{-1}	86.5 ± 11.8^{a}	230.5 ± 28.1^{b}	682.4 ± 139.3^{c}	—	159.0 ± 25.3^{e}	342.0 ± 9.7^{f}	240.0 ± 37.9^{f}	—
t to deposit s^{-1}	36.0 ± 9.8^{a}	102.0 ± 12.8^{b}	416.4 ± 107.2^{c}	—	84.0 ± 15.1^{e}	90.0 ± 13.4^{ef}	240.0 ± 50.0^{g}	—
no. of times touched	2.3 ± 0.3^{a}	10.1 ± 1.0^{c}	5.6 ± 0.6^{b}	1.2 ± 0.1^{d}	4.8 ± 2.0^{f}	14.4 ± 1.9^{e}	10.5 ± 1.6^{ef}	1.8 ± 0.3^{g}
destination	10 N	10 N	25 N, 4 A	10 A	2 N, 8 R	10 R	1 N, 9 R	10 A

The column used was a non-polar methyl silicon capillary column (HP1), 15 m long, internal diameter 0.25 mm with a 0.1 mm film thickness. The carrier gas was helium with nitrogen used as the make-up gas. Programme conditions were: injector port set to follow at 10 °C below oven temperature; starting oven temperature 50 °C, final oven temperature 300 °C; programme of 10 min at 50 °C, ramp at 10 °C min^{-1}, 10 min at 300 °C. The GC–MS employed was a Joel SX102A double focusing magnetic sector mass spectrometer interfaced with an HP5890-II GC. The conditions for GC–MS analysis were: ionization EI (70 eV); ion chamber temperature 230 °C; scan range 40–600 m/z.

The degree of similarity between the hydrocarbon profiles of different extracts was established by calculating their Nei (Ferguson 1980) distances (1, identical; 0, no common chemicals). Dendrograms based on Nei distances were constructed for interspecific comparisons. In these, clustering was based on an unweighted pair-group method, using an arithmetic mean, with the hydrocarbon components arranged on a data matrix consisting of binary attributes (1, present; 0, absent). Ochiai's (Romesburg 1989) resemblance coefficient was used; this takes the same value as the cosine coefficient (Nei's distance) when the data are expressed as a binary attribute matrix.

3. RESULTS

(a) *Behavioural bioassays*

The response of *M. schencki* to *M. rebeli* caterpillars, *M. schencki* kin and non-kin larvae, and control dummies is shown in table 1. Workers quickly recovered conspecific larvae and returned them to the nest, regardless of their origin. However, non-kin larvae took longer to be adopted ($p < 0.01$), because the workers first groomed and examined them with their antennae. *M. rebeli* took significantly longer than *M. schencki* kin or non-kin larvae to be adopted ($p < 0.01$), and after 60 min four out of the 29 caterpillars still remained in the arena. Not all caterpillars were taken directly into the nest (figure 1). Several were first carried around the arena for 10–30 min; and a few were temporarily placed among the rubbish before being retrieved, usually by the same ant. Inside the nest, all larvae and caterpillars were placed beside the ant brood, becoming intimately mixed with it within 24 h. No control dummy was picked up during experiment 1.

In experiment 2, *M. schencki* workers responded positively to extracts of caterpillars and larvae made with all three solvents, demonstrating that chemical signals were involved in recognition. The strongest response was to dummies treated with *M. schencki* and *M. rebeli* hexane extracts, which were invariably picked up and transported (table 1). Ethyl acetate and methanol extracts of *M. rebeli* evoked similar, but less intense, responses, which are not discussed here. Ethyl acetate and methanol extracts of *M. schencki* larvae produced comparatively little response.

Dummies treated with hexane extracts of *M. schencki* kin and non-kin larvae were discovered within 20 s; those with *M. rebeli* extracts (from caterpillars that had never been exposed to ants) took 1–2 min, and control dummies took significantly longer. Kin *M. schencki* hexane extracts were adopted more quickly and with fewer touches than extracts of either *M. schencki* non-kin larvae or *M. rebeli* caterpillars, which showed no significant difference from each other in their pick-up time, time to deposit or in the number of times they were touched (table 1). The final destination of the dummies generally differed from that of live larvae in that the majority, including those with *M. schencki* kin extract, were ultimately deposited on the rubbish rather than in the nest. However, one *M. rebeli*-treated dummy and two *M. schencki*-treated dummies were taken into the nest (figure 1).

M. schencki workers responded to the larvae of four species of *Myrmica* and to dead fourth-instar *M. rebeli* caterpillars in subtly different ways (experiment 3). As in experiment 1, kin larvae were always preferred. *M. ruginodis* larvae evoked mild aggression and were quickly approached, but their pick-up times were slow compared with those for *M. schencki*, *M. sabuleti* and dead *M. rebeli* caterpillars. Overall, there were significant interspecific differences in the times taken to discover and, more importantly, pick up larvae and caterpillars, which were chosen in the following order.

Order of discovery: *M. schencki* > *M. ruginodis* > *M. sabuleti* > dead *M. rebeli* > *M. scabrinodis* (Kruskal–Wallis test, $p < 0.0001$).

Order of pick-up: *M. schencki* > *M. sabuleti* > dead *M. rebeli* > *M. ruginodis* > *M. scabrinodis* ($p < 0.0004$).

(b) *Chemical analyses*

In figure 2 we present chromatograms of final-instar *M. rebeli* caterpillars before their contact with ants;

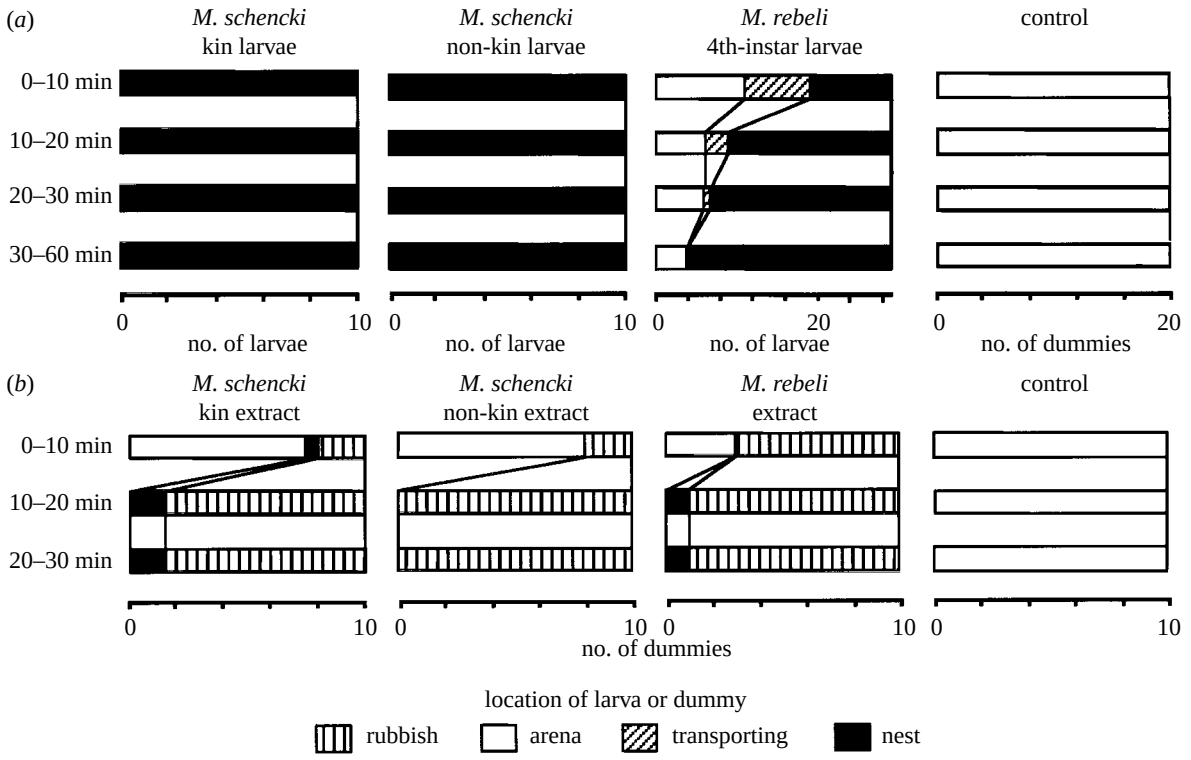

Figure 1. The behavioural response of a *Myrmica schencki* colony to (*a*) whole live kin larvae, non-kin larvae, *Maculinea rebeli* caterpillars and (*b*) their extracts. All *M. rebeli* caterpillars and extracts had never previously been exposed to ants.

M. rebeli caterpillars after seven days inside *M. schencki* nests; and *M. schencki* larvae and workers. The identity and abundance of each chemical in each profile is listed in Appendix A. The profiles of *M. schencki* workers and larvae were almost identical (Nei distance 0.98) and contain a complex mixture of compounds including many hydrocarbons. The chromatogram of pre-adoption *M. rebeli* caterpillars that had never encountered ants was simpler; nevertheless, it contained many compounds similar to those found in *M. schencki* larval and worker extracts (Nei distance 0.32). All had a mixture of non-volatile hydrocarbons. Mass spectrometry revealed that the surfaces of pre-adoption caterpillars and *M. schencki* larvae and workers had several methyl alkanes and *n*-alkanes in common (Appendix A), marked by asterisks in figure 2. They also shared a common terpenoid volatile, provisionally identified as limonene, which was absent from all other *Myrmica* species studied.

The surface chemistry of the *M. rebeli* caterpillar clearly altered after adoption (figure 2*b*; Appendix A). It acquired many of the missing hydrocarbons—probably by adsorption but possibly by biosynthesis—to become an excellent mimic of both *M. schencki* brood and workers (Nei distance 0.85). By comparison, all three conspecific colonies of *M. schencki* had Nei distances (workers) of 0.90.

Hexane extracts of *M. sabuleti*, *M. ruginodis* and *M. scabrinodis* revealed cuticular hydrocarbon profiles rather different from those of *M. schencki* and *M. rebeli*. Nei distances of the similarities between these profiles (table 2) showed that pre- and post-adoption *M. rebeli* caterpillars resembled *M. schencki*, and vice versa, much more closely than any other *Myrmica* species tested, as illustrated in the dendrogram (figure 3).

4. DISCUSSION

The response of *M. schencki* workers to extracts of conspecific larvae and *M. rebeli* (table l; figure l) confirms the hypothesis of Elmes *et al*. (1991) that *M. rebeli* final-instar caterpillars have evolved sufficient chemical similarity to enable them to be mistaken for *M. schencki* brood and be transported into nests. Experiments with glass dummies do, however, suggest that additional cues are required to complete the process. Although dummies treated with either *M. rebeli* or *M. schencki* extracts were recognized and transported, most were ultimately deposited in the rubbish pile rather than retained in nests, exactly as if they were dead brood. Because dummies provide no cues to indicate that they are alive, and lack the hairiness known to reinforce pheromones in *Myrmica* larval recognition (Brian 1975), this result is unsurprising. The application of less than one larval equivalent of extract to the dummies may also have contributed to this result.

The fact that the pick-up times of *M. rebeli* and non-kin *M. schencki* extracts did not differ significantly, but that both were picked up more slowly than were kin extracts, suggests that *M. schencki* also has colony-specific chemical cues but cannot discriminate between caterpillars and non-kin brood. The final bioassay comparing *Myrmica* species was imperfect, because the only uncontaminated *M. rebeli* caterpillars available were dead specimens, which were at an obvious competitive disadvantage to the living *Myrmica* larvae with which they were compared. Nevertheless, even dead caterpillars were picked up by *M. schencki* workers in preference to live larvae of *M. scabrinodis* and *M. ruginodis*, suggesting that *M. rebeli*

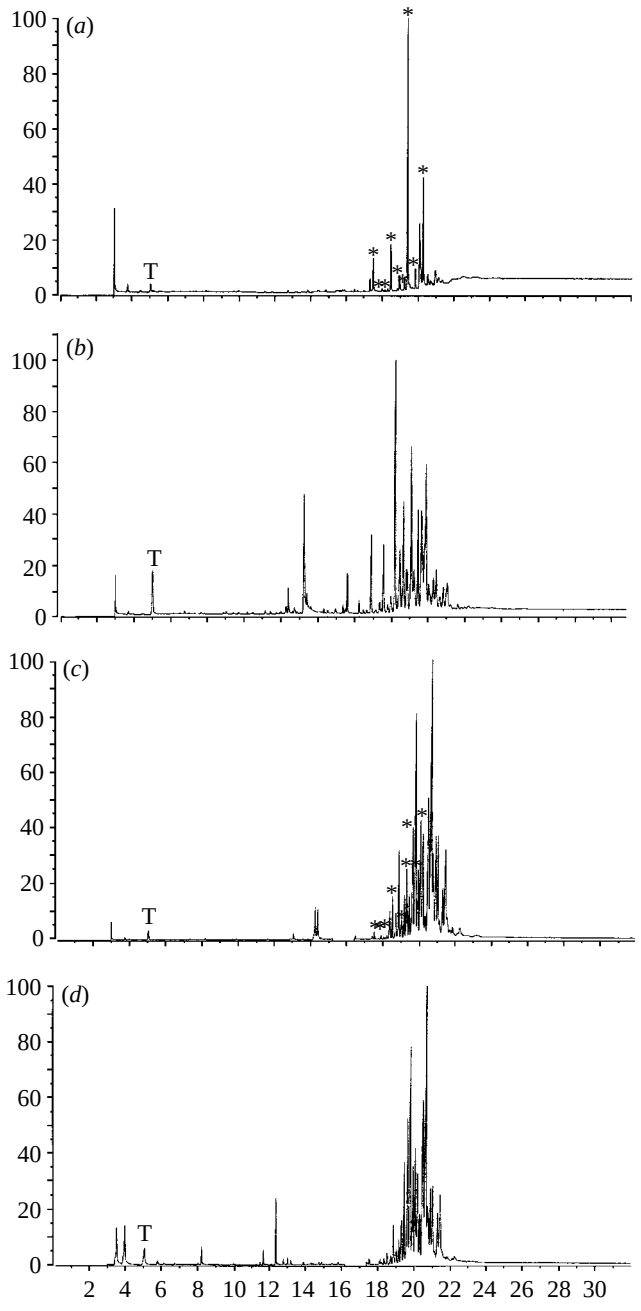

Figure 2. Gas chromatograms of hexane extracts of *Myrmica schencki* and *Maculinea rebeli*. (*a*) Pre-adoption *M. rebeli*; (*b*) post-adoption *M. rebeli*; (*c*) *M. schencki* larva; (*d*) *M. schencki* adult worker. Peaks labelled with an asterisk in (*a*) and (*c*) show hydrocarbons common to both *M. rebeli* caterpillars, before exposure to ants, and *M. schencki* larvae. The terpenoid volatile (limonene) is labelled with a T in all chromatograms.

caterpillars more closely resemble *M. schencki* than do the larvae of at least two of its congeners.

Chemical analysis of these extracts was instructive (figure 2). We showed that *Myrmica* larvae (unlike those of *Formica* and *Lasius*) have surface-recognition chemicals similar to those of adults in their colony, and we confirmed that workers from different *Myrmica* species had different mixtures of chemicals (see, for example, Cammaerts *et al*. 1978; Winterbottom 1980). We also confirmed our second hypothesis that fourth-instar *M. rebeli* caterpillars possess surface hydrocarbons resembling the recognition chemicals of *Myrmica* larvae, before they encounter their hosts. Finally, we confirmed our third hypothesis concerning the host specificity of this mimicry. Although the chemical profile of caterpillars was comparatively simple before exposure to ants, it was significantly closer to the secretions of *M. schencki* than to those of any other *Myrmica* species tested. However, this result should be regarded as a preliminary. Further work is required to determine whether adoption, recognition and caring behaviour are induced by all or just certain of the surface chemicals shown in figure 2 and the appendix, and whether other glandular secretions play a role.

These results explain earlier descriptions of the adoption and host specificity of *M. rebeli*. The manufacture of secretions that most closely mimic *M. schencki* would not stop caterpillars being adopted by other species of *Myrmica* after leaving their gentians, because *Myrmica* odours are sufficiently similar for workers to adopt as their own any 'lost' *Myrmica* larva found in their territory (Brian 1975; Winterbottom 1980; Cammaerts *et al*. 1978). However, closer relatedness (or mimicry) is required if foreign bodies are to compete with the kin brood inside a *Myrmica* colony (Brian 1975; Winterbottom 1980; Elmes & Wardlaw 1983). After the first few days when some caterpillars are killed or neglected by their hosts, the caterpillar acquires the missing chemicals to make it an almost perfect mimic of *M. schencki* larvae (figure 2; Appendix A), so much so that it is thereafter given preferential treatment over kin larvae (Thomas *et al*. 1998). In other words, the caterpillar initially biosynthesizes chemicals that make it an effective mimic of *M. schencki* as a species and later acquires extra odours distinctive to the individual colony. Howard *et al*. (1990) made a distinction between chemical mimicry and chemical camouflage according to the origin of the signal. Mimicry occurs when the organism biosynthesizes mimetic compounds; camouflage when it acquires them from the model. According to this definition, we may not be observing chemical mimicry in the strictest sense in *M. rebeli*, but rather a subtle combination of mimicry and camouflage. We do not know whether the early food plant (Stiefel & Margolies 1998) influences ability of *M. rebeli* to mimic *Myrmica*; whether, for instance, some of the recognition chemicals or their precursors could be obtained from *G. cruciata* while feeding. This requires further study. If certain *M. rebeli* larval cuticular compounds are biosynthesized and others are processed from food sources, this does not alter the fact that the caterpillars secrete these mimetic compounds before any contact with their host ant, *M. schencki*.

The fact that virtually all the *M. rebeli* caterpillars adopted by other species of *Myrmica* are eventually killed suggests that they either continue to biosynthesize chemicals that mimic *M. schencki* inside the nest or produce another species-specific signal. This may not be a liability for several months after adoption, because *Myrmica* colonies tolerate aliens (including the larvae of other *Myrmica* species) in times of plenty, before killing them under stress or food shortage (Winterbottom 1980; Elmes & Wardlaw 1983). Possible reasons for not relying solely on the adsorption of host odours include the following. (i) Volatiles, such as limonene, cannot be obtained through contact with the host. (ii) The

Table 2. *Degree of similarity between larvae of different* Myrmica *species and* Maculinea rebeli *caterpillars before and after adoption*

(Values are Nei distances based on comparisons of hydrocarbon profiles of each larva.)

	M. rebeli post-adoption	*M. schencki*	*M. sabuleti*	*M. scabrinodis*	*M. ruginodis*
Maculinea rebeli pre-adoption	0.61	0.32	0.24	0.25	0.25
Maculinea rebeli post-adoption	—	0.85	0.20	0.36	0.42
Myrmica schencki	—	—	0.14	0.25	0.13
Myrmica sabuleti	—	—	—	0.36	0.43
Myrmica scabrinodis	—	—	—	—	0.62

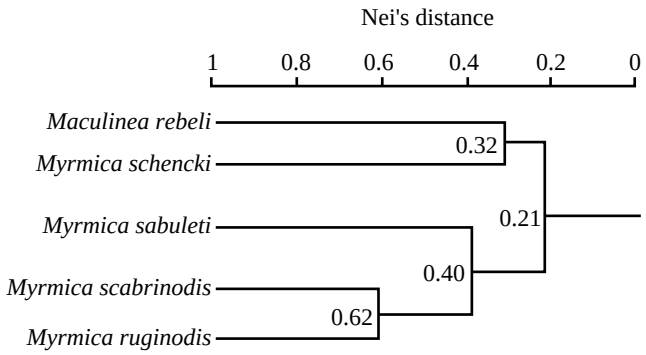

Figure 3. Dendrogram obtained by analysis of Nei's distance as a measure of the degree of similarity between the cuticular hydrocarbon profiles of larvae of *Maculinea rebeli* (pre-adoption) and four species of *Myrmica*.

compounds synthesized by *M. rebeli* include at least one chemical that is present on *M. schencki* larvae but not on the workers. Older caterpillars cannot acquire this chemical because they are kept segregated by size in separate cells by workers (Elmes *et al.* 1991). (iii) Successful *M. rebeli* caterpillars need to boost any acquired chemical signal to compete with ant larvae and other caterpillars for workers' attention.

These results complement other studies of the ways in which arthropods manipulate ant social behaviour. Much work on myrmecophilous butterflies has involved mutualistic or commensal species, the associations of which are achieved through a variety of secretions that agitate ants or that feed and appease them with sugars and amino acids (Maschwitz *et al.* 1975; DeVries 1984, 1988; DeVries & Baker 1989; Fiedler & Maschwitz 1987; Pierce *et al.* 1991). In contrast, Henning (1983) demonstrated that mimetic substances were secreted by the pore cupola of *Lepidochrysops ignota*, the only lepidopteran social parasite hitherto studied in this way. The secretions of *M. rebeli* may also emanate from the pore cupolas; these occur in unusual abundance on the final instar of *Maculinea* caterpillars (Malicky 1969). More generally, these results add one *Maculinea* species to the small number of social parasites known to synthesize mimetic chemicals to penetrate ant societies (Hölldobler & Wilson 1990; Howard *et al.* 1990*a*; Dettner & Liepert 1994). They also extend knowledge in that *M. rebeli* is the first social parasite in which a synthesized mimetic chemical, which was uncontaminated by ants, has been described in sufficient detail to explain the high degree of species-specific host dependency typically found in the field for this type of specialized myrmecophile (Cottrell 1984; Thomas & Elmes 1998; Elmes *et al.* 1999).

APPENDIX A

Comparison of the cuticular hydrocarbon components found in *M. schencki* larvae and adult workers, and *M. rebeli* pre- and post-adoption caterpillars, calculated by percentage area. Pre-adoption caterpillars had never encountered ants.

components	*M. schencki* larvae	*M. schencki* adult	*M. rebeli* post-adoption	*M. rebeli* pre-adoption
nC24	0	0	0.20	0
4MeC24	0	0	0.48	0
nC25	0.03	0.10	2.55	3.74
DiMeC25	0.03	0.08	0	0
nC26	0.04	0.04	0.83	0.61
8MeC26	0.05	0.07	0	0
4MeC26 + DiMeC26	0.32	0.30	0.32	1.12
nC27	0.45	0.80	5.40	7.58
MeC27	0.58	0.56	1.15	0
DiMeC27 + DiMeC27	1.59	2.34	0	0
nC28 + DiMeC27	1.26	1.06	6.59	4.65
MeC28 + DiMeC28	1.23	0.88	0.67	0
MeC28 + DiMeC28	2.54	2.56	1.94	0.94

(*Cont.*)

APPENDIX A (*Cont.*)

components	*M. schencki* larvae	*M. schencki* adult	*M. rebeli* post-adoption	*M. rebeli* pre-adoption
nC29	1.84	3.72	20.24	46.40
MeC29	10.60	9.80	3.55	0
MeC29 + DiMeC29	14.12	13.85	10.14	0
nC30	0.12	0.41	1.55	6.19
DiMeC29	2.91	1.52	1.39	0
MeC30	4.89	5.04	12.74	4.41
DiMeC30	4.56	3.96	2.46	0
nC31	0.66	1.32	8.14	22.16
MeC31	14.66	13.90	8.86	0
DiMeC31	23.32	20.19	9.57	0
TriMeC31	1.13	2.00	0.94	0
nC32	0	0	0	0.70
TriMeC31	3.02	3.75	0.29	0
MeC32	0	0	0	1.50
DiMeC32	2.92	3.91	0	0
MeC33	2.12	2.68	0	0
DiMeC33	5.04	5.18	0	0
	100.00	100.00	100.00	100.00

REFERENCES

Akino, T., Mochizuki, R., Morimoto, M. & Yamaoka, R. 1996 Chemical camouflage of myrmecophilous cricket *Myrmecophilus* sp. to be integrated with several ant species. *Jap. J. Appl. Entomol. Zool.* **40**, 39–46.

Brian, M. V. 1975 Larval recognition by workers of the ant *Myrmica*. *Anim. Behav.* **23**, 745–756.

Cammaerts, M. C., Inwood, M. R., Morgan, E. D., Parry, K. & Tyler, R. C. 1978 Comparative study of the pheromones emitted by workers of the ants *Myrmica rubra* and *Myrmica scabrinodis*. *J. Insect Physiol.* **24**, 207–214.

Cottrell, C. B. 1984 Aphytophagy in butterflies: its relationship to myrmecophily. *Zool. J. Linn. Soc.* **79**, 1–57.

Dettner, K. & Liepert, C. 1994 Chemical mimicry and camouflage. *A. Rev. Entomol.* **39**, 129–154.

DeVries, P. J. 1984 Of crazy ants and the Curetinae: are *Curetis* butterflies tended by ants? *Zool. J. Linn. Soc.* **79**, 59–66.

DeVries, P. J. 1988 The larval ant-organs of *Thisbe irenea* (Riodinidae) and their effects upon attending ants. *Zool. J. Linn. Soc.* **94**, 379–393.

DeVries, P. J. & Baker, I. 1989 Butterfly exploitation of a plant-ant mutualism: adding insult to herbivory. *J. NY Entomol. Soc.* **97**, 332–340.

DeVries, P. J., Cocroft, R. B. & Thomas, J. A. 1993 Comparison of acoustical signals in *Maculinea* butterfly caterpillars and their obligate host *Myrmica* ants. *Biol. J. Linn. Soc.* **49**, 229–238.

Elmes, G. W. & Wardlaw, J. C. 1983 A comparison of the effect of a queen upon the development of large hibernated larvae of six species of the genus *Myrmica* (Hym. Formicidae). *Insectes Soc.* **30**, 134–148.

Elmes, G. W., Thomas J. A. & Wardlaw J. C. 1991 Larvae of *Maculinea rebeli*, a large-blue butterfly and their *Myrmica* host ants: wild adoption and behaviour in ant-nests. *J. Zool.* **223**, 447–460.

Elmes, G. W., Clarke, R. T., Thomas, J. A. & Hochberg, M. E. 1996 Empirical tests of specific predictions made from a spatial model of the population dynamics of *Maculinea rebeli*, a parasitic butterfly of red ant colonies. *Acta Oecol.* **17**, 61–80.

Elmes, G. W., Barr, B., Thomas, J. A. & Clark, R. T. 1999 Extreme host specificty by *Microdon mutabilis* (Diptera, Syrphidae), a social parasite of ants. *Proc. R. Soc. Lond.* B **266**, 447–453.

Ferguson, A. 1980 *Biochemical systematics and evolution*. London: Blackie.

Fiedler, K. & Maschwitz, U. 1987 Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae). III. New aspects of the function of the retractile tentacular organs of Lycaenid larvae. *Zool. Beitr.* **31**, 409–416.

Henning, S. F. 1983 Chemical communication between lycaenid larvae (Lepidoptera: Lycaendiae) and ants (Hymenoptera: Formicidae). *J. Entomol. Soc. S. Afr.* **46**, 341–366.

Hölldobler, B. & Wilson, E. O. 1990 *The ants*. Berlin: Springer.

Howard, R. W., Acre, R. D. & Garnett, W. B. 1990*a* Chemical mimicry of an obligate predator of carpenter ants (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **83**, 607–616.

Howard, R. W., Stanley-Samuelson, D. W. & Akre, R. D. 1990*b* Biosynthesis and chemical mimicry of cuticular hydrocarbons from the obligate predator, *Microdon albicomatus* Novak (Diptera: Syrphidae) and its ant prey, *Mrymica incompleta* Provancher (Hymenoptera: Formicidae). *J. Kansas Entomol. Soc.* **63**, 437–443.

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

Maschwitz, U., Würst, M. & Schurian, K. 1975 Bläulingsraupen als Zuckerlieferanten für Ameisen. *Oecologia* **18**, 17–21.

Pierce, N. E., Nash, D. R., Baylis, M. & Carper, E. R. 1991 Variation in the attractiveness of lycaenid butterfly larvae to ants. In *Ant–plant interactions* (ed. C. R. Huxley & D. F. Cutter), pp. 131–142. Oxford University Press.

Romesburg, H. C. 1989 *Cluster analysis for researchers*. Malabar, FL: R. E. Krieger.

Stiefel, V. L. & Margolies, D. C. 1998 Is host plant choice by a clytrine leaf beetle mediated through interactions with the ant *Crematogaster lineolata*? *Oecologia* **115**, 434–438.

Thomas, J. A. & Elmes, G. W. 1998 Higher productivity at the cost of increased host-specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. *Ecol. Entomol.* **23**, 457–464.

Thomas, J. A., Elmes, G. W. & Wardlaw, J. C. 1998 Polymorphic growth in larvae of the butterfly *Maculinea rebeli*,

a social parasite of *Myrmica* ant colonies. *Proc. R. Soc. Lond.* B **265**, 1895–1901.

Thomas, J. A., Elmes, G. W., Wardlaw, J. C. & Woyciechowski, M. 1989 Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. *Oecologia* **79**, 452–457.

Vander Meer, R. K. and Wojcik, D. P. 1982 Chemical mimicry in a myrmecophilous beetle, *Myrmecophodius excavaticollis*. *Science* **218**, 806–808.

Vander Meer, R. K., Jouvenaz, D. P. & Wojcik, D. P. 1989 Chemical mimicry in a parasitoid (Hymenoptera: Eucharitidae) of fire ants (Hymenoptera: Formicidae). *J. Chem. Ecol*. **15**, 2247–2261.

Wardlaw, J. C., Elmes, G. W. & Thomas, J. A. 1998 Techniques for studying *Maculinea* butterflies. I. Rearing *Maculinea* caterpillars with *Myrmica* ants in the laboratory. *J. Insect Conserv.* **2**, 79–84.

Winterbottom, S. 1980 The chemical basis for species and colony recognition in three species of myrmicine ants (Hymenoptera, Formicidae). PhD thesis, University of Southampton, UK.

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