Chemical disguise as particular caste of host ants in the ant inquiline parasite

*Niphanda fusca* (Lepidoptera: Lycaenidae)

Masaru K. Hojo¹,*, Ayako Wada-Katsumata², Toshiharu Akino¹, Susumu Yamaguchi³, Mamiko Ozaki⁴ and Ryohei Yamaoka¹

¹Division of Applied Biology, Graduate School of Science and Technology, Kyoto Institute of Technology, Kyoto 606-8585, Japan
²Division of Applied Life Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
³Wakamiko, Satama-cho, Hokuto city, Yamanashi 408-0112, Japan
⁴Department of Biology, Graduate School of Science, Kobe University, Kobe 657-8501, Japan

The exploitation of parental care is common in avian and insect ‘cuckoos’ and these species engage in a coevolutionary arms race. Caterpillars of the lycaenid butterfly *Niphanda fusca* develop as parasites inside the nests of host ants (*Camponotus japonicus*) where they grow by feeding on the worker trophallaxis. We hypothesized that *N. fusca* caterpillars chemically mimic host larvae, or some particular castes of the host ant, so that the caterpillars are accepted and cared for by the host workers. Behaviourally, it was observed that the host workers enthusiastically tended glass dummies coated with the cuticular chemicals of larvae or males and those of *N. fusca* caterpillars living together. Cuticular chemical analyses revealed that *N. fusca* caterpillars grown in a host ant nest acquired a colony-specific blend of cuticular hydrocarbons (CHCs). Furthermore, the CHC profiles of the *N. fusca* caterpillars were particularly close to those of the males rather than those of the host larvae and the others. We suggest that *N. fusca* caterpillars exploit worker care by matching their cuticular profile to that of the host males, since the males are fed by trophallaxis with workers in their natal nests for approximately ten months.

**Keywords:** lycaenidae; ants; social parasite; cuticular hydrocarbons; chemical mimicry; brood parasite

1. INTRODUCTION

The exploitation of parental or maternal care is common in avian and insect brood parasites (Wilson 1971; Davies et al. 1989). Such ‘cuckoo’ species and their host engage in a coevolutionary arms race (Dawkins & Krebs 1979; Davies et al. 1989), which involves parasitic adaptations for deceiving host, and host defences as counter adaptations (Soler & Møller 1990; Lotem et al. 1995; Foitzik et al. 2001; Nash et al. 2008). This arms race escalates through the exaggeration of obvious traits for deceiving host and defence, and parasites often use sophisticated strategies to be tolerated and fed by the host species. For example, female birds of avian common cuckoo, *Cuculus canorus* lay striking mimetic eggs to avoid rejection by the host (Brooke & Davies 1988). After hatching, their brood uses the visual signal of gape and exaggerated vocal stimulus to induce feeding by its host foster parents (Davies et al. 1998; Kliner et al. 1999).

Insect brood parasitism is well known in the parasites of social insects (Schmid-Hempel 1998; Thomas et al. 2005). They penetrate and inhabit an insect society to exploit any resource that is valued and protected by the host species. Therefore, like avian cuckoos, they use manipulative signals to avoid rejection and to induce care by host species. The signals used so that they are tolerated by host species have been well studied, especially in parasites of ants and wasps. These signals are principally cuticular hydrocarbons (CHCs), which convey important information in insect societies, such as colony, age, caste and task (reviewed in Howard & Blomquist 2005). By wearing either unnoticeable CHCs (chemical insignificance; Lenoir et al. 2001a) or host-like CHCs (chemical mimicry and camouflage; Dettner & Liepert 1994), the parasites gain access to the host society (Akino et al. 1999; Lenoir et al. 2001a; Sledge et al. 2001). Even after successful accession, the parasites and their brood demand care and food supply from the host workers (Wilson 1971). These requirements are communicated by mechanical and chemical signals (Hölldobler & Wilson 1990; Zimmerli & Mori 1993), although there are only few studies on signals used to get care from the host species in insect cuckoos (Cammaerts 1992; Cervo et al. 2004).

Insect cuckoo species are also found, for example, lycaenid butterflies. These include approximately 6000 species, 200 of which are suspected to be ant parasites (Pierce et al. 2002). After the parasitic lycaenid caterpillars are adopted by the restricted host ant species, they either prey on host ant larvae (predatory) or feed primarily on regurgitations of the ant workers (cuckoos) (Thomas & Elmes 1998). The cuckoo *Maculinea rebeli* caterpillars are adopted by their host *Myrmica* ants, owing to their mimicking of ant larva chemicals (Elmes et al. 1991; Fiedler et al. 1996; Akino et al. 1999). Cuckoo caterpillars...
are preferentially reared by Myrmica ant workers rather than their own ant larvae inside the ant nest (Thomas et al. 1998), as is the case for avian cuckoo species (Soler et al. 1995). This preferential care could stem from additional chemical signals, for example, hydrocarbons, and these chemicals could act as ‘markers’ for a high caste of host ant (Schönrogge et al. 2004).

Here, we examined chemical mimicry in the parasitic lycaenid butterfly, *Niphanda fusca*. It is univoltine, and the newly hatched caterpillars initially feed on excretions of the aphids tended by the Japanese monogygous carpenter ant *Camponotus japonicus*. The caterpillars are carried by *C. japonicus* workers into their nest when they reach the third instar. They are fed by ant worker trophallaxis to grow (figure S1 in the electronic supplementary material) and pupate inside the ant nest after hibernation. As the pupae eclose in the next late spring, they spend approximately 10 months in the host ant nest. The caterpillars survive only when associated with *C. japonicus* (Nagayama 1950), so it is crucial for them to avoid host attacks, as well as to receive worker care.

We hypothesized that the adopted *N. fusca* caterpillars have host colony-specific CHC profiles, because CHC profiles serve as the nest-mate recognition pheromone in *C. japonicus* (Ozaki et al. 2005). Furthermore, since cuticular chemical profiles are also used for recognition of typical caste and tasks in the societies, we hypothesized that *N. fusca* caterpillars induce worker care by matching their cuticular chemical profiles to the typical castes that are fed by worker ants. To test the above hypotheses, we conducted behavioural assays and comparative gas chromatograph analyses on the cuticular chemicals.

### 2. MATERIAL AND METHODS

#### (a) Collection and rearing of study animals

We collected approximately 50 eggs of *N. fusca* with aphids (species not identified) feeding on the Japanese pampas grass *Miscanthus sinensis* and a tending *C. japonicus* colony with workers, broods, males and female sexuals (unmated alates), but no mature queen, at Fujinomiya in Shizuoka prefecture, Japan in 2003. We also collected final (fifth or sixth) instar caterpillars with *C. japonicus* workers, broods, males and female sexuals in 2003, 2004 and 2006 at the same site in Shizuoka, Japan. In the laboratory, the newly hatched caterpillars were reared with the aphids, because they initially feed on the honeydew of aphids. When the caterpillars grew to third instars, they were moved to the foraging arena of the *C. japonicus* colony to be adopted by the workers. The third and final instar caterpillars were reared with *C. japonicus* at room temperature in a plastic box (350 × 250 × 60 mm) serving as a foraging arena, in which a two nest-boxes (110 × 75 × 30 mm) had been placed. The nest-box was covered by a glass plate (120 × 90 × 3 mm) to allow for observations and removal of individuals. Mealworms and 10 per cent sucrose aqueous solution were provided as food in the arena box twice a week.

#### (b) Behavioural bioassays

Immediately before the experiments, one of the nest-boxes was emptied, and then 20 workers were randomly chosen and returned to the nest-box. The nest entrance was sealed with cotton and was later removed from the nest entrance. The workers settled down for 10 min by covering the box with a glass plate. Each test specimen was quietly placed in the centre of the foraging box so as not to disturb the ant workers. Ten minutes after placing the specimen, we recorded all events occurring between the workers and the specimen by a video camera (IXY DV M5; Canon, Japan) for 5 min. All records were reviewed by a single observer for comparative analyses.

To test the ant response to living broods, both *C. japonicus* kin larvae and final instar caterpillars of *N. fusca* were picked up from a single *C. japonicus* colony, and individually tested as specimens. The experiment was conducted on two colonies.

In the same manner, glass bead dummies (3.0 mm in diameter) were tested as specimens after being coated with 0.1 individual equivalents of the corresponding extracts from one of *C. japonicus* worker, male and female sexuals, as well as the *N. fusca* final instar caterpillar. Each was immersed in approximately 2 ml of *n*-hexane for 5 min, and the extracted solutions were individually concentrated to 10 μl for applying them to the dummies. Concerning the ant larvae, one larva equivalent of extract was used to treat the glass dummies, because the amounts of larval CHCs were approximately 10 times lower than those of adult CHCs in our preliminary analyses. As a blank control, solvent-treated glass dummies were also tested. The experiment was conducted on four colonies. The observer was not aware of the treated materials.

#### (c) Solvent extraction and SPME

Five second and three final instar caterpillars were immersed in approximately 2 ml of *n*-hexane for 5 min respectively. No second instar caterpillars had ever been exposed to any ants in the laboratory, while all of the final instars had been cared for by *C. japonicus*. The respective extracts were concentrated and chromatographed on approximately 0.2 g of silica gel (230–400 mesh ASTM, 0.040–0.063 mm, Merck Ltd., Germany), and the hydrocarbons were eluted with 2 ml of *n*-hexane.

Separately, a solid-phase microextraction (SPME) technique was applied for comparison of the cuticular chemical profiles in living ant workers, males, female sexuals, larvae and parasite caterpillars. Immobilized individuals were rubbed 100 times with a SPME fibre coated with a 7 μm PDMS film (SUPELCO, USA). Extraction was conducted on 30 individuals of *N. fusca* (15 third instar and 15 final instar) and 70 *C. japonicus* (34 workers, 8 larvae, 15 males and 13 alate queens) from four colonies. The three instar caterpillars had been adopted by host workers for at least one week and their CHC profiles were not statistically separated from final instar (Wilks’ λ = 0.892, χ² = 2.982, *p* = 0.561).

#### (d) Chemical analyses

The SPME fibre was directly inserted into the injection port of a gas chromatograph (GC-14A; Shimadzu, Japan) equipped with a non-polar capillary column DB-1 (30 m × 0.25 mm × 0.25 μm; J & W Scientific, USA) and a flame ionization detector. Injection was made in the splitless mode for 1 min at 300°C, and the detector was at 300°C. The column temperature was maintained at 80°C for 1 min, programmed to 230°C at a rate of 30°C min⁻¹ and from 230°C to 300°C at 5°C min⁻¹. Helium was used as a carrier gas, and the column head pressure was 50 kPa.

GC-MS analyses were achieved using an HP5890-II GC (Hewlett Packard, USA) interfaced to a JEOL SX102A double focusing magnetic sector mass spectrometer (JEOL Ltd., Japan) in EI mode with 70 eV, and operated by an HP.
model 715/64 computer. The GC was fitted with a DB-1HT column (15 m × 0.25 mm × 0.15 μm; J & W Scientific, USA), programmed from 50°C for 5 min, 20°C min⁻¹ to 380°C and held for 5 min. Helium was used as a carrier gas and the column head pressure was 60 kPa. Injections were made directly onto the capillary column through the cool on-column injector at 53°C and injection temperature was programmed to be oven temperature plus 3°C thereafter. The MS interface temperature was 380°C. Although different temperature profile was used for the GC and GC-MS, the two sets of peaks could be matched up easily, and the relative peak areas were the same in the two analyses.

(e) Statistical analysis

All statistical analyses were performed using SPSS v. 15.0 (SPSS, Inc. 2006). In both behavioural experiments, the workers showed attending behaviour (resting on a specimen) towards ant larvae, parasite caterpillars and glass dummies coated with extracts. During the attending, a licking behaviour was occasionally observed, but in reviewing videotapes, licking time was difficult to discriminate from attending. Therefore, licking time was included in attending time. Aggressive behaviours (for example, bites, opening mandibles or bending abdomen) were not observed. The attending time was normally distributed in both behavioural experiments (one sample Kolmogorov–Smirnov test for normal distribution; broods assay, p = 0.856; extracts assay, p = 0.092). To compare the attending time between treatments, a t-test was used in the broods assay. In the extracts assay, attending time were compared using general linear models (GLM) with extracts and colony nested within extracts as fixed factors. Tukey’s HSD test was used for subsequent multiple comparisons.

Multivariate analysis was performed to determine whether the predefined groups could be discriminated on the basis of the 18 shared CHC peaks of the individuals constituting the groups. Peak areas were transformed according to $Z_{c}=\ln[Y_{c}/g(Y_{c})]$ where $Y_{c}$ is the peak area i for individual $j$ and $g(Y_{c})$ is the geometric mean of all peak areas for individual $j$ (Reymert 1988). To investigate whether hydrocarbon profiles of parasitic caterpillars were assigned to the host colony, we first performed principal component analysis (PCA), and only factors with eigenvalues greater than 1 were used in the subsequent discriminant analysis (DA). Four principal components were extracted and were then used as variables in DA by assigning them to the origin of colony. In this analysis, we excluded data on C. japonicus larvae because nest-mate recognition ability of social insects has been principally studied in adult workers and usually non-kin larvae are accepted by workers (Hölldobler & Wilson 1990). We also performed multivariate analysis to determine whether the five castes (N. fusca caterpillars were included as one caste) could be discriminated on the basis of CHC profiles regardless of their colony of origin. In this analysis, three principal components were extracted and then used as variables in DA.

Chemical distances between individual N. fusca caterpillars and each caste of their host ants were calculated as Mahalanobis distances with 18 of the transformed peak areas. Distances were calculated relative to the centroids of each of five combinations (C. japonicus workers, larva, female sexuals, males and N. fusca caterpillars). Kruskal–Wallis analysis following Mann–Whitney U-test coupled with Bonferroni correction was performed to compare the chemical

Figure 1. Attending behaviour of workers towards glass bead dummies coated with a cuticular component of C. japonicus workers (n=14), female sexuals (n=8), males (n=8), larvae (n=14) and N. fusca caterpillars (n=6). Dummies coated with solvent were used as controls (n=14). Significant differences between columns are indicated by different letters (Tukey–Kramer HSD, p<0.05).

3. RESULTS

(a) Behavioural experiments

The attending times of workers for N. fusca caterpillars did not differ significantly from that for C. japonicus larvae (mean ± s.e.: C. japonicus larvae 214.9 ± 25.8, n=11; N. fusca caterpillars 183.0 ± 23.1, n=11; t = –0.917, d.f.=20, p=0.370). The ant attending times to extracted chemicals were significantly affected by the kind of extract (GLM; extract, F5,46 = 14.001, p<0.001), regardless of the ant colonies tested (colony nested in extract, F12,46 = 1.152, p=0.344). The extracts of the ant larvae, males and parasite caterpillars made the ant attending times significantly longer than those for the solvent, whereas the attending time for the workers and the female sexuals extracts were not different from those for the solvent (figure 1; Tukey’s post hoc test, p<0.05).

(b) Chemical analyses

All cuticular chemical compounds were estimated on the basis of the respective retention indices and mass spectra. The cuticular chemicals of C. japonicus workers mainly consisted of hydrocarbons, which gave a total of 18 peaks in the GC profile (figure 2a). Compounds of the 18 peaks were identified as n-alkenes, n-alkanes, and methyl-branched alkane which contained a total of 20–30 carbons in the molecules. This identification agrees with the report by Ozaki et al. (2005). The larvae, male and female sexuals, as well as workers, had these 18 peaks of the same hydrocarbon compounds (figure 2b–d).

The cuticular chemicals of N. fusca caterpillars mainly consisted of hydrocarbons as well (figure 2e,f). However, the hydrocarbon profiles substantially differed before and after the adoption of the caterpillars by C. japonicus. The pre-adoption caterpillars at the second instar had at least 23 hydrocarbons, 11 of which (peaks 1,2,4,6,8–10,13,14,17 and 18) were common to the hydrocarbons of C. japonicus (Ozaki et al. 2005), but the

Proc. R. Soc. B (2009)
other 12 hydrocarbons were much longer carbon-chained ones that consisted of n-alkanes and methyl-branched alkanes with a total of 31 to 37 carbons in the molecules (peak a-i). The caterpillars possessed these long-chained hydrocarbons in much larger amounts than *C. japonicus*-like hydrocarbons. The post-adoption caterpillars at the third and final instars possessed both complete sets of *C. japonicus*-like hydrocarbons and their specific hydrocarbons. However, their *C. japonicus*-like hydrocarbons were much larger in amounts than their specific hydrocarbons, and their profiles closely resembled those of the *C. japonicus* workers, larvae, males and female sexuals.

DA, performed by assigning each colony using 18 shared hydrocarbons, suggested that *C. japonicus* has colony-specific CHC profiles (Wilks’ λ = 0.121, $\chi^2 = 120.552$, $p < 0.001$; 82.3 per cent of all the individuals are correctly classified), and it correctly assigned the *N. fusca* caterpillars to their host colonies (figure 3a; Wilks’ λ = 0.160, $\chi^2 = 159.484$, $p < 0.001$; 83.7% of all the individuals are correctly classified). This demonstrates that the CHC profiles of the *N. fusca* caterpillars were quantitatively similar to the colony they had parasitized.

By contrast, DA performed by assigning *N. fusca* caterpillars and individual caste of *C. japonicus* (figure 3b) indicates that the CHC profiles differed considerably among *C. japonicus* workers, larvae, males, female sexuals and *N. fusca* caterpillars (Wilks’ λ = 0.101, $\chi^2 = 218.171$, $p < 0.001$; 71.0% of all individuals are correctly classified). Although all categories were statistically separated, *C. japonicus* larvae were distinguished from *C. japonicus* adults by discriminant function 2 (figure 3b). By contrast, discriminant function 2 was almost the same among *C. japonicus* adults, but sexuals and workers were separated by discriminant function 1. Discriminant function 2 of *N. fusca* caterpillars was almost the same as that for adult *C. japonicus*. Furthermore, the parasite caterpillars and *C. japonicus* males were clustered together by both discriminant function 1 and 2.

Chemical distances between pairs of *N. fusca* caterpillars and *C. japonicus* (figure 4a) were significantly different among the ant castes (Kruskal–Wallis, $\chi^2 = 115.191$, $p < 0.001$). The chemical distance between *N. fusca* caterpillars and *C. japonicus* workers was significantly larger than that of inter individuals among *N. fusca* caterpillars (Mann–Whitney U-test with Bonferroni correction,
This was also true for the distances between the caterpillars and ant larvae, and between the caterpillars and female sexuals \((p < 0.05)\), but not between \(N. \text{ fusca}\) caterpillars and ant males \((p > 0.108)\). This indicates that CHC profiles were not quantitatively different between \(N. \text{ fusca}\) caterpillars and male ants. The relative proportions of the three chemical classes of CHCs (figure 4b) also differed among groups. \(Camponotus \text{ japonicus}\) larvae had relatively large amounts of \(n\)-alkanes and lower amounts of \(n\)-alkene than workers (Mann–Whitney U-test with Bonferroni correction, \(p < 0.05\)). By contrast, \(C. \text{ japonicus}\) males, female sexuals and \(N. \text{ fusca}\) caterpillars had relatively large amounts of methyl-branched alkanes and lower amounts of \(n\)-alkane and \(n\)-alkene than workers \((p < 0.05)\).

4. DISCUSSION

This study demonstrates that \(C. \text{ japonicus}\) workers care for \(N. \text{ fusca}\) caterpillars as often as their kin larvae, and that they attended to glass dummies treated with the respective cuticular extracts, as well as the ant male cuticular extract (figure 1). These results suggest that the cuticular chemicals of the caterpillars, ant larvae and males induce such attending behaviour of the workers. We also confirmed that the cuticular chemicals of \(N. \text{ fusca}\) caterpillars were drastically changed before and after ant adoption, and the levels of host ant-like CHCs were increased after adoption. The profiles of such increasing hydrocarbons were shared among colony members, and distinguished from those of foreign colonies, as is often pointed out (Sledge et al. 2001; D’Erritore et al. 2002; Elgar & Allan 2004). When the CHC profiles were compared among the parasites and the four castes of host ant, the post-adoption caterpillars resembled the males, whereas the CHC profiles of ant larvae were quite different. These results suggest that the post-adoption caterpillars exploit worker care by matching their cuticular profiles to host ant males rather than ant larvae.
Since social insects show aggressive behaviour towards non-nest-mates based on differences in the colony odours, the parasites of social insects have the ability to mimic host colony odour (Dettner & Liepert 1994; Lenoir et al. 2001a). Because C. japonicus workers show aggressive behaviour against non-nest-mate CHCs but not nest-mate CHCs (Ozaki et al. 2005), N. fusca caterpillars can avoid aggression of the host workers by wearing colony-specific CHC profiles after adoption. However, CHC profile is not fixed, but changes over time and season in each colony (Nielsen et al. 1999; Liu et al. 2001). Therefore, N. fusca caterpillars probably have the ability to keep adjusting their CHCs to the host colony-specific hydrocarbon profiles. In the host ant nest, N. fusca caterpillars were frequently attended by the workers, and they actively contacted the worker ants. Such physical and social contacts with the host workers will enable parasites to acquire and renew the host colony odour (D’Ettorre et al. 2002), because social interactions, such as allogrooming and trophallaxis, play a key part in the formation of colony odour (Lenoir et al. 2001b).

The CHC profiles of N. fusca caterpillars significantly differed from that of the workers (figure 3b); N. fusca caterpillars had large amounts of methyl-branched hydrocarbons on their cuticles compared with the workers (figure 4b). Because C. japonicus sexuals also have a large amount of methyl-branched hydrocarbons, our results are consistent with the hypothesis that increased hydrocarbons are related to the position of N. fusca caterpillars in the colony’s hierarchy and markers for high caste of the host ant (Schönrogge et al. 2004). A previous study reported that the European parasitic lycaenid butterfly M. rebellis biosynthesizes six typical hydrocarbons after intruding into a host Myrmica ant colony (Schönrogge et al. 2004). In N. fusca, since the caste-specific CHCs are increased after adoption, the simplest explanation of our results is that the parasitic caterpillars passively acquire the host-caste-specific CHCs profile (namely, chemical camouflage). In the artificial nest, however, N. fusca caterpillars were mainly attended by the workers and they not actively contact with the host males. Therefore, there is a possibility that N. fusca caterpillars also actively biosynthesize typical compounds, such as methyl-branched hydrocarbons (namely, chemical mimicry) and further study is needed to clarify this.

Cooperative brood care is a key feature of eusociality (Wilson 1971). Therefore, if the larvae of social parasites mimic the host larvae, they are able to receive effective care by the host workers. Our present study does not support this, as the chemical signature of parasite caterpillars was quite different from that of the host larvae. One possible explanation for our result is that parasite caterpillars avoid elimination from workers by mimicking ant males. In social Hymenoptera, since the female broods can develop into an adult queen or worker, there are potential conflicts over caste determination between broods and adult workers (Bourke & Ratnieks 1999). In such conflicts, the workers have opportunities to manipulate developing females by controlling larval nutrition or eliminating larger larvae. In fact, inquiline parasitic ants, which produce no workers but only sexuals, miniaturize their larval size to avoid elimination (Nonacs & Tobin 1992; Aron et al. 1999; but see Aron et al. 2004). In the case of the parasitic lycaenid butterfly, however, their caterpillars develop obviously larger than the host larvae (figure S2 in the electronic supplementary material). Therefore, if the parasite caterpillars chemically mimic the ant larvae, they would be exposed to the risk of elimination as is suggested in Maculinea arion (Thomas & Wardlaw 1990). Males of social Hymenoptera are also at high risk of being eliminated by workers over sex allocation (Trivers & Hare 1976) and worker reproduction (Ratnieks 1988), but male elimination basically occurred during the brood stage in ants of the genus Camponotus (Nonacs & Carlín 1990; Endler et al. 2004). On the other hand, adult males not only contribute nothing to any social performance, but are also highly competitive in begging for food from female colony members (Wilson 1971). Furthermore, in two Camponotus species, males live for approximately ten months in their natal nests and exchange food through trophallaxis with workers (Hölldoabl 1966). This is also the case for C. japonicus. Both males and female sexuals of C. japonicus appear in late summer and rest in the colony until the next spring, often undergoing trophallaxis with workers (Wang & Liu 1998; M. K. Hojo 2005–2006, personal observation). This sexual production cycle largely corresponds to the parasite cycle of N. fusca caterpillars, which are adopted in summer and pupate the next spring. Therefore, during the course of growing inside the ant nest, N. fusca caterpillars receive effective care by mimicking host males. To date, much of the work on chemical strategy of parasitic lycaenid butterfly has been done on Maculinea butterflies. Because genus Niphanda (Niphanditii) and Maculinea (Polyommattitii) belong to different subtribes, the chemical strategy of N. fusca probably evolved independently from that of Maculinea butterflies.

Although some studies showed that larvae of social parasites are able to obtain more food than host larvae (Hölldoabl 1967; Thomas et al. 1998; Beekman et al. 2000; Cervo et al. 2004), it is not well studied to date how they induce host worker care. Our results demonstrate a novel chemical strategy by the parasitic lycaenid butterfly to induce worker care. Future work in comparative analyses of worker behaviour towards ant larvae, males and N. fusca will clarify how such chemical strategies enable parasites to be preferentially cared for by the host workers.

Because the n-hexane crude extracts were used for behavioural assays, our results show that workers were able to discriminate other castes on the basis of cuticular compounds, but the role of hydrocarbons is controversial. The hexane extracts will probably contain not only hydrocarbons but also other polar lipids. However, for many ant species, there is robust evidence that the hydrocarbon fraction alone is sufficient for the recognition of nest-mates (Lahav et al. 1999; Akino et al. 2004), tasks (Greene & Gordon 2003) and fertility (Dietemann et al. 2003). A previous study showed that a worker of C. japonicus could specifically detect non-nest-mate CHC profiles using a particular chemosensillum on their antennae, although there are slight differences in CHC profiles among nest-mates and non-nest-mates (Ozaki et al. 2005). Therefore, the C. japonicus workers might also use the differences in CHC profiles for caste recognition. Further purification of cuticular chemicals for behavioural and electrophysiological studies is needed to
confirm which compounds are responsible for the worker attending behaviour.

The cuticular chemicals of *N. fusca* caterpillars also contained their own specific hydrocarbons, but these compounds will not induce nursing behaviour in workers. These compounds were also detected from pre-adoption second-instar caterpillars of *N. fusca*, which induce neither adoption nor nursing behaviour by the host workers. Therefore, we suggest that principal cuticular components involved in the exploitation of the worker care are common compounds between the parasites and the host ants, most of which are already induced after adoption. Nevertheless, pre-adoption second-instar caterpillars have some *C. japonicus*-like hydrocarbons. In *M. rebeli*, pre-adoption caterpillars have many compounds similar to those found in host *Myrmica* larvae (Akino et al. 1999). These host-like compounds induce carrying behaviour by host workers, but this has not been investigated in *N. fusca*. More studies are needed to clarify whether host-like compounds also induce adoption behaviour in *N. fusca* caterpillars.

We are grateful to David Nash of the University of Copenhagen and two anonymous referees for their valuable comments on this manuscript, and also thank to our collaborators, Takeshi Takeda, Yuji Satoji, Daisuke Imaeda, Hayato Inui and Daisuke Umemoto for their help in the fieldwork.

REFERENCES


