Entomologia
Experimentalis et Applicata

DOI: 10.1111/eea.12366

A scent shield to survive: identification of the repellent compounds secreted by the male offspring of the cuckoo bumblebee *Bombus vestalis*

Patrick Lhomme[1]*, Manfred Ayasse[2], Irena Valterová[3], Thomas Lecocq[1] &
Pierre Rasmont[1]

[1]Laboratory of Zoology, Research institute of Biosciences, University of Mons, Place du Parc 23, 7000 Mons, Belgium, [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám 2, 166 10 Prague, Czech Republic, and [3]Department of Experimental Ecology, University of Ulm, Albert-Einstein-Allee 11, 89069 Ulm, Germany

Accepted: 3 July 2015

Key words: *Psithyrus*, social parasitism, repellent allomone, tetradecyl acetate, feeding assay, Hymenoptera, Apidae, inquiline, host recognition, GC-EAD, chemical camouflage

Abstract

In social insects, the high cost of parental investment has promoted the evolution of traits that reduce or avoid care for unrelated offspring. They have evolved a sophisticated recognition system which enables them to reject foreign individuals, and therefore prevent their nest's exploitation by parasites and predators. Nonetheless, many social parasites are able to overcome social insect recognition and parasitize their parental care behaviour by laying their eggs in host nests. Although several studies have already been conducted on the strategies used by social parasites to successfully invade host nests, the strategy used by its offspring to evade host rejection is still poorly understood. In bumblebees, previous investigations indicated that male offspring of *Bombus vestalis* (Geoffroy) (Hymenoptera: Apidae), the specific obligate social parasite of *Bombus terrestris* (L.), may use allomones to evade host worker attacks. Following these studies, we performed electrophysiological studies and behavioural experiments to identify the potential repellents. Six compounds elicited electroantennographic responses in host worker antennae. The corresponding mixture of synthetic volatiles as well as pure tetradecyl acetate, and to a lesser extent pure (*Z*)-11-hexadecenyl acetate, showed a repellent effect on starved host workers in a feeding bioassay. We hypothesize that tetradecyl acetate has a key function in the repellent effect of the secretions of *B. vestalis* male offspring towards *B. terrestris* host workers.

Introduction

In social insects, brood care is a costly behavioural trait. It ranges from nest construction and maintenance to feeding and defence of the offspring. These energy-consuming activities affect the relative reproductive success of all colony members because of the greater predation risk and the large amounts of time they allocate (Smiseth et al., 2012).

Because of the high fitness costs of brood care, social insects have evolved traits that reduce or avoid care for alien offspring to save energy or other resources for their own colony relatives (Roldán & Soler, 2011). Most social insects have developed a sophisticated recognition system which enables them to reject foreign individuals and therefore prevent their nest's exploitation by intruders. Nestmate recognition also ensures that altruism is only directed towards colony members. The primary recognition cues consist of shared cuticular hydrocarbons learnt by all the members of a nest (van Zweden & D'Ettorre, 2010). These hydrocarbons have genetic bases and are also acquired from the environment (van Zweden & D'Ettorre, 2010). The ability to discriminate against non-colony members is widespread among social insects. Nonetheless, many species are able to overcome social insect recognition and to parasitize their parental care behaviour by laying their eggs in host nests. These species are called social parasites or inquilines and are widespread among

*Correspondence and current address: P. Lhomme, Swedish University of Agricultural Sciences, Division of Chemical Ecology, Department of Plant Protection Biology, Alnarp, Sweden.
E-mail: patrick.lhomme@slu.se

hymenopterans, especially in social bees, ants and wasps (Lenoir et al., 2001).

Among the social insects, several studies have investigated the chemical strategies evolved by social parasites to confound the host recognition system. Many species use chemical camouflage or mimicry of host recognition signals (Martin et al., 2010; Kreuter et al., 2012), whereas other species use a strategy of 'chemical insignificance', lacking the cuticular hydrocarbons that are recognized by their host (Lenoir et al., 2001). Some species also produce chemical weapons to defend themselves during invasion (D'Ettorre et al., 2000; Zimma et al., 2003).

Until now, the traits that have evolved to prevent parasite offspring discrimination are largely unknown. Very few studies have investigated the strategies used by inquiline offspring to be tolerated by the host workers. Social parasitic wasps of the genus *Polistes* seem to present, at the larval stage, a specific chemical blend which is not recognized by the host colony members (Cervo et al., 2008). After emergence, the chemical signature of the adult offspring also possesses parasite-specific hydrocarbons (Bagnères et al., 1996). Nevertheless, they are still accepted because parasite-female-specific compounds are used to impregnate the host nest and to increase complexity of colony odour and thereby affect the ability of host workers to discriminate non-nestmates (Lorenzi et al., 1999). Lhomme et al. (2012) also showed that bumblebee workers are significantly more permissive towards non-nestmates in parasitized colonies. Another study demonstrated that the wasp *Ichneumon eumerus* Wesmael, a parasitoid of the myrmecophilous caterpillar *Phengaris rebeli* (Hirschke), is not able to mimic host ant recognition cues after hatching. Young parasitoid adults are then recognized but manage to survive by spreading confusing allomones against host workers (Thomas & Elmes, 1993). These examples show that if parasitic offspring cannot deceive the host, an alternative strategy to stay in the nest is to avoid host hostility by producing repellents.

In social parasitic bumblebees of the genus *Bombus*, subgenus *Psithyrus* (Hymenoptera: Apidae), the challenge of avoiding host recognition is especially important for freshly emerged, young parasitic males because of their early production of species-specific cephalic secretions (Lhomme et al., 2012). These secretions are mostly produced in cephalic labial glands that produce the male sexual marking pheromones involved in the pre-mating behaviour (Lecocq et al., 2013a,b).

Lhomme et al. (2012) have recently demonstrated that young parasitic males of *B. vestalis* have evolved an active strategy to survive and to escape the host nest. The parasitic males produce a repellent odour in their cephalic secretions that prevent *B. terrestris* worker's attacks. By using chemical artifices to keep workers at a distance, parasitic males seem to avoid encountering the host recognition system and thereby evade rejection. Many observations have clearly shown that *Bombus terrestris* (L.) is the specific host of *Bombus vestalis* (Geoffroy) (van Honk et al., 1981; Fisher, 1988). Martin et al. (2010) recently demonstrated that *B. vestalis* females mimic *B. terrestris* recognition signals by actively producing the same alkene isomer profile. These results confirm the host specialization of *B. vestalis*.

Despite this specialization, the initial interaction between *B. terrestris* and *B. vestalis* is very aggressive and often causes the death of many host workers (van Honk et al., 1981). The attacks of the workers can last several days before the full acceptance of the intruder. Once in the nest, the inquiline usually avoids interaction with its hosts and hides inside the brood. Nevertheless, it remains relatively aggressive and eliminates all individuals attempting to attack it. The inquiline female is also able to selectively kill older host workers that directly compete with her for reproduction (Sramkova & Ayasse, 2009). After a certain time in the nest, the intruder acquires host colony odour by chemical camouflage (Ayasse & Jarau, 2014). At this time, the parasitic female kills or rejects the host queen out of the nest and usurps its position (van Honk et al., 1981; Fisher, 1988). The female *B. vestalis* then destroy the queen's eggs and exploit the emptied wax cells for her own brood. *Bombus vestalis* is also able of inhibiting worker ovarian development to monopolize reproduction (Vergara et al., 2003). After a few weeks the young social parasites emerge. The host reproductive success is completely eliminated (Lhomme et al., 2013).

The aim of this study is to investigate which semiochemicals are involved in the defensive strategy of the parasitic males of *B. vestalis*, the social parasite of *B. terrestris*. We used gas chromatography coupled with electroantennographic detection (GC-EAD) and behavioural experiments to identify the semiochemicals acting as allomones against host workers. Using GC-EAD, we first identified which compounds produced in *B. vestalis* male cephalic secretions are detected by *B. terrestris* workers. Using a feeding bioassay, we then determined which of these electrophysiologically active volatiles have a repellent effect on host workers.

Materials and methods

Rearing bumblebees

Bombus vestalis vestalis females were collected in spring in the surroundings of Mons, Belgium (50°28′21″N, 3°56′46″E, 30 m a.s.l.). They were introduced into young *B. terrestris terrestris* colonies containing about 15 workers immediately after removing the host queen. *Bombus terrestris* colonies were produced by Biobest (Westerlo, Belgium) from wild queens collected in the surroundings of Westerlo (51°05′23″N, 4°54′51″E, 18 m a.s.l.). We used the rearing method of Lhomme et al. (2013). Ten colonies of *B. terrestris* were successfully parasitized. The bumblebees were reared in plastic boxes (32 × 23 × 12 cm) in a dark room at 26–28 °C and 65% r.h., and supplied with ad libitum sugar syrup (Biogluc®, Biobest) and fresh willow pollen (*Salix* spec.). Five males of *B. vestalis* from various laboratory colonies were collected to sample cephalic secretions. The workers used during these experiments were of different colony origin than the parasitic males used for the cephalic secretion extraction.

Bombus vestalis male's cephalic secretions

The cephalic secretions of *B. vestalis* were extracted from 3-day-old males (intranidal age). Five specimens were first killed by freezing at −20 °C. They were then decapitated and their heads were placed individually in glass vials with 400 μl hexane (>99%; Roth, Karlsruhe, Germany) for compound extraction (e.g., Lecocq et al., 2011, 2015a). The samples were kept for 24 h at room temperature (20 °C) to complete the extraction and then stored until use at −40 °C. The components of the cephalic secretions of *B. vestalis* were identified by Lhomme et al. (2012). Double bound positions were previously determined by Lecocq et al. (2015b).

Origin and purities of the standards

Citral (95%), farnesol (95%), tetradecyl acetate (99%), (*Z*)-9-hexadecenyl acetate (97%), and (*Z*)-11-hexadecenol (99%) were purchased from Sigma-Aldrich (St Louis, MO, USA). (*Z*)-13-octadecenal (94%) and (*Z*)-11-hexadecenyl acetate (95%) were purchased from Bedoukian (Danbury, CT, USA). (*Z*)-11-octadecenal (95%) was synthesized by Wittko Francke (University of Hamburg, Germany).

Gas chromatography coupled with electroantennography (GC-EAD)

Electrophysiological analyses were performed to identify the components in the cephalic secretions of males of *B. vestalis* that can be perceived by *B. terrestris* worker antennae. The GC-EAD system comprised a HP6890 Hewlett-Packard gas chromatograph (Agilent Technologies, Waldbronn, Germany) with a flame-ionization detector (FID) and an electroantennographic detector (EAD) setup (Syntech, Hilversum, The Netherlands). The antenna was cut at the base of the flagellum and at the tip of the terminal antennal segment with a razor blade. The antenna was then mounted between two glass capillaries filled with insect Ringer solution (5 g NaCl, 0.42 g KCl, and 0.19 g $CaCl_2$ in 1 l demineralized water). Hydrogen was used as the carrier gas (2 ml per min, constant flow) and a GC effluent splitter (split ratio 1:1, make-up nitrogen supply 25 ml per min) was used to enable simultaneous recording of the FID and EAD responses. The effluent was added to a humidified and purified air stream (450 ml per min) and directed to the antennal preparation via a glass tube. Male cephalic secretion samples were analysed on a polar DB-Wax GC capillary column (30 m × 0.25 mm × 0.25 μm). Samples (1 μl) were injected in splitless mode at an oven temperature of 50 °C, followed by opening of the split valve after 2 min and programming to 240 °C at 10 °C per min and holding the temperature for 23 min. In addition to the extracts, we also recorded antennal responses to synthetic standards of the identified EAD-active compounds.

We performed a total of 41 successful runs with cephalic secretion extracts of the five *B. vestalis* males used in this study, and three runs with a blend of synthetic references of five identified EAD-active substances [tetradecyl acetate, (*Z*)-11-hexadecenol, (*Z*)-11-octadecenal, (*Z*)-9-hexadecenyl acetate]. Compounds were termed 'electrophysiologically active' when they showed a reproducible EAD response in five or more runs.

Repellent tests

The repellent effect of the cephalic secretions of *B. vestalis* was tested by offering a droplet of sugar water (1:1), surrounded with solvent (hexane) or a solution of chemical substances, to workers of *B. terrestris* that had been starved for 7 h (set-up from Zimma et al., 2003). The workers used were from four colonies. The tests were performed in round plastic dishes (8.5 cm diameter). The sugar water was applied in the centre on a glass cover slip (2 × 2 cm) and surrounded with 20 μl of a solution of (1) parasite cephalic extract, (2) a mixture of all GC-EAD-active compounds (Figure 1), (3) separate GC-EAD-active compounds, (4) citral and (5) farnesol (controls), or (6) hexane as a solvent control. The synthetic blend of the electrophysiologically active compounds was prepared according to the relative concentrations of each of the compounds in the natural head extracts, giving a concentration of one animal-equivalent per 20 μl. Each electrophysiologically active compound was tested according to its relative concentration in the natural sample (Table 1). Solutions of citral (bumblebee repellent; Zimma et al., 2003) and of farnesol (bumblebee attractive flower odour; Zimma et al., 2003) were used as controls at a concentration of 7.5 μg per 20 μl solvent. All tests were carried out under red light at 26 °C. For each test group (n = 11), the experiments were repeated 10×, using 10 workers at a time. We used a total number of 110 workers.

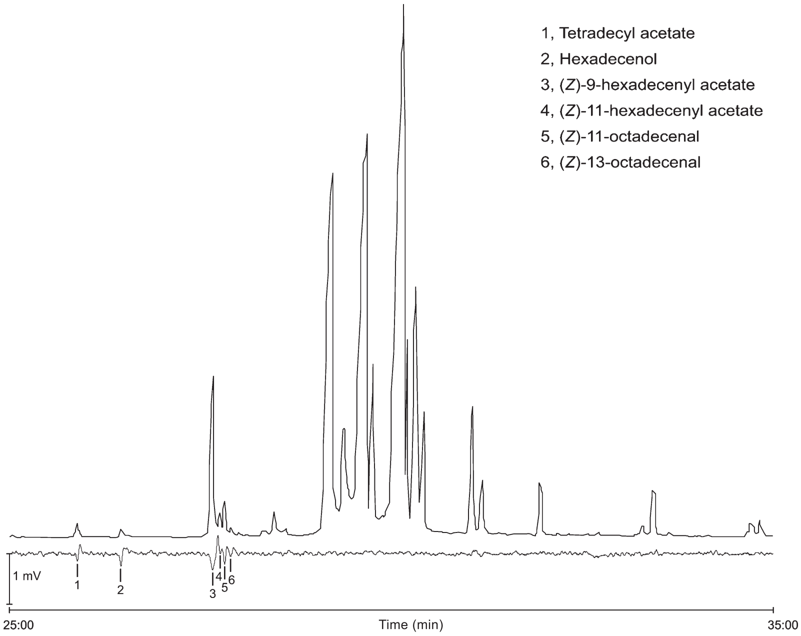

Figure 1 Coupled gas chromatography-electroantennography (GC-EAD) recording of the cephalic extract of a *Bombus vestalis* male using an antenna of a worker of *Bombus terrestris*. Upper trace: FID; lower trace: EAD.

Table 1 Mean (± SD) relative percentage of the compounds in cephalic secretions of *Bombus vestalis* males (n = 5) triggering electrophysiological activity in *Bombus terrestris* worker antennae and percentage of electroantennographic (EAG) responses of the total number of successful runs (n = 41)

Compound	Relative %	% EAG responses
Tetradecyl acetate	14.9 ± 0.6	68.3
Hexadecenol	6.6 ± 1.6	46.3
(*Z*)-9-hexadecenyl acetate	44.7 ± 12.5	78
(*Z*)-11-hexadecenyl acetate	8.5 ± 1.5	58.5
(*Z*)-11-octadecenal	9.7 ± 1.3	65.9
(*Z*)-13-octadecenal	15.4 ± 6.6	63.4

Prior to testing, the bumblebees were acclimated for 1 min in a small glass tube in the arena next to the cover slip. After removal of the glass tube, we recorded the time it took the bees to reach the droplet of sugar water and registered the retreat behaviour (stepping backward) when approaching the area impregnated with test solution. If the bumblebee worker did not feed until the end of the experiment (180 s), it was put in a new plastic dish with an untreated droplet of sugar water to test its starvation status. The data were used for statistical analyses only if the bumblebee fed on the sugar water during this control test. The time limit until feeding was set at 180 s. In nine cases (four with citral, two with tetradecyl acetate, two with the cephalic extracts, and one with the active synthetic blend), the workers never came close to the droplet and stayed away from the impregnated area during the whole experiment. These bees drank the untreated sugar water in the control test and were thus included in the analyses (n = 110 workers). To avoid biased results, the number of retreats was used in statistical analysis only if the worker fed on the droplet during the experiment (n = 101 workers).

Statistical analysis

The statistical analyses were performed using R software (R Development Core Team, Vienna, Austria). Time until feeding was compared among test groups using a generalized linear mixed model (GLMM) based on a gamma error distribution and the number of rejections was compared using a GLMM based on a Poisson error distribution (R function glmer from the R package lme4; Bates et al., 2014). In both statistical models, colony identity was included as a random factor. The mixed models were performed using an ANOVA with χ^2 test as test criterion. Post hoc pair-wise comparisons were made using Tukey's honestly significant differences (HSD) test (R function glht from the R package multcomp; Hothorn et al., 2008).

Results

Electrophysiologically active compounds in the cephalic extracts

In GC-EAD experiments with cephalic extracts of *B. vestalis* males, six compounds elicited electroantennographic responses in *B. terrestris* worker antennae. Chemical compounds belonging to three classes were identified:

three esters [tetradecyl acetate, (*Z*)-9-hexadecenyl acetate, and (*Z*)-11-hexadecenyl acetate], two aldehydes [(*Z*)-11-octadecenal and (*Z*)-13-octadecenal], and one alcohol of unknown stereochemistry (hexadecenol). The reproducibility of all reactions was proven in repeated GC-EAD runs (n = 41; Table 1). Among the electrophysiologically active components, the major one was (*Z*)-9-hexadecenyl acetate, followed by (*Z*)-13-octadecenal and tetradecyl acetate (Table 1). Tetradecyl acetate and (*Z*)-9-hexadecenyl acetate also exhibited the highest percentage of EAG responses, with 68.3 and 78%, respectively (Table 1).

Repellency of synthetic compounds

Time until workers fed from the different tested compounds showed significant differences between treatments (GLMM: χ^2 = 23.35, d.f. = 10, P<0.001; Figure 2). The mean duration of time until workers fed from the droplet of sugar water surrounded with citral was the highest of all test groups. In this test group, four of the 10 tested workers did not cross the odour-impregnated area at all. The behaviour of *B. terrestris* workers in the four test groups of citral, the cephalic extract, the active synthetic blend, and tetradecyl acetate did not differ significantly. Workers of *B. terrestris* took less time to feed on the sugar droplet when exposed to (*Z*)-9-hexadecenyl acetate compared to tetradecyl acetate (Tukey's HSD test: P = 0.02). The mean duration of time did not differ significantly among the six other test groups (Figure 2).

The mean number of rejections (stepping backward) elicited in the workers differed among the 11 test groups (GLMM: χ^2 = 236.04 d.f. = 10, P<0.001; Figure 3). The mean number of rejections was the highest when citral was offered. Worker reactions were not significantly different towards citral than towards the synthetic blend, the cephalic extracts and tetradecyl acetate. These four test groups elicited a significantly higher number of rejections compared to the other test groups (Figure 3), except for (*Z*)-9-hexadecenyl acetate. The mean number of rejections when (*Z*)-9-hexadecenyl acetate was offered was only lower compared to citral (Tukey's HSD test: P = 0.01).

Discussion

Successful survival of the social parasite offspring of *B. vestalis* within the host nest is essential for maximizing its fitness. It is therefore expected that mechanisms evolved for optimizing this process. The results of the present study indicate that the use of compounds present in the cephalic gland secretions of males may provide a defensive line against the host worker attacks, confirming the results of Lhomme et al. (2012). This strategy of allomone production is quite similar to the one used by nest-invading females of social parasitic bumblebees (Zimma et al., 2003). Contrary to cuckoo bumblebee females, parasite males totally lack the morphological weaponry that the females exhibit. Thus, relying on chemical weaponry is an effective strategy for the male parasite offspring.

This study shows that tetradecyl acetate could be the main repellent compound in the defensive secretions of *B. vestalis* males. This compound alone induces the same repellent effect on host workers as the complete blend

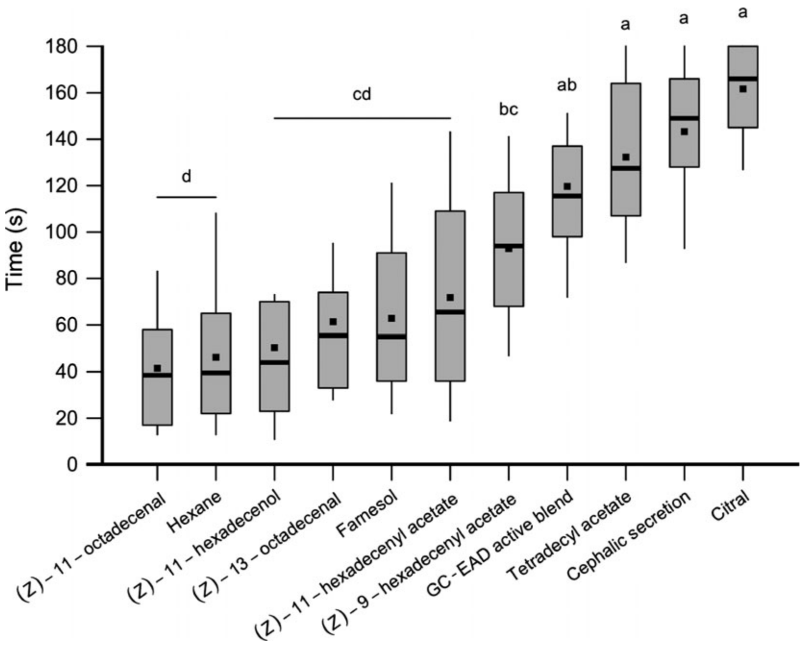

Figure 2 Time (s) until starved *Bombus terrestris* workers began feeding from a sugar droplet surrounded by various chemicals. Box plots show the mean (black square), median (black line), and 25 and 75 percentiles. Whiskers indicate the data ranges. Different letters capping boxes indicate significant differences among treatments (Tukey's HSD test: P<0.05).

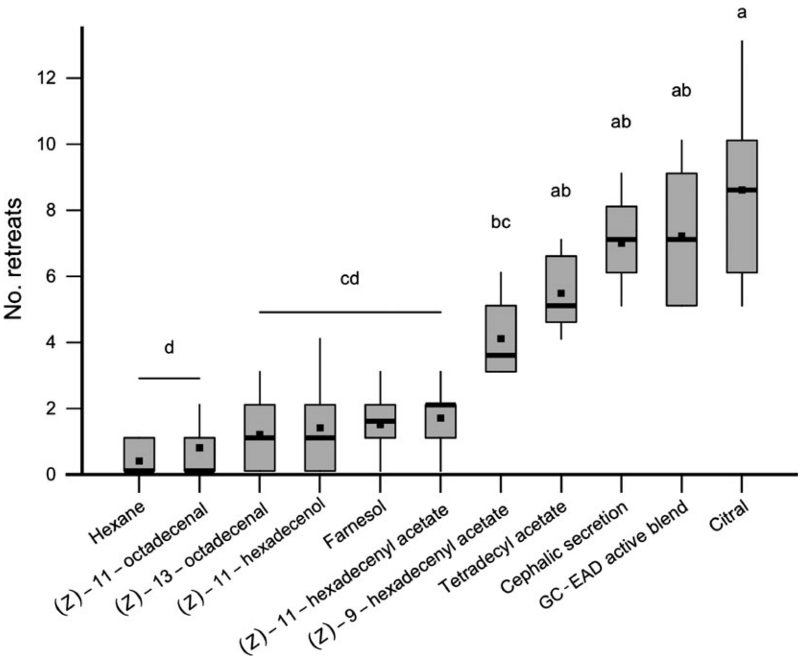

Figure 3 Number of retreats of starved *Bombus terrestris* workers when offered a sugar droplet surrounded by various chemicals during 180 s. Box plots show the mean (black square), median (black line), and 25 and 75 percentiles. Whiskers indicate the data ranges. Different letters capping boxes indicate significant differences among treatments (Tukey's HSD test: P<0.05).

of active compounds and as the cephalic extracts. This compound is quite common in the defensive secretions of hymenopterans. In ants, tetradecyl acetate, together with other acetates, releases alarm-defensive behaviour and serves as a disarming agent during slave raids on other ant colonies in several genera of ants (Wilson & Regnier, 1971). Similar compounds, e.g., decyl acetate, are also found in the enlarged Dufour's gland of slave-making *Polyergus* ants where they act as repellents during raids (D'Ettorre et al., 2000). These acetates are sprayed in large quantities and have a very low evaporation rate. Their active areas are thus larger and they are more persistent (Wilson & Regnier, 1971). Tetradecyl acetate is also found in the cephalic secretions of several fire bees (genera *Trigona* and *Oxytrigona*). These cephalic secretions cause avoidance behaviour in honeybees and are used for plundering their nests (Rinderer et al., 1988). Finally, this compound, together with octadecenal and hexadecenyl acetate, are also present in the Dufour's gland repellent secretions of nest-invading females of the social parasitic bumblebee *Bombus norvegicus* (Sparre-Schneider) and are perceived by its host *Bombus hypnorum* (L.) (Zimma et al., 2003). These compounds do also play a role in the repellent effect of the secretions of *B. norvegicus* females (Zimma et al., 2003).

Although (*Z*)-11-hexadecenyl acetate and (*Z*)-13-hexadecenyl acetate seem to induce a slight repellent effect on host workers, it is not the case in (*Z*)-11-octadecenal, (*Z*)-13-octadecenal, and hexadecenol. However, these compounds did elicit electroantennographic responses from *B. terrestris* in GC-EAD experiments, suggesting they may have another communicative function that was not tested in our experiments. It can as well be that they are not behaviourally active when present individually, but contribute to the overall repellent effect of the blend. Although not significant, the GC-EAD-active blend induces a slightly higher number of retreats in the host workers compared to tetradecyl acetate alone. This indicates that the other compounds potentially play a secondary role in the repellent effect of the blend. Further studies are needed to evaluate the specific roles of the other compounds by testing their effect in various binary mixtures to identify whether there is any synergistic effect of the compounds when blended. However, considering that tetradecyl acetate alone induces the same behavioural reaction on host workers than the active blend or the cephalic extracts, we can expect that the other compounds play no or a minor role in the repellent effect of these secretions. These compounds might be part of the sex pheromone blend produced by males to attract conspecific females.

The evolution of this chemical defensive strategy could be explained by the type of relation that both host and social parasite share. *Bombus vestalis* females are queen-intolerant inquilines meaning that they kill the resident queen during colony take-over. In queenless colonies, the only way for the host to gain some fitness is through host worker reproduction. Therefore, hosts parasitized by queen-intolerant inquilines should be selected to become more aggressive and to refine their alien brood discrimination abilities. This is in accordance with previous studies showing that *B. terrestris* workers exhibit highly aggressive behaviour towards *B. vestalis* females and are also able to identify and destroy their brood (van Honk et al., 1981; Fisher, 1988). In ants, behavioural experiments have also demonstrated that higher aggression directed towards the social parasites results in a higher proportion of the brood being rescued by invaded host colonies (Foitzik et al., 2001). On the contrary, some other species of cuckoo bumblebees are queen-tolerant inquilines and thus allow host queen reproduction. It is the case of the cuckoo bumblebee *Bombus sylvestris* (Lepeletier) hosted by *Bombus pratorum* (L.) (Lhomme et al., 2013). In colonies where both host queen and parasitic female reproduce, reciprocal selective pressures should be weaker because host reproduction success is not eliminated. In fact, *B. pratorum* host workers do not exhibit aggressive behaviour towards *B. sylvestris* females and the interaction is quite peaceful (Lhomme et al., 2013). Interestingly there is no acetate in the cephalic secretions of *B. sylvestris* males (Urbanová et al., 2004). The production of a repellent odour, mediated by a blend of acetates, could thus be a counter adaptation evolved by *B. vestalis* male offspring to escape from host aggressiveness. Further experiments are needed to confirm this hypothesis by testing the effect of *B. sylvestris* cephalic extracts on *B. pratorum* host worker behaviour.

More than half of the esters (mostly acetates) known to be involved in insect chemical defences are produced by hymenopterans, mainly bees and ants (Blum, 1978). They are usually produced in low amounts and considered to play a secondary role, as wetting agents, to facilitate the penetration of the toxic compounds produced by the venom glands (Blum, 1978). We can argue that these social hymenopterans may have evolved an ability to detect and avoid these particular compounds generally associated with aggressive behaviour. Social parasites may then have evolved a deceptive strategy enabling them to hijack this avoidance behaviour to facilitate host nest infiltration or exfiltration. Although speculative, this could explain why the use of these esters as chemical weapons, a very widespread trait among social parasite hymenoterans, may have evolved multiple times and potentially helped transition to parasitic lifestyle.

In conclusion, we confirm here that the cephalic secretions of *B. vestalis* males have a repellent effect on *B. terrestris* workers. The compound that has the major repellent effect is tetradecyl acetate, although we cannot exclude a potential role of the other compounds on the overall effect. However, as tetradecyl acetate alone has the same effect as the total blend of active compounds, we suggest that this compound has a key function in the repellent effect of the cephalic secretion of *B. vestalis* males.

Acknowledgments

The authors acknowledge the Fonds pour la Recherche dans l'Industrie et l'Agriculture (FRIA), the German Science Foundation (AY 12/2-1, AY 12/2-2), and the Institute of Organic Chemistry and biochemistry of the Academy of Sciences of the Czech Republic (research program RVO: 61388963) for financial support. We are also grateful to Biobest for providing the colonies of *Bombus terrestris*, Klára Urbanová for her help with chemical compound identification, and Wittko Francke for the synthesis of the compounds.

References

Ayasse M & Jarau S (2014) Chemical ecology of bumble bees. Annual Review of Entomology 59: 299–319.

Bagnères A-G, Lorenzi MC, Dusticier G, Turillazzi S & Clément JL (1996) Chemical usurpation of a nest by paper wasp parasites. Science 272: 889–892.

Bates D, Maechler M, Bolker B & Walker S (2014) lme4: Linear Mixed-Effects Models Using Eigen and S4. R Package Version 1.1-7. Available at: http://CRAN.R-project.org/package=lme4 (accessed 3 May 2015).

Blum MS (1978) Biochemical defenses of insects. Biochemistry of Insects (ed. by M Rockstein), pp. 465–513. Academic Press, London, UK.

Cervo R, Dani FR, Cotoneschi C, Scala C, Lotti I et al. (2008) Why are larvae of the social parasite wasp *Polistes sulcifer* not removed from the host nest? Behavioral Ecology and Sociobiology 62: 1319–1331.

D'Ettorre P, Errard C, Ibarra F, Francke W & Hefetz A (2000) Sneak in or repel your enemy: Dufour's gland repellent as a strategy for successful usurpation in the slave-maker *Polyergus rufescens*. Chemoecology 10: 135–142.

Fisher RM (1988) Observations on the behaviours of three European cuckoo bumble bee species (*Psithyrus*). Insectes Sociaux 35: 341–354.

Foitzik S, Deheer CJ, Hunjan DN & Herbers JM (2001) Coevolution in host–parasite systems: behavioural strategies of slave-making ants and their hosts. Proceedings of the Royal Society of London B 268: 1139–1146.

van Honk CGJ, Röseler PF, Velthuis H & Malotaux M (1981) The conquest of a *Bombus terrestris* colony by a *Psithyrus vestalis* female. Apidologie 12: 57–68.

Hothorn T, Bretz F & Westfall P (2008) Simultaneous inference in general parametric models. Biometrical Journal 50: 346–363.

Kreuter K, Bunk E, Lückemeyer A, Twele R, Francke W & Ayasse M (2012) How the social parasitic bumblebee *Bombus bohemicus* sneaks into power of reproduction. Behavioral Ecology and Sociobiology 66: 475–486.

Lecocq T, Lhomme P, Michez D, Dellicour S, Valterová I & Rasmont P (2011) Molecular and chemical characters to evaluate species status of two cuckoo bumblebees: *Bombus barbutellus* and *Bombus maxillosus* (Hymenoptera, Apidae, Bombini). Systematic Entomology 36: 453–469.

Lecocq T, Dellicour S, Michez D, Lhomme P, Vanderplanck M et al. (2013a) Scent of a break-up: phylogeography and reproductive trait divergences in the red-tailed bumblebee (*Bombus lapidarius*). BMC Evolutionary Biology 13: 263.

Lecocq T, Vereecken NJ, Michez D, Dellicour S, Lhomme P et al. (2013b) Patterns of genetic and reproductive traits differentiation in mainland vs. corsican populations of bumblebees. PLoS ONE 8: e65642.

Lecocq T, Coppée A, Mathy T, Lhomme P, Cammaerts M-C et al. (2015a) Subspecific differentiation in male reproductive traits and virgin queen preferences, in *Bombus terrestris*. Apidologie 46: 595–605.

Lecocq T, Brasero N, De Meulemeester T, Michez D, Dellicour S et al. (2015b) An integrative taxonomic approach to assess the status of Corsican bumblebees: implications for conservation. Animal Conservation 18: 236–248.

Lenoir A, D'Ettorre P, Errard C & Hefetz A (2001) Chemical ecology and social parasitism in ants. Annual Review of Entomology 46: 573–599.

Lhomme P, Ayasse M, Valterova I & Rasmont P (2012) Born in an alien nest: how do social parasite male offspring escape from host aggression? PLoS ONE 7: e43053.

Lhomme P, Sramkova A, Kreuter K, Lecocq T, Rasmont P & Ayasse M (2013) A method for year-round rearing of cuckoo bumblebees (subgenus *Psithyrus*). Annales de la Société Entomologique de France 49: 117–125.

Lorenzi MC, Cometto I & Marchisio G (1999) Species and colony components in the recognition odor of young social wasps: their expression and learning (*Polistes biglumis* and *P. atrimandibularis*; Hymenoptera: Vespidae). Journal of Insect Behavior 12: 147–158.

Martin SJ, Carruthers JM, Williams PH & Drijfhout FP (2010) Host specific social parasites (*Psithyrus*) reveal evolution of chemical recognition system in bumblebees. Journal of Chemical Ecology 36: 855–863.

Rinderer TE, Blum MS, Fles HM, Bian Z, Jones TH et al. (1988) Nest plundering allomones of the fire bee *Trigona* (*Oxytrigona*) *mellicolor*. Journal of Chemical Ecology 14: 495–501.

Roldán M & Soler M (2011) Parental-care parasitism: how do unrelated offspring attain acceptance by foster parents? Behavioral Ecology 22: 679–691.

Smiseth PT, Kölliker M & Royle NJ (2012) What is Parental care? The Evolution of Parental Care (ed. by NJ Royle, PT Smiseth & M Kölliker), pp. 1–14. Oxford University Press, Oxford, UK.

Sramkova A & Ayasse M (2009) Chemical ecology involved in invasion success of the cuckoo bumblebee *Bombus vestalis* and workers of its host *Bombus terrestris*. Chemoecology 19: 55–62.

Thomas JA & Elmes GW (1993) Specialized searching and the hostile use of allomones by a parasitoid whose host, the butterfly *Maculinea rebeli*, inhabits ant nests. Animal Behavior 45: 593–602.

Urbanová K, Halík J, Hovorka O, Kindl J & Valterová I (2004) Marking pheromones of the cuckoo bumblebee males (Hymenoptera, Apoidea, *Bombus* Latreille): compositions of labial gland secretions of six species occurring in the Czech Republic. Biochemical Systematics and Ecology 32: 1025–1045.

Vergara CH, Schroder S, Almanza MT & Wittmann D (2003) Suppression of ovarian development of *Bombus terrestris* workers by *B. terrestris* queens, *Psithyrus vestalis* and *Psithyrus bohemicus* females. Apidologie 34: 563–568.

Wilson EO & Regnier FE (1971) The evolution of the alarm-defense system in the formicine ants. American Naturalist 105: 279–289.

Zimma BO, Ayasse M, Tengö J, Ibarra F, Schultz C & Francke W (2003) Do social parasitic bumblebees use chemical weapons? Journal of Comparative Physiology A 189: 769–775.

van Zweden JS & D'Ettorre P (2010) Nestmate recognition in social insects and the role of hydrocarbons. Insect Hydrocarbons (ed. by GJ Blomquist & AG Bagnères), pp. 222–243. Cambridge University Press, Cambridge, UK.