J Chem Ecol (2010) 36:855–863
DOI 10.1007/s10886-010-9805-3

Host Specific Social Parasites (*Psithyrus*) Indicate Chemical Recognition System in Bumblebees

Stephen J. Martin · Jonathan M. Carruthers · Paul H. Williams · Falko P. Drijfhout

Received: 22 March 2010 / Revised: 13 May 2010 / Accepted: 19 May 2010 / Published online: 28 May 2010

Abstract Semiochemicals influence many aspects of insect behavior, including interactions between parasites and their hosts. We studied the chemical recognition system of bumblebees (*Bombus*) by examining the cuticular hydrocarbon cues of 14 species, including five species of social parasites, known as cuckoo bees (subgenus *Psithyrus*). We found that bumblebees possess species-specific alkene positional isomer profiles that are stable over large geographical regions and are mimicked by three host-specific cuckoo parasites. In three host-cuckoo associations where mimicry is poor, possibly due to recent host shifts, these cuckoos produce dodecyl acetate a known chemical repellent that allows the cuckoos to invade their host colonies. Our findings indicate cuckoos use two chemical mechanisms, mimicry and repellents, to invade their hosts, and this may reflect different stages of an ongoing dynamic arms race.

Key Words Recognition · Cuticular hydrocarbons · Isomers · *Bombus* · Cuckoos

Electronic supplementary material The online version of this article (doi:10.1007/s10886-010-9805-3) contains supplementary material, which is available to authorized users.

S. J. Martin (✉) · J. M. Carruthers
Department of Animal and Plant Sciences,
University of Sheffield,
Sheffield S10 2TN, UK
e-mail: s.j.martin@sheffield.ac.uk

P. H. Williams
Department of Entomology, The Natural History Museum,
Cromwell Road,
London SW7 5BD, UK

F. P. Drijfhout
Chemical Ecology Group, School of Physical and Geographical Sciences, Lennard-Jones Laboratory, Keele University,
Keele ST5 5BG, UK

Introduction

The first line of defence in an insect society is its sophisticated cuticular hydrocarbon recognition system. Colonies usually can recognize their nest-mates and detect intruders, whom they often attack vigorously. Many social parasites, however, are able to break into the fortresses of their hosts and take advantage of them for extended periods without being harmed. Parasites have evolved some remarkable strategies, such as chemical mimicry (Lenoir et al. 2001) that enable them to evade the colony's recognition system. These adaptations provide a way of studying the chemical recognition systems of social insects.

Recent studies have begun to reveal the groups of hydrocarbons (Greene and Gordon 2007; Martin et al. 2008a) and mechanisms (Ozaki et al. 2005; Martin et al. 2008b) involved in the recognition systems used by social insects. These studies show the importance of looking at relationships within and between groups of hydrocarbons, rather than assuming that all hydrocarbons form part of the signal. By using this new approach, rather than the standard multivariate statistical methods that compare all components simultaneously (Martin and Drijfhout 2009a), we studied the host specific *Psithyrus* cuckoo bees as a tool to search for the bumblebee species recognition system. Hydrocarbons are synthesized in the oenocytes and transported to the cuticle and Dufour's gland where they are secreted. These hydrocarbons are present as a thin oily layer that covers the insects' entire surface and that acts primarily as an anti-desiccation agent. In bumblebees, these hydrocarbons are dominated by *n*-alkanes and alkenes. The alkenes are unusual in that they are rich in (*Z*) positional isomers, which include all positions from 5 to 15 (Lanne et al. 1987; Tengö et al. 1991; Ayasse et al. 1995, 1999). This unique richness is likely to have a functional role, since it should be costly to maintain biochemical mechanisms to produce a variety of isomers (Morgan 2004), and bees are able to detect and recognize different isomers (Châline et al. 2005; Blažytė-Čereškienė and Būda 2007).

There are over 250 species of bumblebees, *Bombus*, of which 30 have evolved into social parasites known as cuckoo bumblebees (Williams 1998). For simplicity, all cuckoo bumblebees are referred to as *Psithyrus,* although they are now considered a subgenus within *Bombus* (Williams 1998), while all remaining non-parasitic bumblebee species are referred to as *Bombus*. The *Psithyrus* female (queen) invades an established nest of its host bumblebee species and lays eggs that are reared by host workers into new *Psithyrus* sexuals, since all *Psithyrus* species lack a worker caste. Typically, *Psithyrus* females of a given species only parasitize nests of one, or a few, host species (Richards 1927; Reinig 1935; Alford 1975; Fisher 1987; Williams 2008), but their host invasion behavior can be highly variable (Kupper and Schwammberger 1995; Frehn and Schwammberger 2001).

Psithyrus females are well adapted to their parasitic life style, having thicker cuticles, longer stings, tougher inter-segmental membranes, sharper more powerful, mandibles, and a larger venom sac and Dufour's gland than their hosts (Richards 1927; Free and Butler 1959; Alford 1975). These features facilitate the successful usurpation of the host nest (Fisher and Sampson 1992) and are found also in socially parasitic wasps (Edwards 1980) and ants (Tsuneoka and Akino 2009), both distantly related but socially similar taxa. The *Psithyrus* females often visually mimic their host color patterns, especially in Europe (Williams 2008), and employ a variety of chemical mechanisms that facilitate nest invasion. For example, *B. (Ps.) norvegicus* secrete dodecyl acetate from their enlarged Dufour's gland, which repels host workers (Zimma et al. 2003). *Psithyrus* females are thought to locate their host colony by species-specific olfactory cues (Sladen 1912; Fisher 1983), since *Psithyrus* species can distinguish host from non-host species based on their Dufour's gland extract (Fisher et al. 1993).

Free and Butler (1959) suggested that, to aid in nest invasion, *Psithyrus* species may have evolved similar recognition cues to their host species that could explain their high host-specificity. Here, we tested the hypothesis that *Psithyrus* species mimic the chemical profiles of their host species. In addition, we investigated whether the presence of the repellent dodecyl acetate is a general feature of *Psithyrus* bumblebees, since the production of a repellent appears counter-productive for species thought to use chemical mimicry.

Methods and Materials

Sample Collection Between April and June 2007, bumblebee queens were caught while feeding on flowers or searching for nest-sites in the Hanko region of Southern Finland and the Sheffield region of South Yorkshire, UK. Queens also were collected from the Burren in Western Ireland during early June 2006. All samples were killed by freezing and stored at −20°C. Across the three sites, 76 queens were collected representing 14 species. These comprised nine nest-building species belonging to five different sub-genera, and five *Psithyrus*-cuckoo species (Fig. 1). An additional 19 dried *Psithyrus* queens were obtained from three different private collections of bumblebees collected mainly in Leicestershire and Buckinghamshire, England (Fig. 1).

Chemical Analyses Cuticular hydrocarbon extracts from each bumblebee were prepared by removing one pair of wings and immersing them in a vial containing 80 µl of HPLC grade hexane at room temperature for 15 min. Wings were used in order to minimize potential contamination from glandular secretions. The extracts were evaporated and stored at 5°C. A Dufour's gland extract of each bumblebee (excluding the 19 dried *Psithyrus* samples) also was obtained following the same procedure used for the wings. The Dufour's gland contains only internally produced hydrocarbons. The remaining pairs of wings then were pooled for each species providing a stronger extract that allowed better resolution of the positional isomers after dimethyl disulfide (DMDS) derivatization (see below). The cuticular hydrocarbon extracts from the 19 dried *Psithyrus* samples were obtained by immersing the entire body and wings in a small glass dish containing 1 ml of hexane for 5 min before transferring the hexane to a vial where it was evaporated. This ensured that sufficient extract was available for DMDS derivatization. Just prior to analysis, 30 µl of hexane were added to the vials, and the samples were analyzed on an HP6890 gas chromatograph (GC) equipped with an HP-5MS column (length: 30 m; internal diam: 0.25 mm; film thickness: 0.25 µm) connected to an HP5973 quadrupole mass spectrometer (MS) with 70 eV electron impact ionization. Samples were injected in the splitless mode, and the oven was programmed from 70°C to 200°C at 40°C/min, and then from 200°C to 320°C at 15°C/min, and held for 2 min at 320°C. Helium was used as carrier gas, at a constant flow rate of 1.0 ml/min. Compounds were characterized by the use of standard MS databases, diagnostic ions, and their Kovats indices. Pooled wing extracts, individual body washes, and individual Dufour's gland samples were subject to DMDS derivatization in order to determine the alkene double bond positions (Carlson et al. 1989) and re-analyzed on the GC-MS under the same conditions as the non-derivatizated samples.

Data Analyses The analysis of the three main groups of compounds (acetates, *n*-alkanes, and alkenes) were con-

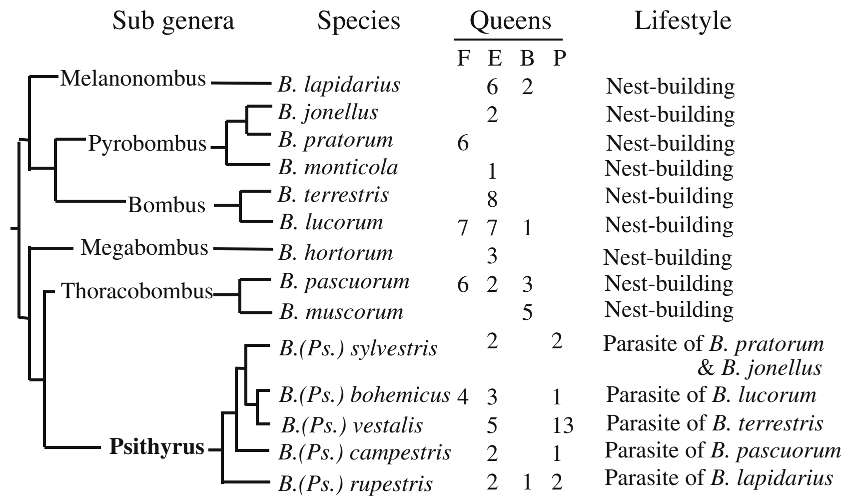

Fig. 1 The simplified sub-generic classification of the bumblebees studied adapted from Williams et al. (2008) showing the phylogenetic relationships among the 14 species studied. The number of *Bombus* queens and *Psithyrus* females of each species collected from Finland (*F*), England (*E*), the Burren in Ireland (*B*) or from private English collections (*P*). Their lifestyle also is presented

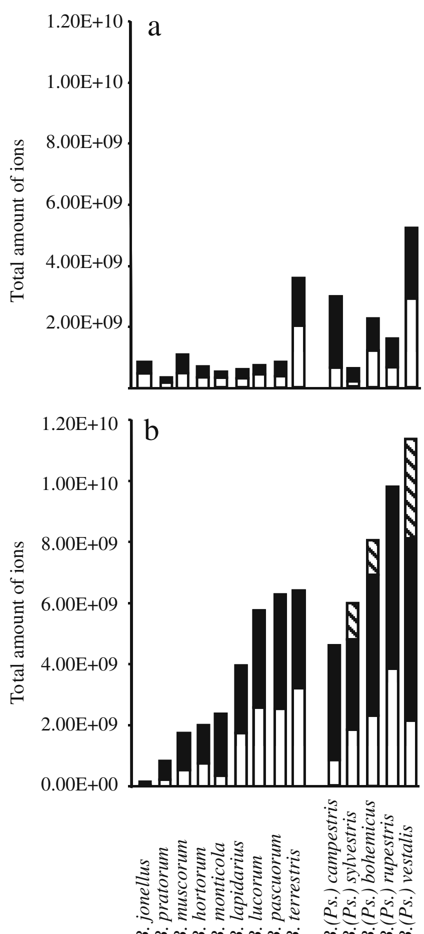

Fig. 2 The average relative amount of *n*-alkanes (white), alkenes (black), and dodecyl acetate (stripes) extracted from **a** wings and **b** the Dufour's gland of 14 bumblebee species. Error bars are not shown for clarity

ducted separately, as there is growing evidence (Châline et al. 2005; Greene and Gordon 2007; Martin and Drijfhout 2009a) that the profile perceived by the insect and that produced by the GC-MS are not congruent. For acetates and *n*-alkanes, peak areas were integrated from the original (i.e., non-derivatized) individual Dufour's gland and wing gas chromatograms. Data were log-transformed in order to meet the assumption of equal variances before conducting *t*-tests in SPSS v14. For the alkenes, the presence of several isomers, often with overlapping retention times, meant accurate integration from the original gas chromatograms was not possible. Therefore, the proportion of each alkene isomer present was calculated from the derivatized sample chromatograms as follows. By using the characteristic ions for each isomer at each chain length, the amounts of these ions were individually integrated using the 'extract ion' function in MSD Chem-Station. This produced a table of ion counts for each isomer at each chain-length for each individual. This was converted to a percentage of the overall count, and any compound (i.e., an isomer at a specific chain length) that represented less than 0.5% was excluded. The percentage of the remaining isomers was re-calculated.

We then investigated whether any patterns existed that connected the positional isomers to chain-length before calculating the proportion of each isomer irrespective of its chain-length for each individual. Initially, the proportion of each isomer was calculated in the normal way, i.e., total (*Z*)-10 = (*Z*)-10C_{23} + (*Z*)-10C_{25} + (*Z*)-10C_{27}. This produced an unusual isomer pattern in *B. hortorum* (see "Results"). However, the apparent anomaly might be explained better from a functional viewpoint since isomers potentially can be read from either end of the molecule. Therefore, we calculated the proportion of each isomer so that only *odd* isomers were present e.g., (*Z*)-13 = (*Z*)-10C_{23} + (*Z*)-12C_{25} + (*Z*)-13C_{27}. This approach is supported by the facts that even positional isomers (e.g., [*Z*]-8 and [*Z*]-10) are a biochemical rarity, since (*Z*)-9-oleic acid is the key precursor, and in insects Δ desaturases are normally odd-numbered (e.g., 5, 9, 11, and 11) (Byers 2006).

Results

General Findings Figure 2 shows that non-polar compounds extracted from the bumblebee wings and their Dufour's glands are almost exclusively (>99%) composed of *n*-alkanes (C_{21}–C_{31}) and alkenes (various isomers) except in *B. (Ps.) campestris,* in which it is >90% due the presence of methylated compounds that are rare (<1%) in all other species. In addition to the hydrocarbons, dodecyl acetate, a volatile compound, was detected in the Dufour's gland of three of the five *Psithyrus* species (Fig. 2). The hydrocarbon ion counts (*n*-alkanes and alkenes) extracted from the wings (Fig. 2a) and the Dufour's gland (Fig. 2b) are significantly higher among *Psithyrus* than the non-parasitic *Bombus* (wings $P=0.018$, $t=-2.729$ $df=12$; Dufour's gland $P=0.018$, $t=-2.735$ $df=12$).

n-Alkanes For all species, the *n*-alkanes account for 23–54% of the hydrocarbons in the wing extracts and 14–50% of the Dufour's gland extracts (Figs. 2a, b). Comparison of the *n*-alkane profiles shows high variability between individuals. Consequently, no distinct differences between species, and no clear associations between the *n*-alkane profiles of *Psithyrus* and their hosts were apparent in either the wing or the Dufour's gland samples (Supplemental Fig. 1). This is further supported by Discriminant Analysis (DA) in which the *n*-alkanes only separate one of the non-parasitic *Bombus* species, whereas the alkenes separate all of the non-parasitic *Bombus* species (Fig. 3).

Alkenes The alkenes are the most abundant group of compounds detected in the wing, Dufour's gland, and whole body extracts with a wide range of isomers detected, even within the profile of a single species. These include all isomers from (*Z*)-5 to (*Z*)-14. All three *B. hortorum* queens possessed the same rich and highly unusual isomer pattern, with respect to which isomer was prevalent at each chain-length (Fig. 4). However, when double bond positions are viewed differently see ("Methods"), the apparently unusual isomer pattern of *B. hortorum* becomes clear; i.e., the production of mainly the (*Z*)-13 isomer (Fig. 4).

Although we detected odd and even isomers from (*Z*)-5 to (*Z*)-14, our re-interpretation of the results is that odd isomers from (*Z*)-5 to (*Z*)-21 were detected (as shown in subsequent figures). The same overall conclusions are reached, irrespective of the method used to calculate the isomer profiles, but fewer odd isomers are implied.

We were able to separate all of the non-parasitic *Bombus* species by using only the observed variation between species in their alkene isomer profile (Fig. 3). The only exceptions were three samples from the *B. terrestris*-group that all had distinctive isomer profiles (Fig. 5). This chemo-group (*B. terrestris II*) was composed of a Finnish *B. lucorum*, and two *B. terrestris* from Sheffield that were morphologically indistinguishable from their conspecifics.

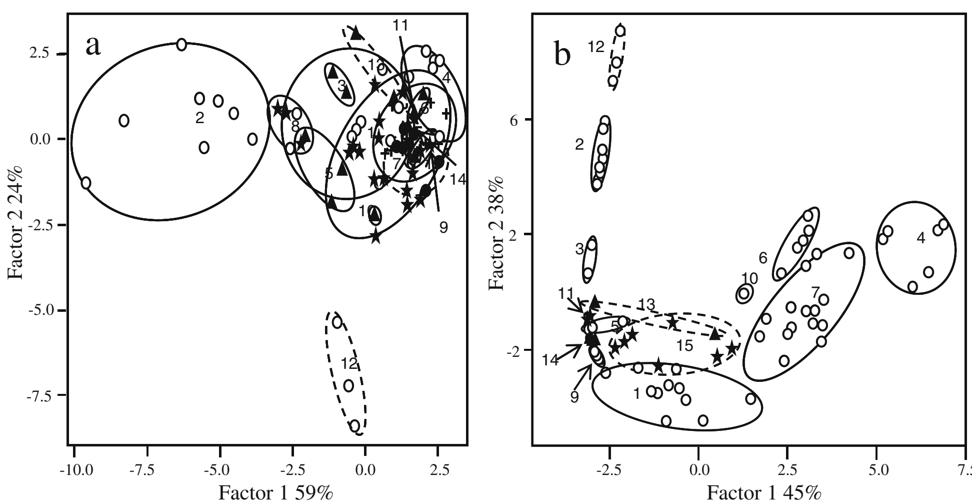

Fig. 3 Comparison of the discriminant analysis using SPSS based on the transformed proportions (for method see Martin and Drijfhout 2009a) for **a** alkanes and **b** alkene isomers from the Dufour's gland of each bumblebee queen. Different symbols are used where ellipses that enclose all individuals of that species overlap. Ellipses for the cuckoo species are dashed. The two factors indicate the amount of variation explained by each discriminate component. The isomer profile of *Bombus hortorum* (No. 8) was so different from all other species that it was removed from the analysis otherwise it was difficult to visualize the data. 1.*B. pascuorum*; 2. *B. lapidarius*; 3. *B. jonellus*; 4. *B. pratorum*; 5.*B. terrestris II*; 6. *B. terrestris*; 7. *B. lucorum*; 8.*B. hortorum*; 9. *B. muscorum*; 10. *B. monticola*; 11. *B. (Ps.) campestris*; 12. *B. (Ps.) rupestris*; 13. *B. (Ps.) sylvestris*;14. *B. (Ps.) vestalis*; 15. *B. (Ps.) bohemicus*

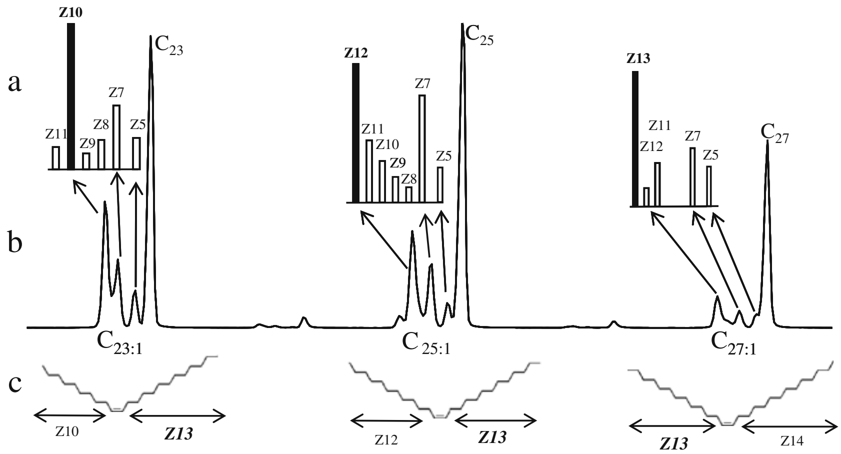

Fig. 4 **b** The gas chromatogram of *Bombus hortorum* showing at each chain length three alkene peaks that contain up to seven different co-eluting isomers and a single *n*-alkane peak. **a** The proportion of each isomer after derivatization that revealed an unusual isomer pattern. **c** However, the most abundant isomer at each chain length (*Z*)-10-$C_{23:1}$, (*Z*)-12-$C_{25:1}$, and (*Z*)-13-$C_{27:1}$ are in fact all (*Z*)-13 if position is determined from either end of the molecule

In two species (*B. pascuorum* and *B. lucorum*), conspecific queens were collected from three different countries (Fig. 1). Despite this, all 11 *B. pascuorum* queens had similar alkene isomer profiles, as shown by the small amount of variation (Fig. 5) and forming a single cluster (Fig. 3b). Although the 15 *B. lucorum* queens also form a single cluster (Fig. 3b), there were small but consistent differences in the isomer profiles between the Sheffield and Finnish populations (Fig. 5).

Mimicry of Hosts' Species Specific Alkene Isomer Patterns by Psithyrus The nine non-parasitic *Bombus* species all had species-specific alkene isomer patterns in their Dufour's gland extracts (Fig. 5) that mirrored their pooled wing extracts (Fig. 5). The (*Z*)-7 and (*Z*)-9 alkene isomer ratios of several *Psithyrus* species showed a remarkable resemblance to those of their hosts (Figs. 5, 6), irrespective of whether wing, entire body, or Dufour's gland extracts were compared (Fig. 5). For example, the (*Z*)-7: (*Z*)-9 ratio is dominated by (*Z*)-9 in both *B. pascuorum* and its cuckoo *B. (Ps.) campestris*, while it is dominated by the (*Z*)-7 isomer in *B. lapidarius* and its cuckoo *B. (Ps.) rupestris*. This chemical similarity between the cuckoos and their hosts is even more remarkable considering that the hosts are spread across four sub-genera (Fig. 1) with greatly differing (*Z*)-7: (*Z*)-9 ratios (Fig. 6). *Bombus (Ps.) vestalis* is a close mimic of a rare chemo-type detected within the *terrestris*-group, while all other *B. terrestris* possess a very different isomer ratio. Furthermore, the match between host and cuckoo in the case of *B. lucorum* and *B. (Ps.) bohemicus* is closer in the UK population than the Finnish population, as the UK *B. (Ps.) bohemicus* queens are the only *Psithyrus* species to produce isomers other than (*Z*)-7 and (*Z*)-9 in any appreciable amounts (Fig. 5).

Presence of Dodecyl Acetate The presence of volatile repellent dodecyl acetate was not detected on any of the wing samples ($N=76$) (Fig. 2a) nor the Dufour's gland extracts of any non-parasitic *Bombus* species studied (Fig. 2b). However, dodecyl acetate was a major compound detected in the Dufour's gland of all *B. (Ps.) bohemicus* ($N=6$), *B. (Ps.) sylvestris* ($N=3$) and *B. (Ps.) vestalis* ($N=2$) females (Fig. 2b). These are all species that invade hosts with slightly different alkene isomer patterns from their own (Fig. 5). In these species, dodecyl acetate represented an average of 21% of the non-polar compounds extracted from the Dufour's gland. Dodecyl acetate was absent from the Dufour's glands of all *B. (Ps.) campestris* ($N=2$) and *B. (Ps.) rupestris* ($N=3$) (Fig. 2b), both species that mimic closely the isomer profiles of their hosts (Fig. 5).

Discussion

This study has revealed an additional level of detail contained within an insect's cuticular hydrocarbon profile that is a potential candidate for encoding the recognition cues used by bumblebees. Among the insects, bumblebees are unusual in the variety of isomers they produce, as shown by this and previous studies (Lanne et al. 1987; Tengö et al. 1991; Ayasse et al. 1995, 1999; Urbanová et al. 2004). The nine non-parasitic *Bombus* species in this study and four additional species (*B. ignitus, B. diversus, B. hypocrita,* and *B. deuteronymus*; authors' unpublished data) all have species-specific alkene isomer patterns. The high isomer diversity and stable species-specific patterns are consistent with a functional role in recognition, which is supported by the mimicry of these isomer profiles by the three corresponding

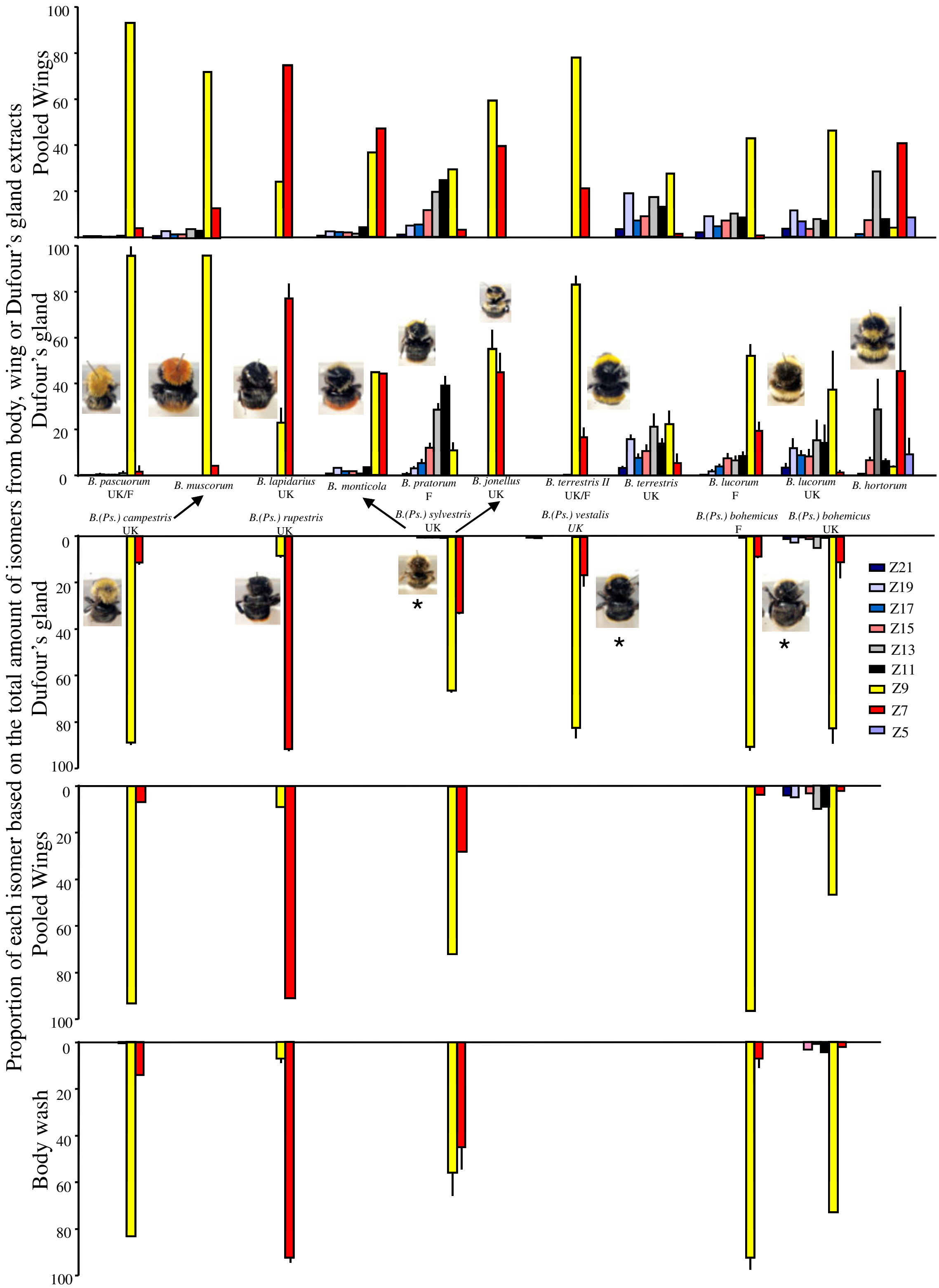

Fig. 5 Species-specific alkene isomer patterns determined from the pooled wing, Dufour's gland and entire body extracts for 14 bumblebee species. The top two graphs represent the non-parasitic *Bombus* species, while the three lower inverted graphs present the data for the corresponding *Psithyrus*-cuckoo species. Arrows indicate alternative hosts. The error bars represent one SD. No error bars are present for the pooled wing samples as sample sizes are one. The asterisk indicates species that produce the repellent dodecyl acetate

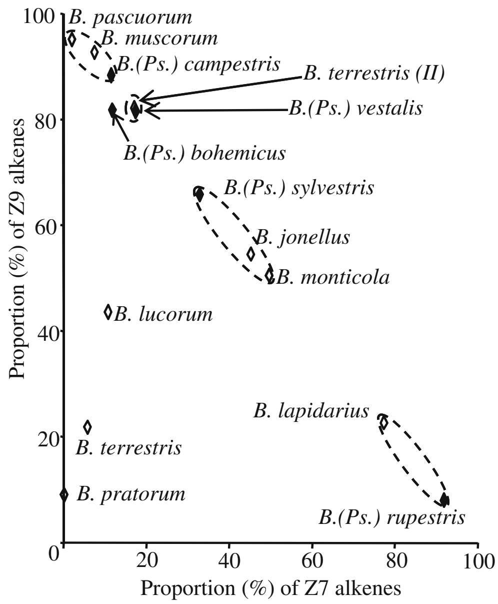

Fig. 6 The matching of a wide range of host (*Z*)-7: (*Z*)-9 alkene isomer profiles occurring across four sub-genera by five *Psithyrus* species that all belong to the same sub-genus (see Fig. 1). The dotted ellipses link the host and cuckoo species

species of social parasite (i.e., cuckoos). In contrast, in those cases where the host and cuckoo isomer profiles do not match, the cuckoos produce a known worker repellent, dodecyl acetate, that represents an alternative strategy for invading colonies. Within each species, the variability of alkene isomers remained small, even where specimens were collected from three countries and had different color forms e.g., *B. pascuorum*. Species-specific alkene isomer profiles found in this study also can be seen in *B. terrestris* collected from Sweden, Israel, and Germany (Tengö et al. 1991) and *B. lapidarius* collected from Sweden (Tengö et al. 1991). This stability of discrimination signals within a species across coarse geographical scales also has been found previously in *Drosophila* flies (Rouault et al. 2001), bark beetles (Symonds and Elgar 2004), and *Formica* ants (Martin et al. 2008c). Conversely the *n*-alkanes show high variability both among individuals within species and among species, resulting in no species-specific profiles. Alkane production is influenced by environmental factors, such as temperature, humidity, and task (Wagner et al. 2001; Martin and Drijfhout 2009b), which may explain this variation.

Hydrocarbons identified in the Dufour's gland of bumblebees also are present on their eggs (Ayasse et al. 1999) as well as on their cuticles (Oldham et al. 1994; Ayasse et al. 1995; this study). This may help "fool" host workers into rearing *Psithyrus* eggs. The recent finding that *Psithyrus* females have retained their ability to produce wax also may help explain their chemical integration into the colony (Sramkova and Ayasse 2008).

The possibility that the cuckoos' profiles have been acquired from either the hosts that raised them or that they will invade is small, because 82 out of the 95 *Psithyrus* females were caught searching for nests rather than being removed from host colonies. As there is a close match between the external (entire body or wing extracts) and internal (Dufour's gland extract) hydrocarbon profiles, *Psithyrus* females may be examples of mimics that biosynthesize their chemical disguise prior to invasion rather than acquire it from their hosts after invasion (Lenoir et al. 2001).

The production of dodecyl acetate by three of the five *Psithyrus* species appears inconsistent, but it is produced only by *Psithyrus* species that fail to closely mimic their host in terms of alkenes. Dodecyl acetate is a known bumblebee worker repellent (Zimma et al. 2003) and represents an alternative way of invading colonies. Limited behavioral observations show that host-cuckoo interactions that involve the two cuckoo species lacking dodecyl acetate (*B. (Ps.) rupestris* and *B. (Ps.) campestris*) are non-aggressive (Hoffer 1888; Sladen 1912; Fisher 1988), whereas aggressive and non-aggressive interactions have been observed against cuckoos that produce dodecyl acetate (Sladen 1912; Van Honk et al. 1981; Küpper and Schwammberger 1995). Similar compounds (e.g., decyl-acetate and decyl butyrate) also are found in the enlarged Dufour's glands of slave-making *Formica* and *Polyergus* ants, where they act as repellents during raids (Regnier and Wilson 1971; Graham et al. 1979; D'Ettorre et al. 2000; Tsuneoka and Akino 2009).

Co-evolution is a dynamic process that may be at different stages for different cuckoo-host associations. For example, *B. pascuorum* and *B. lapidarius* are both common species, and their cuckoos (*B. (Ps.) campestris* and *B. (Ps.) rupestris*) are close color and chemical mimics. *Bombus (Ps.) sylvestris* is a close chemical mimic of *B. jonellus* and *B. monticola,* both uncommon species in Britain, but its other host there is *B. pratorum,* a species with a similar ecology (e.g., early nesting, pollen-stores, small colony size (Sladen 1912)) and that is common and widespread (Williams 2007). This may indicate that *B. (Ps.) sylvestris* has undergone a host shift. This is supported by the facts that *B. (Ps.) sylvestris* produces the worker repellent dodecyl acetate and remains a much closer color mimic of *B. jonellus* than *B. pratorum.* The UK *B. (Ps.) bohemicus* population is unique in its ability to produce other isomers besides (*Z*)-7 and (*Z*)-9 in substantial amounts (Fig. 5). This may result from the cuckoo tracking the profiles of different species within the *lucorum*-complex, and could be evidence of an ongoing "arms race".

The presence of two distinct chemo-types within the *terrestris*-group (Fig. 5) is supported by Pamilo et al. (1984) who also suggested the presence of two sibling species of *B. lucorum* with a wide distribution in

Fennoscandia based on phosphoglucomutase (PGM) gene variation. German laboratory colonies of *B. lucorum* also had isomer profiles similar to our *B. terrestris* II group (Tengö et al. 1991), whereas, field caught *B. terrestris* (around Bonn) have isomers similar to UK *B. terrestris* and *B. lucorum* (Sramkova and Ayasse 2009). The *B. lucorum-terrestris* species complex is poorly resolved taxonomically, and additional morphological, chemical, and genetic data are needed before clear species boundaries can be defined.

The invasion behavior of *Psithyrus* females ranges from highly aggressive to passive (Dronnet et al. 2005), as an invading queen may usurp the host queen (Wilson 1971) or live in peaceful cohabitation with her (Fisher 1987, 1988; Carvell et al. 2008). This might arise from different chemical mechanisms being employed (i.e., mimicry or repellents) by different *Psithyrus* species.

Acknowledgments Thanks go to Matthew Heard of CEH Wallingford, Dave Goulson of Stirling University, and Maggie Frankum of Leicester for providing additional *Psithyrus* queens. Also, thanks to Roger Butlin, Paul Brakefield, and Duncan Jackson of Sheffield University for detailed comments, in addition to the helpful comments by two anonymous referees. Funding was provided by NERC (NE/C512310/1 and NE/F018355/1).

References

ALFORD, D. V. 1975. *Bumblebees*. London: Davis-Poynter. 352 pp.

AYASSE, M., MARLOVITS, T., TENGÖ, J., TAGHIZADEH, T., and FRANCKE, W. 1995. Are there pheromonal dominance signals in the bumblebee *Bombus hypnorum* L (Hymenoptera, Apidae)? *Apidologie* 26:163–180.

AYASSE, M., BIRNBAUM, J., TENGÖ, VAN DOORN, A., TAGHIZADEH, T., and FRANCKE, W. 1999. Caste- and colony-specific chemical signals on eggs of the bumble bee, *Bombus terrestris*. *Chemoecology* 9:119–126.

BLAŽYTĖ-ČEREŠKIENĖ, L., and BŪDA, V. 2007. Ability of honey bees to detect and recognise isomers of cresol. *Ekologija* 53:16–21.

BYERS, J. A. 2006. Pheromone component patterns of moth evolution revealed by computer analysis of the Pherolist. *J. Anim. Ecol.* 75:399–407.

CARLSON, D. A., ROAN, C-S., YOST, R. A., and HECTOR, J. 1989. Dimethyl disulfide derivatives of long chain alkenes, alkadienes, and alkatrienes for gas chromatography/mass spectrometry. *Anal. Chem.* 61:1564–1571.

CARVELL, C., ROTHERY, P., PYWELL, R. F., and HEARD, M. S. 2008. Effects of resource availability and social parasite invasion on field colonies of *Bombus terrestris*. *Eco. Entomol*. 33:321–327.

CHÂLINE, N., SANDOZ, J. C., MARTIN, S. J., RATNIEKS, F. L. W., and JONES, G. R. 2005. Learning and discrimination of individual cuticular hydrocarbons by honey bees (*Apis mellifera*). *Chem. Senses* 30:327–333.

D'ETTORRE, P., ERRARD, C., IBARRA, F., FRANCKE, W., and 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* 10:35–142.

DRONNET, S., SIMON, X., VERHAEGHE, J. C., RASMONT, P., and ERRARD, C. 2005. Bumblebee inquilinism in *Bombus (Fernaldaepsithyrus) sylvestris* (Hymenoptera, Apidae): behavioural and chemical analyses of host-parasite interactions. *Apidologie* 36:59–70.

EDWARDS, E. 1980. Social Wasps: Their Biology and Control. Rentokil Libr., East Grinstead. 398 pp.

FISHER, R. M. 1983. Recognition of host nest odour by the bumble bee social parasite *Psithyrus ashtoni* (Hymenoptera: Apidae), *J. N. Y. Entomol. Soc.* 91:503–507.

FISHER, R. M. 1987. Queen-worker conflict and social parasitism in bumblebees. *Anim. Behav.* 35:1026–1036.

FISHER, R. M. 1988. Observations on the behaviours of three European cuckoo bumble species. *Insect. Soc.* 35:341–354.

FISHER, R. M., and SAMPSON, B. J. 1992. Morphological specializations of the bumble bee social parasite *Psithyrus ashtoni*. *Can. Entomol*. 124:69–77.

FISHER, R. M., GREENWOOD, D. R., and SHAW, G. J. 1993. Host recognition and the study of a chemical basis for attraction by cuckoo bumble bees (Hymenoptera: Apidae). *J. Chem. Ecol.* 19:771–786.

FREE, J. B., and BUTLER, C. G. 1959. *Bumblebees*. London. 208 pp.

FREHN, E., and SCHWAMMBERGER, K. H. 2001. Social parasitism of *Psithyrus vestalis* in Free-foraging Colonies of *Bombus terrestris* (Hymenoptera: Apidae). *Entomol. Gen*. 25:103–105.

GRAHAM, R. A., BRAND, J. M., and MARKOVETZ, A. J. 1979. Decyl acetate synthesis in the ant *Formica schaufussi* (Hymenoptera: Formicidae). *Insect Biochem*. 9:331–333.

GREENE, M. J., and GORDON, D. M. 2007. Structural complexity of chemical recognition cues affects the perception of group membership in the ants *Linephithema humile* and *Aphaenogaster cockerelli*. *J. Exp. Biol.* 210:897–905.

HOFFER, E. 1888. Die schmarotzerhummeln Steiermarks. Lebensgeschichte und beschreibung derselben.*Mitt. naturw. Ver. Steiermark*, 25:82–158.

KÜPPER, G., and SCHWAMMBERGER, K. H. 1995. Social parasitism in bumble bees (Hymenoptera, Apidae): observations of *Psithyrus sylvestris* in *Bombus pratorum* nests. *Apidologie* 26:245–254.

LANNE, B. S., BERGSTRÖM, G., WASSGREN, A-B., and TÖRNBÄCK, B. 1987. Biogenetic patterns of straight chain marking compounds in male bumblebee. *Comp. Biochem. Physiol.* 88B: 631–636.

LENOIR, A., D'ETTORRE, P., and ERRARD, C., 2001. Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol*. 46:573–599.

MARTIN, S. J., and DRIJFHOUT, F. P. 2009a. How reliable is the analysis of complex cuticular hydrocarbon profiles by multivariate statistical methods? *J. Chem. Ecol*. 35:375–382.

MARTIN, S. J., and DRIJFHOUT, F. P. 2009b. Nestmate and task cues are influenced and encoded differently within ant cuticular hydrocarbon profiles. *J. Chem. Ecol*. 35:368–374.

MARTIN, S. J., VITIKAINEN, E., HELANTERÄ, H., and DRIJFHOUT, F. P. 2008a. Chemical basis of nestmate recognition in the ant *Formica exsecta*. *Proc. R. Soc. B*. 275:1271–1278.

MARTIN, S. J., HELANTERÄ, H., and DRIJFHOUT, F. P. 2008b. Colony-specific hydrocarbons identify nest mates in two species of *Formica* ant. *J. Chem Ecol.* 34:1072–1080.

MARTIN, S. J., HELANTERÄ, H., and DRIJFHOUT, F. P. 2008c. Evolution of Species-Specific Cuticular Hydrocarbon Patterns in *Formica* Ants. *Bio. J. Linn. Soc*. 95:131–140.

MORGAN, E. D. 2004. Biosynthesis in Insects. Royal Society of Chemistry, Cambridge.

OLDHAM, N. J., BILLEN, J., and MORGAN, E. D. 1994. On the similarity of the Dufour gland secretion and the cuticular hydrocarbons of some bumblebees. *Physiol. Entomol.* 19:115–123.

Ozaki, M., Wada-katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., Nisimura, T., and Yamaoka. R. 2005. Ant nest mate and non-nest mate discrimination by a chemosensory sensillium. *Science* 309:311–315.

Pamilo, P., Varvio-aho, S-L., and Pekkarinen, A. 1984. Genetic variation in bumblebees (*Bombus, Psithyrus*) and putative sibling species of *Bombus lucorum*. *Hereditas* 101:245–251.

Regnier, T. E., and Wilson, E. O. 1971. Chemical communication and 'propaganda' in slave-making ants. *Science* 172:267–269.

Reinig, W. F. 1935. On the variation of *Bombus lapidarius* L. and its cuckoo, *Psithyrus rupestris* Fabr., with notes on mimetic similarity. *J. Genetics* 30:321–356.

Richards, O. W. 1927. The specific characters of the British humblebees (Hymenoptera). *Trans. Entomol. Soc. Lond.* 75:233–268.

Rouault, J., Capy, P., and Jallon, J-M. 2001. Variations of male cuticular hydrocarbons with geoclimatic variables: an adaptative mechanism in *Drosophila melanogaster*? *Genetica* 110:117–130.

Sladen, F. W. L. 1912. The Humble-bee, Its Life History and How to Domesticate it, with Descriptions of All the British Species of *Bombus* and *Psithyrus*. London: MacMillan. 283 p. [Republished in 1989, Woonton (Herefordshire): Logaston Press. 273 p.]

Sramkova, A., and Ayasse, M. 2008. *Psithyrus* females do possess wax glands. *Insect. Soc*. 55:404–406.

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

Symonds, M. R. E., and Elgar, M. A. 2004. The mode of pheromone evolution: evidence from bark beetles. *Proc. R. Soc. B.* 271:839–846.

Tengö, J., Hefetz, A., Bertsch, A., Schmitt, U., Lübke, G., and Francke, W. 1991. Species specificity and complexity of Dufour's gland secretion of bumblebees. *Comp. Biochem. Physiol*. 99B: 641–646.

Tsuneoka, Y., and Akino, T. 2009. Repellent effect on host *Formica* workers of queen Dufour's gland secretion of the obligatory social parasite ant, *Polyergus samurai* (Hymenoptera: Formicidae). *Appl. Entomol. Zool*. 44:133–141.

Urbanová, K., Halík, J., Hovorka, O., Kindl, J., and Valterová, I. 2004. Marking pheromones of the cuckoo bumblebee males (Hym, Apoidea, *Bombus*): compositions of labial gland secretions of six species found in the Czech Republic. *Biochem. Sys. Ecol*. 32:1025–1045.

Van honk, C. G. J., Roseler, P. F., Velthuis, H., and Malotaux, M. 1981.The onquest of a *Bombus terrestris* colony by a *Psithyrus vestalis* female. *Apidologie* 12:57–67.

Wagner, D., Tissot, M., and Gordon, D. M. 2001. Task-related environment alters the cuticular hydrocarbon composition of Harvester ants. J. Chem. Ecol. 27:1805–1819.

Williams, P. H. 1998. An annotated checklist of bumble bees with an analysis of patterns of description (Hymenoptera: Apidae, Bombini). *Bull. Nat. His. Mus*. (Entomol.) 67:79–152.

Williams, P. H. 2007. Bumble bees - and their decline in Britain, pp. 161–176, in NL Carreck and TW Johnson (eds.). Aspects of sociality in insects. Central Association of Bee-Keepers: Ilford.

Williams, P. H. 2008. Do the parasitic *Psithyrus* resemble their host bumblebees in colour pattern? *Apidologie* 39:637–649.

Williams, P. H., Cameron S. A., Hines, H. M., Cederberg, B., and Rasmont, P. 2008. A simplified subgeneric classification of the bumblebees (genus *Bombus*). *Apidologie* 39:46–74.

Wilson, E. O. 1971. The Insect Societies. Harvard Univ. Press, Cambridge, Mass, 548 pp.

Zimma, B. O., Ayasse, M., Tengö, J., Ibarra, F., Schulz, C., and Francke, W. 2003. Do social parasitic bumblebees use chemical weapons? (Hymenoptera, Apidae). *J. Comp. Physiol. A* 189:769–775.