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Labial Gland Chemistry of Three Species of Bumblebees (Hymenoptera: Apidae) from North America

Gunnar Bergström,[a] Peter Bergman,[a*] Monica Appelgren[a] and Justin O. Schmidt[b]

[a]*Department of Chemical Ecology, Botanical Institute, Göteborg University, Carl Skottsbergs Gata 22, S-413 19, Göteborg, Sweden*

[b]*Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, AZ 85719, U.S.A.*

Abstract—The volatile secretion emanating from the cephalic part of the paired labial gland in male bumblebees of three North American species, viz. *Bombus sonorus* Say, *Bombus huntii* Greene and *Psithyrus insularis* (F. Smith) has been analysed by GC–MS and GC–FTIR. The secretion, which is deposited as a marking secretion on various objects along a repetitive flight path, is composed of isoprenoids, (acyclic sesqui- and diterpenes) and straight-chain fatty acid derivatives (alcohols containing 14, 16 and 18 carbon atoms, and odd numbered hydrocarbons ranging from 23 to 27 carbons). The secretions are species-specific with just a few major components. In *B. sonorus* (*Z*)-11-octadecen-1-ol is the main compound accompanied by geranylgeraniol and tetradecan-1-ol; in *B. huntii* the dominating compound is *trans*-2,3-dihydrofarnesol and it also contains (*Z*)-11-octadecen-1-ol and hexadecan-1-ol; *P. insularis* is characterized by geranylcitronellol, together with the (*Z*)-11-octadecen-1-ol and hexadecan-1-ol. These results agree with analyses of 36 species and two forms of Scandinavian bumblebees analysed previously. Copyright © 1996 Elsevier Science Ltd

Introduction

North America contains a rich and diverse fauna of bumblebees. The genus *Bombus* consists of approximately 39 species in 10 subgenera, and the genus *Psithyrus* contains six species in three subgenera.[1] Many of these species overlap temporally and geographically; thus there is a possibility of interspecific mating. In Scandinavia, male bumblebees are well known to establish flight routes; males scent mark during a couple of hours in the morning, whereafter they patrol their scent marked route for the rest of the day.[2–4] During the scent marking behaviour, different objects, such as small twigs, leaf edges, grass and tree trunks at different heights in the their environment are scent marked.[3,5] The scent marks are made with a secretion from the cephalic part of the labial glands,[2] with each species possessing its own idiosyncratic chemical blend.[5–8] The chemicals deposited while scent marking are likely to be important for species recognition and hence avoiding nonconspecific mating.

Although behavioural studies have been carried out on North American bumblebees,[9–12] little is known about the chemistry of male scent marking in these bumblebee species.[12] Some North American species, like *B. sonorus* and *B. fervidus*, differ from the Scandinavian ones in having another male premating behaviour. In these species, males gather just outside a bumblebee nest entrance where they try to reach a favorable position from which they can grasp and mount a virgin queen emerging from the nest.[9,11]

The purpose of this investigation was to discover the nature of male cephalic labial gland secretions of two North American species of *Bombus*, *B. sonorus* and *B. huntii*, and one species of *Psithyrus*, *P. insularis*, and to compare these species chemically with the previously analysed Scandinavian species.

Key words: Labial gland secretion, *Bombus*, *Psithyrus*, isoprenoids, fatty acid derivatives, GC–MS.

Results and Discussion

As in the case of earlier analysed Scandinavian bumblebees, the male labial gland secretion in the present three investigated species is composed of acyclic isoprenoids (sesqui- and diterpenes) and straight-chain fatty acid derivatives. Typical GC for each investigated species are given in Figures 1(a)–(c). The combination of compounds in each secretion makes it species-specific and the individual differences are relatively small, qualitatively as well as quantitatively. There are a few major components characterizing each secretion and many smaller components.

The major compound characteristic for *B. sonorus* [Fig. 1(a)] is (*Z*)-11-octadecen-1-ol and in addition appreciable amounts of geranylgeraniol and tetradecan-1-ol (Fig. 2). *Bombus huntii* [Fig. 1(b)] is characterized by dominating amounts of *trans*-2,3-dihydrofarnesol and it also has quite large amounts of (*Z*)-11-octadecen-1-ol

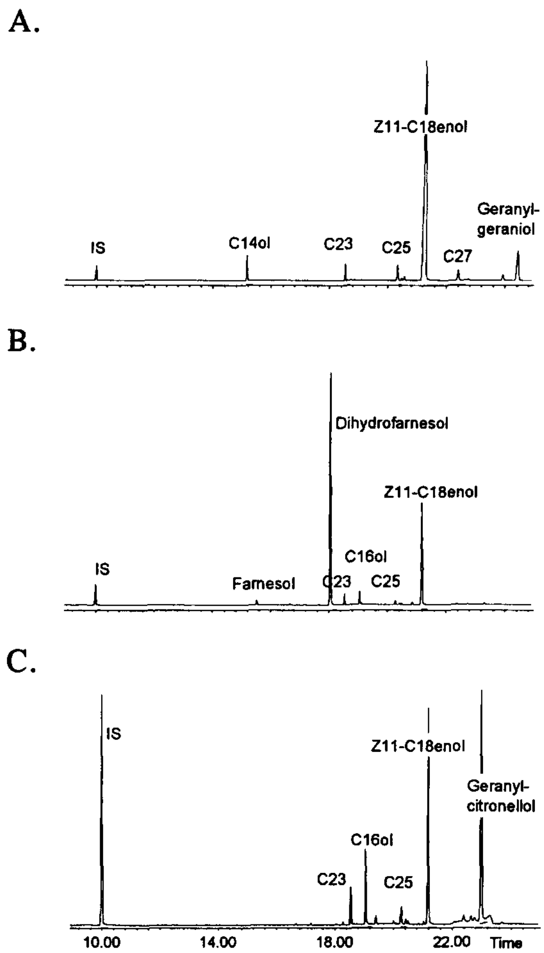

Figure 1. GC of a single individual male labial gland of (a) *B. sonorus*, (b) *B. huntii* and (c) *P. insularis*. IS = pentadecane added as an internal quantification standard; C23, C25 and C27 denotes tricosane, pentacosane and heptacosane, respectively.

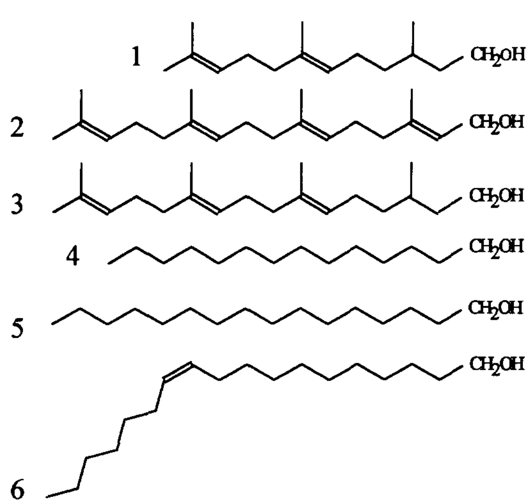

Figure 2. Structural formulas of the major components found in the three bumblebee species. The upper three are isoprenoids: (1) *trans*-2,3-dihydrofarnesol, (2) geranylgeraniol, (3) geranylcitronellol and the latter three are fatty acid derivatives, (4) tetradecan-1-ol, (5) hexadecan-1-ol and (6) (*Z*)-11-octadecen-1-ol.

and hexadecan-1-ol (Fig. 2). In *P. insularis* [Fig. 1(c)] the major component is geranylcitronellol with (*Z*)-11-octadecen-1-ol as the second one, followed by, like in *B. huntii*, hexadecan-1-ol (Fig. 2).

In addition to the species-specific blends, there seem to be interspecific quantitative differences in the amount of labial gland secretion produced. *Bombus sonorus*, which is a large species, has close to 0.5 mg of volatiles in the labial gland secretion; *B. huntii*, which is a much smaller species, has somewhat less, about 350 μg per individual; *P. insularis*, on the other hand, has only about 25 μg per individual on average. Relative mean amounts of compounds found in all the investigated individuals are shown for each species in Figs 3(a)–(c).

All secretions possess straight-chain saturated and mono-unsaturated, uneven numbered hydrocarbons ranging from tricosane (C23:ane) to heptacosane (27:ane). Of these hydrocarbons, tricosane and penta-

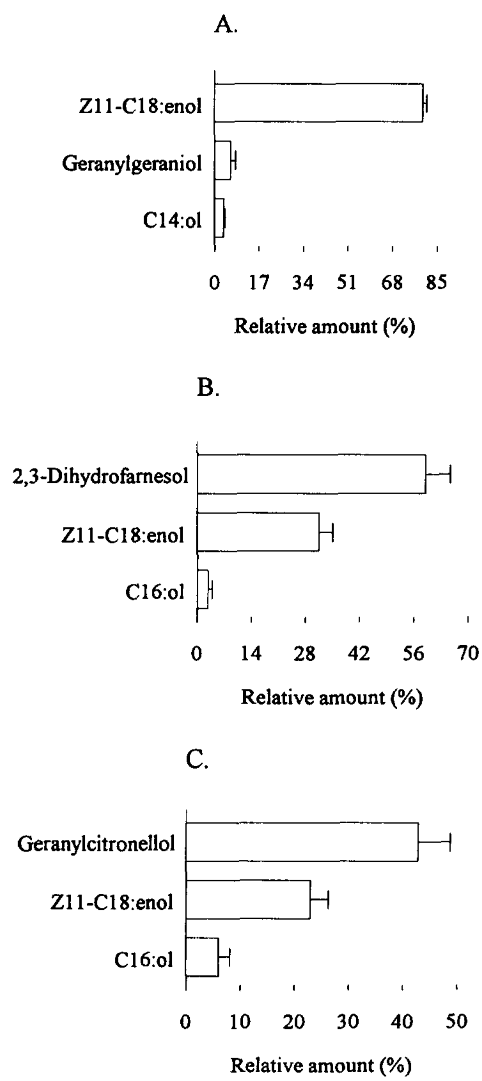

Figure 3. Mean percentage and upper standard error value of the components found in the labial gland samples of male (a) *B. sonorus*, (b) *B. huntii* and (c) *P. insularis*.

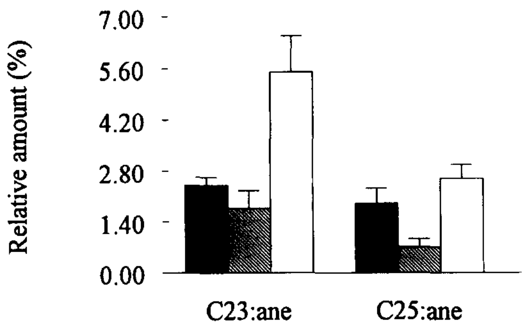

Figure 4. Mean percentage and upper standard error value of the two straight chain hydrocarbons, tricosane (C23:ane) and pentacosane (C25:ane), found in largest amounts in all labial gland samples of male *B. sonorus* (black bars), *B. huntii* (oblique bars) and *P. insularis* (open bars).

cosane were present in all of the males analysed, regardless of species (Fig. 4).

The results of the chemical analyses of the three North American species agree well with our earlier analyses of 36 species and two forms of Scandinavian bumblebees.[5,7,13–15] In these cases some were found to be predominantly 'isoprenoid' species, others 'fatty acid derivative' species and still others were 'mixed'. The three North American species now analysed all fall into this third category; they all contain both isoprenoids and fatty acid derivatives.

The presently investigated species of *Bombus* are placed taxonomically in two different subgenera: *B. sonorus* belongs to *Fervidobombus* and *B. huntii* to *Pyrobombus*.[1] The investigated *Psithyrus* species, *P. insularis*, is placed in the subgenera *Citrinopsithyrus*.[16] Of these three subgenera, only *Pyrobombus* is represented in the Scandinavian bumblebee fauna and there represented by six species.[17] A comparison shows that *B. huntii* has clear group similarities with the Scandinavian *Pyrobombus* species. The main component, *trans*-2,3-dihydrofarnesol, has been found as the main component also in three Scandinavian species, two of which belong to the *Pyrobombus* subgenus: *B. jonellus* and *B. cingulatus*.[8] Also the two fatty acid derivatives (*Z*)-11-octadecen-1-ol and hexadecan-1-ol and the sesquiterpene farnesol found in *B. huntii*, are common in the Scandinavian *Pyrobombus* species. Hexadecan-1-ol has been found to be particularly common in Scandinavian species, occurring in nine species as a large component. Four of these are *Pyrobombus* and in one of them, *B. hypnorum*, it is the main component.

As for *B. sonorus* this species is unique due to its main component, since the (*Z*)-11-octadecen-1-ol has not been identified as a main component before, although it is present as a large component in two Scandinavian species, *B. pratorum* (subgenus *Pyrobombus*) and *B. humilis* (subgenus *Thoracobombus*). Of the other components in *B. sonorus*, both geranylgeraniol and tetradecan-1-ol have been found in Scandinavian species, but never in large amounts.

The nine Scandinavian *Psithyrus* species have relatively few isoprenoids and in that respect *P. insularis* is most similar to *P. vestalis* (subgenus *Asthonipsithyrus*) which has geranylcitronellol as a second component, and *P. rupestris* (subgenus *Psithyrus* s. str.) which contains geranylgeranyl acetate. On the other hand, geranylcitronellol is the major component in two Scandinavian *Bombus* species, *B. hypnorum* and *B. lapponicus* (both *Pyrobombus*), and present in still three other species, *B. balteatus* and *B. hyperboreus* (both *Alpinobombus*), *B. terrestris* (*Bombus* s. str.).[8] In one individual of *P. insularis* we found smaller amounts of saturated and mono-unsaturated ethyl esters with 16 and 18 carbon atoms. This individual did not seem to differ morphologically from the others of this species. It may represent a true form of the species or it may be an age effect. In the Scandinavian bumblebees we have encountered ethyl esters in species belonging to the *Bombus* s. str. subgenus, such as *B. lucorum*, *B. terrestris* and *B. patagiatus*,[7] and also in two *Psithyrus* species, *P. sylvestris* and *P. flavidus*.[5,18]

A special study was made earlier to compare the occurrence of fatty acid derivatives, especially unsatured ones, among the Scandinavian bumblebees.[18] Different double-bond positions occurred and in all where it was determined the unsaturation was *cis* (*Z*). In this context, none of the American bumblebee species differed since they all had (*Z*)-11-octadecen-1-ol in the labial secretion. Further, it should be noted that all the major compounds identified in the American species, apart from the hydrocarbons, are alcohols. This is true both for the isoprenoids and the fatty acid derivatives. As in the present study, straight-chain hydrocarbons, both saturated and mono-unsaturated, have been reported in all previous analyses of labial secretions in Scandinavian *Bombus* and *Psithyrus* species.[5,7,18] These hydrocarbons are also common constituents of the insect cuticle.

Of the three North American species investigated in this study, only one has been studied from a behavioural point of view; *B. sonorus*.[11] However, males of this species were never observed exhibiting any scent marking behaviour. It was rather proposed that the premating behaviour applied by males of the Scandinavian bumblebee species, viz. the establishing of a scent marked flight route, was not used by the *B. sonorus* males. Instead they were "congregating at a nest site, presumably awaiting the emergence of virgin gynes".[11] Nevertheless, with regard to their labial gland secretion in comparison to these found in the Scandinavian species that establish scent marked flight routes, the *B. sonorus* males do seem capable of scent marking. A reasonable suggestion would be that the males establish flight routes but often incorporate a nest within their route and that they tend to aggregate at the nest site. If this really is the case, or if the males use their labial gland secretion for some other purposes must however be elucidated by further field observations of the species. In any case, the major results of this study clearly demonstrate the resemblance in labial gland chemistry between North American and Scandinavian bumblebees of the genera *Bombus* and *Psithyrus*.

Experimental

Bumblebees

Bombus sonorus Say [=*B. pennsylvanicus sonorus* Say] and *B. huntii* Greene are allopatric species that differ greatly in size and belong in different subgenera. *Bombus sonorus,* a very large sized species in the *Fervidobombus* subgenus, is essentially a Mexican species that lives in the desert regions of southern and central California, Arizona, New Mexico, Texas and much of central and northern Mexico. *Bombus huntii,* a small sized species in the *Pyrobombus* subgenus, inhabits much of the Great Basin area of Western U.S.A. with isolated populations near the mountain tops in Southern Arizona. Although the two species were collected in the same mountain range, they were isolated by nearly 1000 m elevation and a short spatial distance (20–30 km). *Psithyrus insularis* (F. Smith) was not previously known from this mountain range and the specimens vary from the more typical California specimens in having a lighter coloration and greater density of coat. Exact taxonomy of this population is currently under investigation.

Most material was collected from the Santa Catalina Mountains (32° 20′N latitude), in Pima County, southern Arizona between 2 and 10 Oct. 1993 and 30 Sept. to 5 Oct. 1994. *Bombus sonorus* were collected at 1350 m elevation in the Molino Basin area and *B. huntii* and *P. insularis* were collected at 2800 m elevation at the top of the mountains. Some *Psithyrus* were also collected from the Huachna Moutains (31° 20′N latitude) in Cochise Co, southern Arizona between 28 Sept. and 2 Oct. 1994 at an elevation of 2400 m. All captured individuals were chilled on ice and transported to the laboratory. Labial glands were dissected and placed individually, or as pools of several individuals, in small quantities of hexane containing pentadecane as internal standard in vials with Teflon® lined caps. Glands were taken up from the solvent after 48 h.

Chemical analysis

All chemical analyses were made by combined GC–MS on a HP 5890 gas chromatograph connected to a HP 5972 mass selective detector. The GC was equipped with a OV-351 column (30 m × 0.25 mm, GeneTec). Helium was used as carrier gas at a constant flow 0.6 mL/min (32 cm/s at 50 °C). The injector temperature was 220 °C. After sample injection, the GC-oven was kept at 50 °C for 2 min and the temperature then increased with 10 °C/min to 240 °C. The maximum temperature was kept for 40 min.

Volatile compounds were identified by comparison of GC retention values and MS data with those of reference compounds. The *cis/trans* isomerism was determined by GC–FTIR analyses using a HP 5890 gas chromatograph connected to a gas phase HP 5965B IR detector.[19] The double-bond position of the unsaturated (*Z*)-11-octadecen-1-ol was determined by ozonolysis.[20]

Statistical analysis

The quantity of an identified compound was determined by comparing its area in the gas chromatogram with that of the internal quantification standard, pentadecane. No comparison to an absolute standard curve was made and all quantitative data are relative amounts given as percentage of totals. Since such data must usually be arcsine transformed to calculate means and intervals of standard errors (SE),[21] the percentage values were transformed before calculating the means, SE, and then transformed back to percentages. Due to these transformations the upper and lower SE values are not necessarily symmetrical around the mean.[21]

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