Chemotaxonomical characterisation of males of *Bombus lucorum* (Hymenoptera: Apidae) collected in the Czech Republic

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**Abstract.** Labial gland secretions of 26 males of the bumblebee *Bombus lucorum* (L.), collected in the Czech Republic, were analysed. The secretions consisted of 60 compounds; ethyl (Z)-9-tetradecenoate was the main component (average 53%). Although the males varied in colour, their labial gland secretions were similar in composition, which indicated they belonged to one species. Chemically the *B. lucorum* occurring in the Czech Republic correspond to the earlier described “blonde form” of this species.

**INTRODUCTION**

The species of bumblebees (*Bombus* Latreille, 1802) belonging to the subgenus *Bombus* s.str. are often difficult to determine. Colouration is very variable and other morphological differences are indistinct. Individuals of the "lucorum species group" are especially difficult to distinguish morphologically (Krüger, 1954). In West and Central Europe this group consists of *Bombus* (s.str.) *lucorum* (Linnaeus, 1761) and two other closely related species - *B. (s.str.) magnus* Vogt, 1911 and *B. (s.str.) cryptarum* Fabricius, 1775.

*Bombus lucorum* in the broadest sense (the taxonomy and nomenclature is very confused - see Williams, 1991, p. 81–85) is a widely distributed bumblebee species, which occurs throughout the Palaearctic. It is one of the commonest bumblebee species in Central and Northern Europe (Rasmont, 1984). The males are very variable in colour throughout their distribution. Due to their morphological similarity *B. cryptarum* (Rasmont, 1984) and *B. magnus* (Loken, 1973) have either been regarded as distinct species or have been synonymised with *B. lucorum*, sometimes even with *B. terrestris* (Warncke, 1981, 1986). This has led several authors to use characteristics other than morphological and morphometrical ones to separate these taxa. Species can be distinguished using enzyme electrophoresis (Scholl et al., 1983, 1992) or the chemical composition of the marking pheromone of the males (Pamilo et al., 1997; Bertsch, 1997a, b). The second characteristic is known to be species-specific. In Scandinavia, the labial gland secretions of males were used to identify two forms of *Bombus lapponicus* (Bergström & Svensson, 1973), which were later classified as distinct species, *Bombus lapponicus* (blonde form) and *Bombus scandiavicus* (dark form) (Svensson & Bergström, 1977) as (1979, 1980) later synonymised *Bombus scandiavicus* with *Bombus monticola*.

The first analysis of the chemical composition of the labial gland secretion of *B. lucorum* males was reported by Calam (1969). He identified the main component (ethyl 9-tetradecenoate) and several of the minor components. *B. lucorum* occurring in Sweden was studied extensively by Kullenberg et al. (1970) and Bergström et al. (1973). The labial gland secretions of individuals of a wide variety of colour, ranging from light yellow to dark, from the island Oland were analysed. While that of the blonde form contained mainly ethyl (Z)-9-tetradecenoate, the dark form produced mainly ethyl dodecanoate (Kullenberg et al., 1979). The “dark” form was later transferred to *B. cryptarum* (Rasmont et al., 1986; Bertsch, 1997a, b).

Bumblebees belonging to the “*B. lucorum* species group” occur in Czech Republic (Tkalcí, 1974, 1999), but the chemical composition of the males’ labial gland secretion is unknown. The purpose of this study was to make a detailed analysis of the labial secretions of specimens collected in different localities in the Czech Republic, to determine the species on the basis of both morphological and chemical traits and to compare our results with the data in the literature.

**MATERIAL AND METHODS**

**Insects.** Males (26 individuals) of the bumblebee species *Bombus lucorum* were collected in the summers of 1994–1999 at seven different localities in the Czech Republic (both in Bohemia and Moravia, Table 1). Some of the males came from colonies that were established artificially using mated females from the previous year. The insect material is deposited in the collection of one of the authors (O.H.).

For the chemical analyses, living insects were transported to the laboratory and then kept in a freezer until the labial glands were dissected. The glands were extracted with hexane (50 µl per gland). After 15 minutes of shaking and 2 h standing in the refrigerator, the hexane extract was filtered and stored in a freezer before analysis. Each sample was analysed separately.

**Gas chromatography.** The extracts were analysed using a gas chromatograph with a splitless injector (200°C) and a mass detector (Fisons MD 800). A BPX5 column (5% phenyl methyl silicone; 30 m × 0.22 mm, film thickness 0.25 µm) and helium (flow 0.55 ml/min at 50°C) were used for the separations. The temperature program started at 70°C (2 minutes delay) after which the temperature was increased to 140°C at a rate of 40°C/min, then to 240°C at a rate of 2°C/min, and finally to 300°C at a rate of 5°C/min. Compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology Library (NIST, U.S.A.) and by co-chromatography with synthetic or commercially available standards.

The double bond positions were determined from mass spectra of dimethyl disulphide (DMDS) adducts of unsaturated components. The configurations of the double bonds in the following compounds were determined (after their chroma-
RESULTS

All the males belonged to the "blonde" form, in particular the medium blonde form as figured in Bergström et al. (1973, Plate 1, Figs 2–4). The material was slightly variable. The variability in the colouration of the Czech males corresponds to that cited for *B. lucorum* males by Rasmont et al. (1986).

The composition of the labial gland secretion of *B. lucorum* males is summarised in Table 2. The positions of the double bonds are specified in Table 2 except for minor components (content < 0.1%) and polysaturated compounds where the DMDS adducts were not found. The configurations of the double bonds, as determined either from the co-chromatography with standards or from infrared spectra (absence of the band 965 cm⁻¹, which is intensive and typical for E-isomers), were Z in all cases.

In all the Czech samples, ethyl (Z)-9-tetradecenoate dominated (mean value 52.9%) but was present in variable quantities. Present in the medium-abundant category (3–10%) were ethyl dodecanoate (5.9%), hexadecanoate (3.6%), ethyl 9-hexadecenoate (3.6%), and tricosane (5.4%). In some samples, relatively high proportions of tetradecenoic acid (2.5%) and hexadecyl tetradecenoate (5.3%) were identified. In the minor category (1–2%) were ethyl tetradecanoate, 7-hexadecenol, 11-octadecenol, ethyl 9-octadecenoate, 9-pentacosene, pentacosane, and tetradecenyl octadecatrienol.

With the exception of 7-hexadecenol, 11-octadecenol and the wax-type esters, all of the above mentioned compounds were identified in the labial gland secretion of the Scandinavian "blonde" form (Bergström et al., 1973). Many trace components (less than 1%) not reported in the literature for *B. lucorum*, were detected in the Czech samples.

Traces of the sesquiterpenic alcohols farnesol and 2,3-dihydrofarnesol (below 0.1%) were detected in Czech bumblebees. The Scandinavian blonde form did not contain any iso-prenoids (Bergström et al., 1973). We also detected several compounds with larger molecules than pentacosane. The majority were present in trace amounts, but substantial quantities of 9-heptacosene and wax-type esters were present, especially in some of the samples. It is possible that these compounds were not analysable by the older techniques, which may account for why they were not recorded previously.

The principal components analysis (PCA) showed a slight tendency for the data to separate into two groups, one containing the males collected in 1997 and one containing all the other males. However, cross-validation revealed no significant components (CSV/SD(1) = 0.97). A PCA, in which hydrocar-
bons and fatty acids were excluded, resulted in a model with one component on the limit of significance (CSV/SD = 0.94, variance explained (1) = 22%) that showed the same pattern of groups. Hydrocarbons present in the gland extracts are not considered to be the active components (Bergman, 1997). Free fatty acids are most probably precursors of the components in the secretion (Bergman, personal communication). Therefore, hydrocarbons and free fatty acids were excluded from the following analysis.

A PLS-DA (for males 1997 Y = -1 and for the other males Y = 1) resulted in one significant component (CSV/SD = 0.69, variance explained in X = 21%, variance explained in Y = 58%). The loading plot showed that the most important constituents separating the males collected in 1997 from the rest were the wax-type esters. They produced higher quantities of hexadecyl dodecanoate (0.4–1.2%), octadecadienyl dodecanoate (0.2–0.7%), hexadecyl tetradecenoate (4.5–22.5%), and tetradecenyl octadecatrienoate (2.4–7.7%). The function of these “heavy” molecules in the secretion of some males remains unknown. No grouping based on origin was observed.

## DISCUSSION

Although the males collected in 1997 differed from the rest of the males, the order of difference is very small compared to that between the Scandinavian dark and blonde forms described by Bergström et al. (1973). Therefore, *B. lucorum* in the Czech Republic are all chemically similar and more similar to the Scandinavian blonde form than to the dark form (Bergström et al., 1973). All the major components we found were also found by Bergström in the blonde form. Compounds reported only from the dark form (ethyl decanoate, ethyl octadecadienoate, geranylgeraniol, and geranylgeranyl acetate) were not found in the Czech males. The literature on "lucorum species group" and our results point to the conclusion that the Czech specimens belonged to *B. (s.str.) lucorum* and not to *B. cryptarum* or *B. magnus* (Bertsch, 1997a). Differences between individuals were most probably due to differences in age or physiological conditions rather than their origin.

The composition of the labial gland secretion of *B. cryptarum* (Bertsch, 1997a) corresponds to that of the "dark form" of *B. magnus* and not to *B. lucorum* (s.str.) (Bertsch, 1997a). The composition of the labial gland secretion of *B. cryptarum* (Bertsch, 1997a) corresponds to that of the "dark form" of *B. magnus*.
lucorum (Bergström et al., 1973). The components of the marking pheromone of B. magnus have not been described in detail. Bertsch (1997a) mentions the two main components (9,12-octadecadienol and 9,12,15-octadecatrienol) of the secretion collected from artificially reared B. magnus, that originated from Scotland. Czech B. lucorum males produced these two compounds, too, but both in small amounts (0.8% and 1.3%, respectively).

B. lucorum is the most common species of the "lucorum species group" in the localities where we collected our specimens. This agrees with the data in the literature. B. cryptarum, B. magnus, B. lapponicus and B. scandinavicus of Bombus lapponicus Fabr. (Hymenoptera, Apidae) gives in the blonde form (semiquantitative data), n.d. = not determined because of the low content of the component

**REFERENCES**

**Fatty Acids**

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<tr>
<th>Fatty Acid</th>
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<td>Dodecanoic acid</td>
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<td>Tetradecenoic acid</td>
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**Hydrocarbons**

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*1 Z-configuration in all cases as determined either by co-chromatographic with standards or from infrared spectra

*2 reported by Bergström et al. (1973) in the blonde form (semiquantitative data), n.d. = not determined because of the low content of the component

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**REFERENCES**


Svensson B.G. 1979: Pyrobombus lapponicus auct. in Europe recognized as two species: P. lapponicus (Fabricius, 1793) and P. monticola (Smith, 1849) (Hymenoptera, Apoidea, Bombinae). Entomol. Scand. 10: 275–296.


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