Volatile Compounds from Cephalic Secretions of Females in two Cleptoparasite Bee Genera, *Epeolus* (Hym., Anthophoridae) and *Coelioxys* (Hym., Megachilidae)

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Dedicated to Professor Holger Erdtman *on the Occasion of His 80th Birthday*

Epeolus, Coelioxys, Cephalic Secretions, Pyrazines, Spiroacetals

Volatile cephalic secretions from females of four cleptoparasitic bee species, *Epeolus cruciger* (Pz.), *E. variegatus,* (L.) (Anthophoridae) and *Coelioxys* (*Coelioxys*) *quadridentata* (L.), *C.* (*Schizocoelioxys*) *mandibularis* Nyl. (Megachilidae) were analysed by combined gas chromatography/mass spectroscopy. There are considerable similarities between the two species within each genus, and also some qualitative correspondences between the two genera. The *Epeolus* species contain spiroacetals, 2-alkanols and pyrazines while the *Coelioxys* species contain spiroacetals, 3-alkanols and 3-ketones along with unsaturated alcohols. Two of the spiroacetals are first found in nature.

Introduction

Cleptoparasitism is a parasitoid relationship in which a female of one species appropriates food resources intended for the offspring of another usually non-conspecific female, to rear her own brood. Mostly she seeks out and enters the host nest, where she lays an egg in a brood cell. In some parasites the female destroys the host egg or larva, while in others the parasitoid larva does.

The evolution of cleptoparasitism in bees and wasps has been treated by Bohart [1], Eickwort [2], and Wheeler [3]. Studies of specific host-cleptoparasite pairs have been carried out (parasite genus given in brackets) by Eickwort and Eickwort [4], Legewie [5], Ordway [6] (*Sphecodes*), Rust and Thorp [7] (*Stelis*), Rozen and Favreau [8] (*Dioxys*), Rozen *et al.* [9] (*Protepeolus*), Rozen and Favreau [10] (*Epeolus*), Thorp [11] (*Melecta*), Eickwort and Abrams [12] and Linsley and MacSwain [13] (*Nomada*). Social parasitism, a closely related phenomenon, is dealt with in books by Michener [14] and Wilson [15].

Most cleptoparasites are host specific and attack single species or a group of closely related species. Such a coexistence involves the development of special behavioral adaptations: in the host they may function to defend the nest and in the cleptoparasite to evade the defence of the host and to facilitate the attack. The importance of odours to release this behaviour has been suggested. That volatile compounds play a role in the parasite localization of the host nest was already observed by Graenicher [16]. On the other hand it can also contribute in the direct interactions between the host and the cleptoparasite females: In many cases it has been observed that solitary bees behave aggressively towards conspecific females while they do not pay attention to their cleptoparasite [17]; again odours might play a role in this connection. In the cleptoparasite genus *Nomada* host odour mimetism is hypothesized to act in the non-aggressive relation between host and parasite females [18]. Ordway [6] suggests that odorous cues emitted by the parasite *Sphecodes pimpinellae* play a role in the intimidation of the host, causing the females of the social nest to stop working or to leave it.

A comprehensive study on the behaviour-releasing and population regulating mechanisms in cleptoparasitism is in progress. The present report is concerned with the identification of volatile compounds in the female cephalic secretion of four cleptoparasite species, *Epeolus cruciger* (Pz.) and *E. variegatus* (L.) such as *Coelioxys (Coelioxys quadridentata)* (L.) and *C. (Schizocoelioxys) mandibularis* Nyl.

Reprint requests to Dr. J. Tengö.

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Materials and Methods

All bees used in this study were collected on the Swedish island of Öland and on the mainland close to it. Taxonomic determinations of the species were kindly checked by Dr. Klaus Warncke.

Methods for isolation and identification of volatile components from cephalic secretions of solitary bees have been described earlier [19, 20]. Synthetic substances were used as reference compounds.

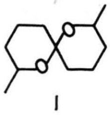
Samples of *Epeolus* have been collected and analysed in 1974, 1978, 1979, 1980 and 1981, *Coelioxys* were collected and analysed in 1974, 1975, 1976, 1978, 1979 and 1981. During the period 1974–1979 a gas chromatographic precolumn was used and separation was achieved with the aid of glass capillary columns coated with OV-101, SE-30, OV-17 and OV-17/FFAP as stationary phases. In 1980 and 1981 samples were introduced into the gaschromatograph by solvent injection (pentane) and WG-11 glass capillary columns were used for separation. Single or a few heads were used in each analysis.

Results

A) *Epeolus:* The results of the isolation and identification work have been summarized in Table I. The main compound in both *Epeolus cruciger* and *E. variegatus* was identified as the known *E,E*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**I**) [21]. Another spiroacetal, present in *E. cruciger*, was identified as *E*-2-methyl-1,7-dioxaspiro[5.5]undecane (**II**). The fragmentation patterns of this new compound follow general rules [19, 22], a plotted spectrum is given in Fig. 1.

Two alcohols, 2-nonanol and 2-undecanol, were found in appreciable amounts in both species. Apart from these compounds the secretion contained pyrazines, which were identified as: 2,5-dimethyl-3-ethylpyrazine (**V**) (trace amounts in *E. cruciger*), 2,5-dimethyl-3-isobutylpyrazine (**VI**) (present in appreciable amounts in both species), 2,5-dimethyl-3-isopentylpyrazine (**VII**) (small amounts in *E. cruciger*) and 2,5-dimethyl-3-(2-methyl)-butylpyrazine (**VIII**) (small amounts in *E. cruciger* and appreciable amounts in *E. variegatus*).

Mass spectral fragmentation patterns of alkylpyrazines have been described [23], the spectra of our *Epeolus* pyrazines completely matched those reported in the literature [24–26]. Synthetic pyrazines have been prepared for reference according to the method of Bramwell [27].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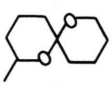
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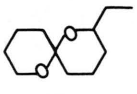
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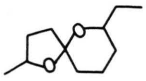
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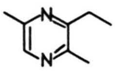
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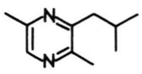
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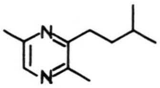
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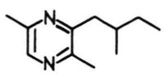
VIII

B) *Coelioxys:* Both *Coelioxys quadridentata* and *C. mandibularis* contained *E*-2-ethyl-1,7-dioxaspiro[5.5]undecane (**III**) (mass spectrum of this new spiroacetal see Fig. 1) together with the known *E,Z*-isomers of 7-ethyl-2-methyl-1,6-dioxaspiro[4.5]decane (**IV**) [22].

Both species also contained secondary alcohols and ketones: 3-nonanol, 3-undecanol, 1-nonen-3-ol, 1-undecen-3-ol, 3-nonanone and 3-undecanone. In

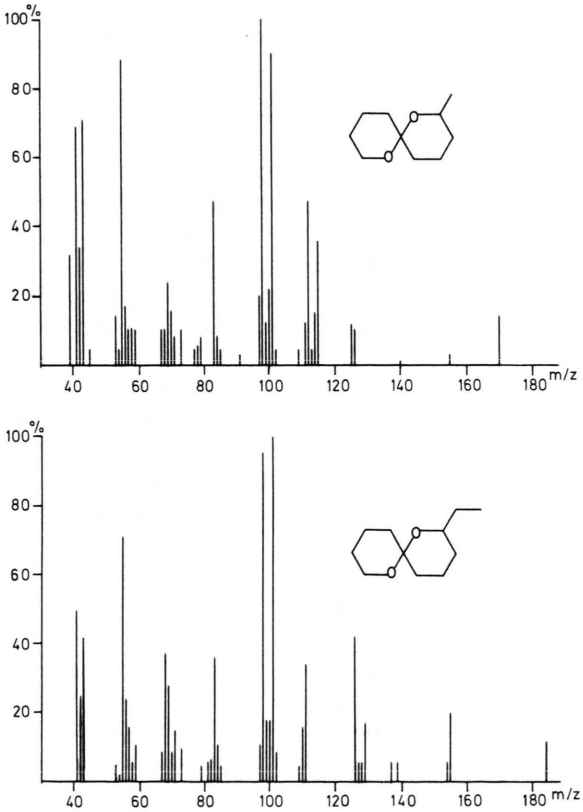

Fig. 1. 70 eV mass spectra of *E*-2-methyl-1,7-dioxaspiro[5.5]undecane and *E*-2-ethyl-1,7-dioxaspiro[5.5]undecane.

Table I. Identified volatile compounds in cephalic secretions of females of *Epeolus* and *Coelioxys* ($\underline{\times}$ Main compound; × present; (×) minor amounts, not consistently found).

Compounds	M	E. c.	E. v.	C. q.	C. m.
Spiroacetals					
E,*E*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane	184	$\underline{\times}$	$\underline{\times}$		
E-2-methyl-1,7-dioxaspiro[5.5]undecane	170	×			
E-2-ethyl-1,7-dioxaspiro[5.5]undecane	184			×	×
E-7-ethyl-*E*-2-methyl-1,6-dioxaspiro[4.5]decane	184			×	×
E-7-ethyl-*Z* 2-methyl-1,6-dioxaspiro[4.5]decane	184			×	×
Acyclic alcohols and ketones					
2-nonanol	144	×	×		
2-undecanol	172	×	×		
3-nonanol	144			×	×
3-undecanol	172			×	×
1-nonen-3-ol	142			×	$\underline{\times}$
1-undecan-3-ol	170			×	×
E-2 nonen-1-ol	142				(×)
3-nonanone	142			×	×
3-undecanone	170			(×)	(×)
Pyrazines					
2,5 dimethyl-3-ethyl-pyrazine	136	(×)			
2,5-dimethyl-3-isobutylpyrazine	164	×	×		
2,5-dimethyl-3-isopentylpyrazine	178	(×)	×		
2,5-dimethyl-3-(2-methyl)-butylpyrazine	178	(×)	×		
Hydrocarbons					
Heneicosane	296	×	×	×	×
Tricosane	324	×	×	×	×

addition traces of *E*-2-nonen-1-ol were sometimes present in *C. mandibularis.*

Vinylcarbinols have been prepared by reaction of acrolein and alkylmagnesiumhalides, 2-nonen-1-ol was obtained by reduction of ethyl-2-nonenoate. Ethylketones and the corresponding secondary vinylcarbinols furnish very similar mass spectra [28], but are well separated by GLC. On many stationary phases the vinylcarbinol appears after the ethylcarbinols while the ketone elutes in front.

All spiroacetals were synthesized by known methods [29].

In both *Epeolus* and *Coelioxys* the ubiquitious hydrocarbons heneicosane and tricosane occur in pretty large amounts.

Discussion

Despite their separate origins and relatively distant relatedness, the genera *Coelioxys* (family Megachilidae) and *Epeolus* (family Anthophoridae) (*cf.* Fig. 2) share many ethological, ecological and morphological characteristics. Their host nest search pattern is very similar, they lay eggs in host cells being in a certain stage of development and their 1st larval instar is heavily armed with long pointed mandibles. Many of the common characteristics are the result of convergence through the parasitic behaviour pattern of the two genera.

Inspection of Table I shows that within the genera both species of *Epeolus* and *Coelioxys* closely

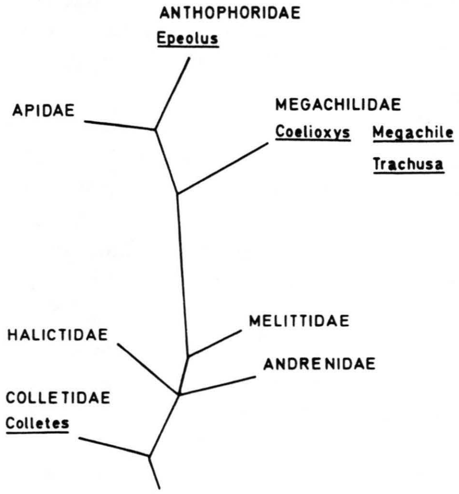

Fig. 2. Dendrogram on Apoidea – only genera dealt with in this report are given (simplified after Michener [14]).

resemble each other in their composition of the female volatile cephalic secretions. Between the two genera, on the other hand, there are distinct differences but also striking similarities. They both contain spiroacetals, but these are of different structures in each genus. *Epeolus* has 2-alkanols in the cephalic secretion, whereas *Coelioxys* possesses 3-alkanols, the corresponding vinylcarbinols and ethylketones. Finally, only *Epeolus* contains pyrazines. Thus the volatile secretions from mandibular glands of *Epeolus* and *Coelioxys* females achieve their characteristic composition by having a blend of at least 7 to 12 compounds, which belong to five different classes: spiroacetals, acyclic alcohols, ketones, hydrocarbons and pyrazines.

Spiroacetals also occur in some species of the cleptoparasitic genus *Nomada* (to be published), which belong to the subfamily Nomadinae as does *Epeolus*. They are, however, frequent in *Andrena* bees (Andrenidae) and in common wasps. *E,E*-2,7-Dimethyl-1,7-dioxaspiro[5.5]undecane is one of the main components in *Andrena wilkella* [21] while the *E,Z*-isomers of 7-ethyl-2-methyl-1,6-dioxaspiro[4.5]-decane do not only occur in the cephalic secretions of solitary bees [19] but also in the abdomina of common wasps [30]. *E*-2-Methyl-1,7-dioxaspiro[5.5]-undecane is besides *E*-2-propyl-1,7-dioxaspiro[5.5]-undecane [21] the second insect-spiroacetal with an even numbered unbranched carbon chain.

Secondary alcohols and ketones are repeatedly found as biologically active compounds in insects. 1-Nonen-3-ol has been identified from the flower stalk of *Petasites japonicus* [31] while 3-nonanone and 1-nonen-3-one are components of the defense secretion of the tenebrionid beetle *Eleodes beameri* [32]. Structural relationships between the latter substances are the same as in the *Coelioxys* compounds. The two nonenols found in the cephalic secretions might well be regarded as a pair, derived from the same precursor. Even the spiroacetals which contain 11 carbon atoms and an oxygen function in "position 3" may originate from the same pool as the openchained compounds. It should be noted, that the *Epeolus* sprioacetals contain an oxygen in the same position as the acyclic alcohols present in the same secretion. Several vinylketones have been found in termites [33]; biogenetic relationships between these compounds and β-ketoaldehydes – which too occur in termites – have been discussed [34].

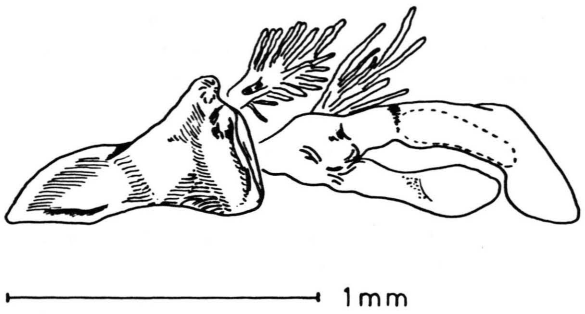

Fig. 3. Excised right mandible and gland reservoir of *E. variegatus* female.

Alkyl-2,5-dimethylpyrazines have been found as flavour constituents of roasted or cocked foods but also occur in insects [35]. 3-Ethyl-2,5-dimethylpyrazine has been reported to act as a trail pheromone in several ant species [36]. While 2,5-dimethyl-3-isopentylpyrazine frequently occurs in the mandibular glands of ants [25, 26, 37], 2,5-dimethyl-3-isobutylpyrazine is more scarce, whereas 2,5-dimethyl-3-(2-methyl)-butylpyrazine to our knowledge has been identified only in an Australian formicine ant of the genus *Calomyrmex* [26], the pyrazines of which are qualitatively the same as in *Epeolus*. Odour correspondences in host-cleptoparasite relationships are found in *Nysson* (parasite) and *Argogorytes* (host), both Sphecidae wasps, which share 2,5-dimethyl-3-isopentylpyrazine in the volatile secretions [38].

It seems very likely that at least the nonhydrocarbons identified above are secreted from the mandibular gland systems. An excised right mandible of *Epeolus variegatus* female with a glandular reservoir is shown in Fig. 3.

In Apoidea cleptoparasitism has arosen at least 16 times as judged from existing parasitic lines [1]. In several cases the host and the parasite, in the relationships occurring in recent days, are taxonomically closely related. The genus *Coelioxys* represents this type. It has arosen within the subfamily *Megachilinae* [14] and parasitizes genera like *Megachile* and *Trachusa* belonging to the same group.

On the other hand, within the subfamily Nomadinae of the family Anthophoridae, a large group of cleptoparasitic genera occurs, most of which are parasites of bees belonging to other families; *Epeolus* is an example of this type. Definite host records of the species reported here are unknown. Indisputable host data can be established only through rearing of the parasite from host cells. These relationships are presently under investigation.

Acknowledgement

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