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STUDIES ON SEX PHEROMONE GLAND MORPHOLOGY AND PHEROMONE COMPONENTS IN FEMALE ELATERID BEETLES *AGRIOTES OBSCURUS* L. AND *AGRIOTES LINEATUS* L. (*COLEOPTERA, ELATERIDAE*)

Abstract. The present paper gives a brief morphological description of the female reproductive system of the elaterid beetle *Agriotes obscurus*. The paired pheromone gland is located in VIII segment of the female abdomen. The length of cistern-like reservoirs of the gland was 0.9—1.3 mm, their width 0.25—0.35 mm. The amount of secretion in two reservoirs of one female pheromone gland, obtained with a fine glass capillary, was 30—40 nl.

Gas liquid chromatography showed that the secretion of the female pheromone gland of *A. obscurus* caught in Estonia consisted of two main components: geranyl hexanoate and geranyl oclanoate whose ratio varies mainly between 0.3—0.7 (average 0.44). However, in a few cases this may reach up to 1.6. The result is in agreement with earlier literature data. Thus, no essential differences seem to occur in the sex pheromone composition of beetles from different geographical localities (North Caucasus, Estonia, Sweden).

The only main component of the pheromone gland secretion of *A. lineatus* beetles caught in Estonia is geranyl octanoate. Geranyl hexanoate and evidently also farnesyl acetate occur as minor components. The pheromone gland secretion of beetles caught in North Caucasus consists of two main components and over ten minor components. One main component is farnesyl acetate, but the other could not be identified by comparison with standard compounds. Geranyl hexanoate and geranyl octanoate occur here as minor components.

Thus, there are significant differences in the sex pheromone composition of female *A. lineatus* beetles originating from North Caucasus and Estonia. Probably, this is the case of two subspecies. Such a conclusion would also clear up contradictory literature data on the pheromone composition of *A. lineatus*.

Introduction

Insect sex pheromone is produced by specialized exocrine cells or glands whose location and structure depend on the systematic belonging of the species (Percy and Weatherston, 1974; Джекобсон, 1976). In the case of elaterid beetles, two types of pheromone glands have been morphologically described. In the species *Selatosomus latus* F. the gland is located on an inter-segmental membrane between VIII and IX abdominal segments as a thickened folded plate. The secretion reservoir of the gland

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is lacking (Иващенко и Адаменко, 1980). A different structure of the pheromone gland has been described in the species *Agriotes litigiosus* (Иващенко, Адаменко, 1971) and *A. gurgistanus* Fald. (Иващенко, Олещенко, 1974) in which the gland is located as two sacklike formations in the female abdomen. It is supposed that the existence of a more or less formed gland reservoir is characteristic of all female beetles of the species belonging to the subfamily *Elaterinae* (Орлов, Исмаилов, 1986).

However, more detailed morphological data on the localization and structure of the pheromone glands of *A. obscurus* and *A. lineatus* are lacking. Such data are necessary for the study and solution of physiological and ethological problems of pheromone excretion, and for the collection of the amount of pheromone sufficient for identification. The present work serves as an attempt to fill this gap. Since it became evident, in the course of work, that the morphological difference in the reproductive systems of the two species under study is not significant, only a brief description of the female reproductive system of *A. obscurus* will be presented.

According to literature data, the sex pheromone of *A. obscurus* consists of two main components: geranyl hexanoate and geranyl octanoate (Borg-Karlson et al., 1988; Олещенко et al., 1986) and a number of minor components (Яцынин et al., 1988). It is shown that there are differences in the pheromone composition of individuals from various geographical regions, which are reflected in differences between the ratio of the two main components and in the presence or absence of some minor components (Яцынин et al., 1988).

As to the pheromone composition of *A. lineatus*, literature data are strongly divergent (Borg-Karlson et al., 1988; Яцынин et al., 1980; Яцынин, Лебедева, 1984), which is evidently due to the fact that the female beetles used have been collected from different geographical regions. The clarification of the differences in the pheromone composition of the beetles of one and the same species inhabiting geographically different regions is of theoretical as well as of practical interest. The present paper gives a gas liquid chromatographic analysis of the pheromone composition of female beetles caught in Estonia (*A. obscurus* and *A. lineatus*) and North Caucasus (*A. lineatus*), and makes an attempt to compare them with each other and with earlier literature data.

Material and methods

The beetles used in the morphological part of the work were collected from the soil of South Estonian fields in September-October 1986—1990. At that time female beetles of *A. obscurus* and *A. lineatus* are inactive and start overwintering in the pupal cradle. For undergoing the reproductive diapause beetles were kept in a refrigerator at low above-zero temperatures until spring. 15 sexually mature and 15 sexually immature female beetles of either species were intersected by means of microinstruments under a binocular microscope in a water vessel. Preparations of the reproductive system were stained with eosin in a drop of water. Fresh preparations were observed under an optical microscope (magnification up to 250×), the necessary measurements, photographs and drawings were made.

When analysing sex pheromone sexually mature beetles were used which were collected in May—June 1988—1990 in South Estonia (*A. obscurus*, 21 individuals; *A. lineatus*, 2 individuals) and in North Caucasus (*A. lineatus*, 2 individuals). The secretion was collected by

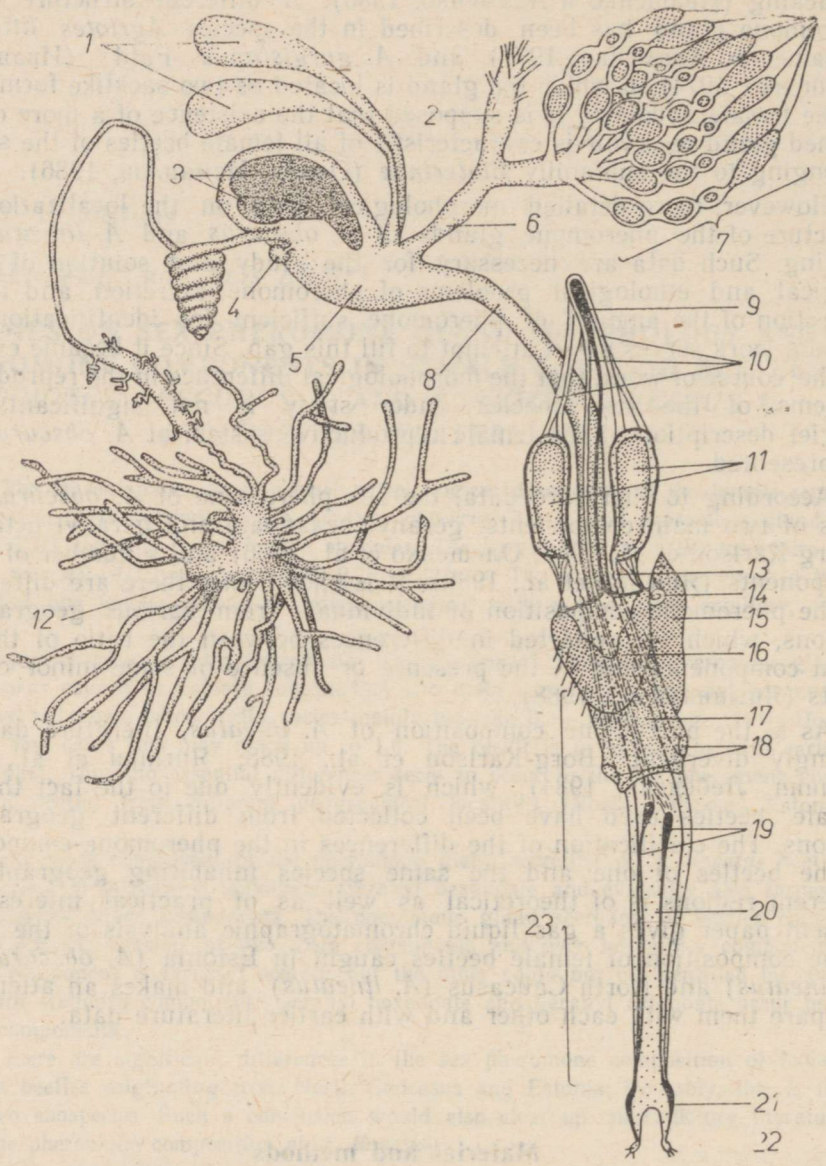

Fig. 1. Reproductive system of sexually mature female *A. obscurus*. *1* — paired accessory gland; *2* — oviduct; *3* — chitin plates; *4* — spermatheca; *5* — bursa copulatrix; *6* — common oviduct; *7* — ovary; *8* — vagina; *9* — appendage of VIII sternite; *10* — muscles of the pheromone gland and pseudo-ovipositor; *11* — pheromone gland; *12* — azygous accessory gland; *13* — excretory duct of the pheromone gland; *14* — stigma; *15* — VIII tergite; *16* — VIII sternite; *17* — thickened part of the intersegmental membrane; *18* — openings of the excretory ducts of the pheromone gland (pseudo-ovipositor pockets); *19* — appendages of IX sternite; *20* — thin transparent part of the intersegmental membrane; *21* — IX sternite; *22* — terminal (X) abdominal segment (tentacle-like appendages of the pseudo-ovipositor); *23* — telescopiform protruding pseudo-ovipositor.

means of a fine glass capillary directly from the reservoirs of the female pheromone gland. This method allows to study individual peculiarities of the pheromone composition, since the amount of secretion collected from one female beetle (about 30—40 nl) is sufficient for gas liquid chromatography. The secretion is a colourless liquid with a weak pleasant smell, which a female beetle excretes into environment as pheromone. The secretion does not contain any ballast materials and therefore does not need special purification. The pheromone separated in this way has high biological activity.

Gas liquid chromatography was performed by means of the chromatograph "Chrom 5" (Laboratorne Pristroje, Czechoslovakia) with a flame ionization detector. A 23 m quartz capillary (with the inner diameter 0.22 mm) was used which was coated with OV-101 on the inside. Helium (1.0 cm^3/min) was used as eluent gas. The temperature of the column was 160 °C, and that of the evaporator 230 °C.

Results and discussion

Morphology of the reproductive system in female *A. obscurus*

The reproductive system of female *A. obscurus* consists of ovaries, genital ducts together with accessory glands and a pseudo-ovipositor. A paired pheromone gland also belongs to the ovipositor (Fig. 1).

In elaterid beetles the development, maturation and secretion activity of ovaries are under the control of the endocrine system. It has been demonstrated that affecting diapausing female beetles of *Agriotes sputator*, *A. proximus*, *Ampedus ochropterus*, *A. coenobita* with a juvenile hormone analogue interrupts the diapause and activates the development of the whole reproductive system (Орлов, Исмаилов, 1986). In natural conditions reactivation occurs as a result of low above-zero temperatures. In autumn, before overwintering in soil, the ovaries and pheromone glands of female beetles of both species investigated were not developed (reservoirs of the pheromone gland were empty).

In spring, overwintered female beetles emerge from soil a few weeks later (in May) than male beetles. Their ovaries are not fully developed yet, but reservoirs of the pheromone gland are filled up (Fig. 1). Later, as eggs are maturing, ovaries expand and occupy a greater part of the abdominal cavity. The number of ovarioles in 15 female beetles studied ranged between 51 and 67, and they were divided between the right and left duct in most cases unequally. The average number of eggs per one female beetle was 338, in a few cases it amounted to more than 400.

The genital ducts of female *A. obscurus* consist of paired lateral oviducts and a common oviduct. The oviducts are surrounded by a muscular shell which is especially well developed in the common oviduct. The common oviduct falls into bursa copulatrix. The latter contains chitin plates covered with multiple short thorns. A strongly branching azygous accessory gland is attached to the spermatheca by means of an excretory duct. The paired accessory gland falls into bursa copulatrix near the common oviduct. According to literature data, the paired accessory gland is absent in *Selatosomus latus* (Иващенко, Адаменко, 1980) but is present in *Agriotes gurgistanus* (Иващенко, Олещенко, 1974).

A telescopiform protruding pseudo-ovipositor is formed by VIII, IX and X abdominal segments. The tip of the ovipositor consists of two small cylindrical segments which correspond to the abdominal terminal (X)

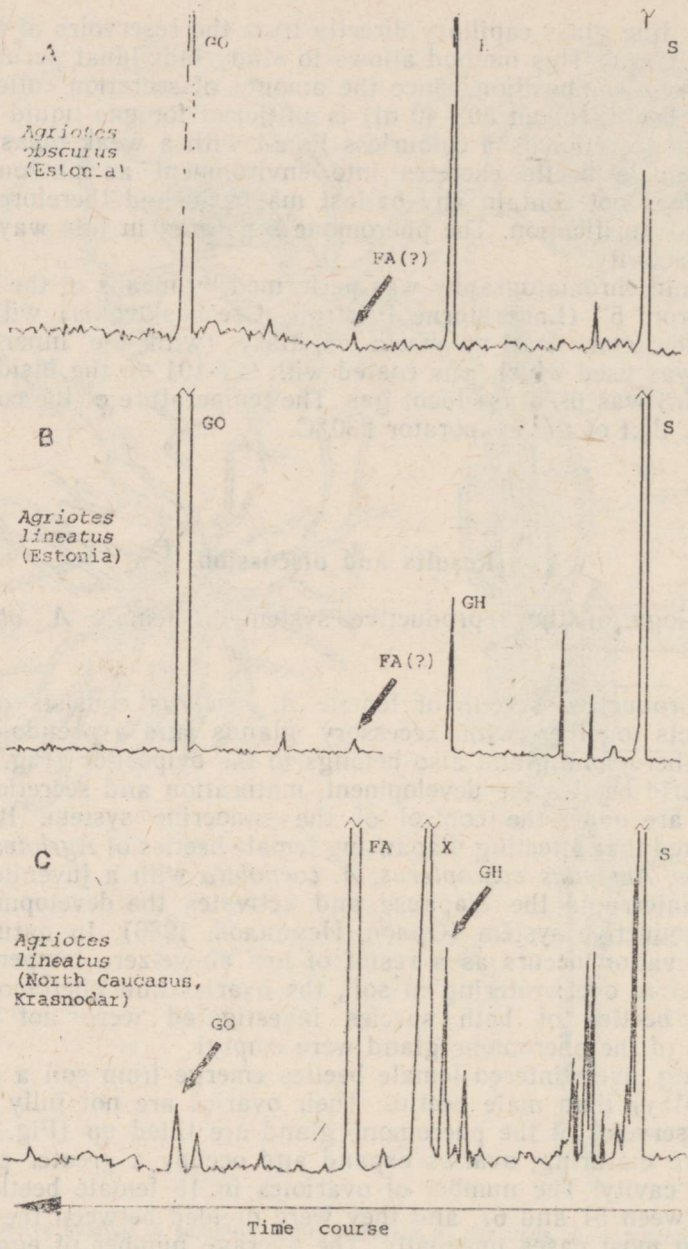

Fig. 2. Gas liquid chromatograms of the secretion of the pheromone gland of female *A. obscurus* and *A. lineatus*. *A*, *B* — beetles caught in Estonia; *C* — beetles caught in North Caucasus.

S — solvent; GH — geranyl hexanoate; GO — geranyl octanoate; FA — farnesyl acetate; X — unknown component.

sclerite. The long thin chitin appendages of VIII and IX sternites form the skeleton of the pseudo-ovipositor. To these are attached the greater part of pseudo-ovipositor muscles. Between the sclerites of VIII and IX segments is located an extensive intersegmental membrane. Its anterior part represents a whitish non-transparent thickened fold. The cell contours of the one-layer epidermis are visible under the light microscope. The intersegmental membrane is thinner in its posterior part and is wholly transparent. The cell structure is not observable.

The paired pheromone gland is located in VIII abdominal segment and is attached to the sternite with muscular fibres. The reservoirs of the gland are connected with the intersegmental membrane by means of thin winding excretory ducts which are dilated before opening on the body surface, forming pseudo-ovipositor pockets. The excretory ducts are spirally surrounded by thin muscular fibres which are functionally evidently related to the excretion of secretion. The length of gland reservoirs in sexually mature females was 0.9—1.3 mm, width 0.25—0.35 mm. The amount of secretion in two reservoirs of one female pheromone gland was 30—40 nl. Behavioural experiments with male beetles showed that the secretion of the gland has high biological activity.

Gas liquid chromatography of the sex pheromone of *A. obscurus* and *A. lineatus*

Gas liquid chromatography showed that the pheromone of female *A. obscurus* caught in Estonia consists of two main components and several minor components (Fig. 2*A*). Comparing the chromatograms of synthetic standard compounds and the pheromone of *A. obscurus*, the total temporal coincidence of the peaks of geranyl hexanoate and geranyl octanoate, and those of two main components of the pheromone under study was observed. Such a result is in agreement with earlier literature data (Borg-Karlson et al., 1988; Олещенко et al., 1986). It can be supposed that one of the minor components is farnesyl acetate, since its peak on the chromatogram coincides temporally with the peak of the corresponding standard compound. However, a small farnesyl acetate peak occurred on the chromatogram of only every fourth female pheromone.

The comparison of the chromatograms of 21 female pheromones showed that the ratio of the main components, geranyl hexanoate and geranyl octanoate, varies mainly between 0.3—0.7 (average 0.44). However, in a few cases this may reach up to 1.6.

The female pheromone of *A. lineatus* beetles caught in Estonia consisted of only one main component and 6—7 minor components (Fig. 2*B*). When comparing the chromatograms of the pheromone and the corresponding standard compounds, it became evident that the main pheromone component of the Estonian population of *A. lineatus* is geranyl octanoate. Of minor components we succeeded in identifying, in the same way, geranyl hexanoate and probably also farnesyl acetate. The ratio of geranyl hexanoate and geranyl octanoate in the pheromone was 0.02.

The female pheromone of *A. lineatus* caught in North Caucasus consists of two main components and over ten minor ones (Fig. 2*C*). It was found that in the present case, too, geranyl hexanoate and geranyl octanoate belong to the composition of the pheromone but both as minor components. The peak of one main pheromone component on the chromatogram coincided temporally with that of farnesyl acetate which served as a standard compound. The other main component (Fig. 2*C*, X) could not be identified by comparison with standard compounds.

Literature data pertaining to the pheromone composition of *A. lineatus* are contradictory. It has been demonstrated (Яцынин et al., 1980) that the pheromone of *A. lineatus* consists of more than ten components two of which were identified as *trans,trans*-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol acetate (*trans, trans*-farnesyl acetate) and *cis*-3,7-dimethyl-2,6-octadien-1-ol-3-methyl butanoate (neryl isovaleriate). Four years later it was established (Яцынин, Лебедева, 1984) that besides farnesyl acetate (62.4%) as the other main component, the pheromone of *A. lineatus* contains a substance (34.4%) whose structure is *trans, trans*-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol octanate (*trans, trans*-farnesyl caprilate). In addition to the structure of the two main components, that of seven minor components was determined as well; geranyl hexanoate and geranyl octanoate were not mentioned among them. Unfortunately, the above-mentioned two papers do not refer to the geographical regions from where the beetles were collected but evidently they originate from North Caucasus. According to the data of Swedish researchers (Borg-Karlson et al., 1988) the pheromone of *A. lineatus* consists of only one component — geranyl octanoate. Different results are probably due to the fact that the beetles used in analysis had been collected in different geographical regions of the distribution area.

Thus, basing on our data, the composition of the female pheromone of *A. obscurus* caught in Estonia does not significantly differ from that reported in earlier literature data. The pheromone composition of beetles inhabiting various geographical regions can reveal only insignificant differences which do not evidently disturb the odour communication between female and male beetles originating from different parts of the distribution area.

The composition of the pheromone in *A. lineatus* beetles collected in North Caucasus coincided with literature data (Яцынин et al., 1980; Яцынин, Лебедева, 1984) only with respect to one main component, farnesyl acetate. The other main component is, according to our data, evidently not farnesyl caprilate since its retention time is longer than in the case of farnesyl acetate (Яцынин, Лебедева, 1984), while the retention time of the other main component is shorter than in the case of farnesyl acetate (Fig. 2*C*, X).

The composition of the pheromone of *A. lineatus* beetles caught in Estonia is in agreement with the results of Swedish researchers with respect to the main component (Borg-Karlson et al., 1988).

Thus, there occur drastic differences in the pheromone composition of female *A. lineatus* beetles caught in Estonia and North Caucasus. Probably, this is the case of at least two subspecies. The composition of the female pheromone of the Estonian population of *A. lineatus* resembles more the pheromone of the species *A. obscurus* than the female pheromone of the North Caucasian population (Fig. 2).

Our results are also supported by field experiments performed with pheromone traps (Яцынин et al., 1980; Мяхар, 1985). The pheromone traps of *A. lineatus* with an attractive mixture (1 mg per trap) consisting of synthetic neryl isovaleriate (1%) and *trans, trans*-farnesyl acetate (99%), which were used in North Caucasus (Krasnodar), were not less effective than the traps baited with female beetles. The traps with the same mixture, used in Estonia, caught few male beetles. The problem why a few male beetles of the Estonian population of *A. lineatus* still fell into traps during these experiments, remains unsolved. The composition of the female pheromone of the Estonian population of this species is, after all, quite different from the mixture used in traps. A possible explanation could be that synthetic substances are not perfectly pure, and the pheromone preparation could contain, as an undesirable addition, some com-

ponent belonging to the composition of the female pheromone of the Estonian population of this species. It can also be supposed that although the components of the synthetic attractive mixture are lacking in the female pheromone composition of the Estonian population, they still exert a weak attractive effect on the male beetles of the Estonian population. The circumstance that in Estonian conditions, besides a few male beetles of *A. lineatus*, also the male beetles of *A. obscurus* fell into traps, and even in a greater number (Мяхар, 1985), supports these two different suppositions.

The fact that the natural pheromone of the Estonian population of *A. lineatus* and female *A. obscurus* beetles contains common components, is confirmed by field experiments with pheromone traps carried out in Estonia (Мяхар, 1985). The experiments showed that the pheromone traps of *A. obscurus* (the attractive mixture consisted of synthetic geranyl hexanoate and geranyl octanoate) caught also male *A. lineatus* beetles. Moreover, the traps of *A. obscurus* caught more male *A. lineatus* beetles than the traps of *A. lineatus* themselves.

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TUME-VILJANAKSURI *AGRIOTES OBSCURUS* L. JA TRIIBULISE VILJANAKSURI *AGRIOTES LINEATUS* L. (*COLEOPTERA, ELATERIDAE*) FEROMOONINÄÄRME MORFOLOOGIA JA SUGUFEROMOONI KOMPONENTIDE UURIMINE

On antud naksurlase *Agriotes obscurus* emasmardika reproduktiivse süsteemi morfoloogiline lühikirjeldus. Paariline feromooninääre asub emasmardika tagakeha VIII segmendis. Näärme tsisternjate reservuaaride pikkus on 0,9—1,3 mm ja laius 0,25—0,35 mm. Peene klaaskapillaari abil kättesaadava sekreedi hulk ühe mardika feromooninäärme kahes reservuaaris kokku on 30—40 nl.

Gaasikromatograafiline analüüs näitas, et liigi *A. obscurus* Eestist püütud mardikate feromooninäärme sekreet koosneb kahest põhikomponendist: geranüülheksanoaadist ja geranüüloktanoaadist, mille omavaheline suhe muutub peamiselt vahemikus 0,3—0,7 (keskmiselt 0,44), üksikutel juhtudel võib ulatuda kuni 1,6. Saadud tulemus on kooskõlas kirjanduses varem avaldatud andmetega. Seega liigi *A. obscurus* erinevatest geograafilistest piirkondadest (Põhja-Kaukaasia, Eesti, Rootsi) pärinevate mardikate suguferomooni koostises ei näi esinevat olulisi erinevusi.

Liigi *A. lineatus* Eestist püütud mardikate feromooninäärme sekreedi ainsaks põhikomponendiks on geranüüloktanoaat. Geranüülheksanoaat ja tõenäoliselt ka farnesüülatsetaat esinevad minoorsete komponentidena. Põhja-Kaukaasiast püütud mardikate feromooninäärme sekreet koosneb kahest põhikomponendist ja rohkem kui kümnest minoorsest komponendist. Üheks põhikomponendiks on farnesüülatsetaat, teist ei õnnestunud etalonainetega võrdlemisel identifitseerida. Geranüülheksanoaat ja geranüüloktanoaat esinevad minoorsete komponentidena.

Seega liigi *A. lineatus* Põhja-Kaukaasiast ja Eestist pärinevate mardikate suguferomooni koostises on olulisi erinevusi. Tõenäoliselt on siin tegemist kahe alamliigiga. Selline järeldus tooks selgust ka kirjanduses avaldatud andmete vasturääkivusele liigi *A. lineatus* feromooni koostise osas.

Энно МЕРИВЕЭ, Антс ЭРМ

ИЗУЧЕНИЕ МОРФОЛОГИИ ФЕРОМОННОЙ ЖЕЛЕЗЫ И КОМПОНЕНТОВ ПОЛОВОГО ФЕРОМОНА ЩЕЛКУНОВ ТЕМНОГО (*AGRIOTES OBSCURUS* L.) И ПОЛОСАТОГО (*AGRIOTES LINEATUS* L.) (*COLEOPTERA, ELATERIDAE*)

Парная феромонная железа находится в восьмом абдоминальном сегменте самки. Количество секрета, полученного из двух резервуаров феромонной железы одной самки при помощи тонкого стеклянного капилляра, составляло 30—40 нл.

Методом газожидкостной хроматографии в составе феромона щелкуна темного, выловленного в Эстонии, основными компонентами идентифицированы геранилгексаноат и геранилоктаноат. Соотношение этих двух компонентов в основном изменялось в пределах 0,3—0,7 (в среднем 0,44), в отдельных случаях достигало 1,6. Полученный результат соответствует данным литературы. Таким образом, в составе полового феромона самок щелкуна темного, обитающего в разных географических районах (Северный Кавказ, Эстония, Швеция), не наблюдается существенных различий.

Единственным основным компонентом секрета феромонной железы самок щелкуна полосатого (*A. lineatus*), обитающего в Эстонии, идентифицирован геранилоктаноат. Геранилгексаноат, а вероятно, и фарнезилацетат наблюдаются в качестве минорных компонентов. Секрет феромонной железы особей, пойманных на Северном Кавказе, состоит из двух основных компонентов и более десяти минорных компонентов. Один основной компонент был идентифицирован как фарнезилацетат, второй компонент не удалось идентифицировать методом сравнивания хроматографических пиков феромона и эталонных веществ. В качестве минорных компонентов наблюдались геранилгексаноат и геранилоктаноат.

Таким образом, можно утверждать, что в составе полового феромона самок щелкуна полосатого, обитающего на Северном Кавказе и в Эстонии, имеются существенные различия. Вероятно, мы имеем дело с двумя подвидами. Такой вывод может послужить объяснением для имеющихся в литературе противоречий, касающихся состава феромона щелкуна полосатого.