A comparative study of sex pheromone reception in the Hadeninae (Lepidoptera: Noctuidae)

MICHEL RENOU, PHILIPPE LUCAS, JEAN-CHRISTOPHE DORE,* BERNARD LALANNE-CASSOU,[†] JEAN-PIERRE CHAMBON and CHRISTIAN COCQUEMPOT[‡] INRA Laboratoire des Médiateurs Chimiques, Magny les Hameaux, *Laboratoire de Chimie Appliquée aux Corps Organisés, U.A. 401 CNRS, Muséum National d'Histoire Naturelle, Paris, [†]INRA, Station de Zoologie, and [‡]Laboratoire de Faunistique, CNRA, Versailles

Abstract. A comparative study of the olfactory responses to pheromone compounds was performed in twenty-four species of Hadeninae. Electroantennograms (EAG) were recorded on male moths in response to thirty compounds and the response profiles of each species were analysed by factorial correspondence analysis. A limited number of molecules were found active and the most effective stimuli were Z9 tetradecenes and Z11 hexadecenes. The species of the *Mythimna* genus were most sensitive to aldehydes. *Tholera decimalis* did not respond to any of the molecules tested. The species belonging to the *Mamestra* and the *Orthosia* genera responded to a variety of molecules and no simple correlation was found between the genus and the EAG sensibility. Pheromone reception is discussed in relation to the taxonomy and the evolution of olfactory communication in Hadeninae.

Key words. Lepidoptera, Noctuidae, Hadeninae, sex pheromones, electroantennography, multivariate analysis.

Introduction

The antennae of male moths bear numerous olfactory neurones narrowly tuned to sex pheromone compounds. Global antennal responses to pheromone components (electroantennograms or EAGs; Schneider, 1962) have been extensively recorded. Commonly, EAG screenings of series of synthetic analogs or isomers are performed to draw the response profile of a moth species. Peaks of activity on the

EAG response profile reveal the most active molecules, among which a key compound corresponds with the major constituent of the pheromone secretion. Thus, EAG technique has been extensively used for both characterization and identification of the sex pheromone components of moths (Roelofs, 1984).

However, there are very few comparative analyses of EAG responses of several moth species. Although biochemical and behavioural studies are needed to determine the composition of the multicomponent pheromone blends, all the behaviourally relevant compounds are necessarily detected through sensory neurones and a good knowledge of the peripheral olfactory system is essential. A description of the differences and the similarities in the responses

Correspondence: Dr M. Renou, INRA, Laboratoire des Médiateurs Chimiques, Domaine de Brouessy, F78114 Magny les Hameaux, France.

of the olfactory receptors of related moth species to a common set of pheromone compounds might provide information on many important aspects of pheromone communication in a taxonomic group: the chemical nature of detected pheromone compounds and their specificity, the sensory equipment and sensory coding in relation to pheromone blends, the evolutionary trends in the pheromone reception system. EAG recording procedures might be standardized and comparative studies are facilitated by multivariate methods in analysis of response-profiles (Renou *et al.*, 1988).

In this paper we report a comparative study of the antennal sensitivity of a sample of palae-arctic species belonging to a Noctuidae sub-family, the Hadeninae. Hadeninae are triline noctuid moths characterized by their hairy eyes and the absence of spines on their tibia. They are active at night and polyphagous in general, but some of them can be more specialized. Larvae eat the aerial part of plants. Generally plurivoltines, they overwinter as pupae or larvae. Several species damage crops and integrated programmes including sex attractant trapping have been developed to control their populations. A better knowledge of pheromone signal processing by the olfactory system is needed to improve effectiveness and specificity of synthetic pheromone blends.

Materials and Methods

Insects. Native males were caught by a light trap illuminated with a 160 W mercury vapour lamp. The trap was made of a tin funnel which led the moths to a gauze sack where they were retained. They were transferred into plastic boxes and fed with sugar solution until electro-antennograms were recorded, within 3 days after their capture. The offspring of mated females of some species were laboratory reared on an artificial diet.

Most of the species were caught in Ile de France with a light trap permanently installed from March to October in the immediate vicinity of the laboratory, in an area of deciduous forest and field. Different genera of Hadeninae succeeded each other in the trap from early spring to late summer: *Orthosia* spp. in March and April were followed by *Mamestra* Ochsenheimer and *Mythimna* Ochsenheimer from June

to September. Some other Hadeninae species were caught by episodic light trapping in various localities of France. Identification of the specimens was carried out by three of us (J.-P.C., C.C. and B.L.-C.) after the EAGs had been performed by careful examination of the male genitalia. Specimens belonging to twenty-four different species were caught in sufficient number to enable quantitative analysis. The species are listed in the appendix according to the order proposed by Leraut (1980) and are followed by the place and the month of their catch.

Compounds. Thirty pheromone compounds were chosen after consultation of the literature on pheromones or sex attractants of Hadeninae (Arn *et al.*, 1986). These included true pheromone components or attractants and closely related structures (Fig. 1). Purity, checked by gas chromatography, was better than 95%. Formulae of tested molecules are in the form commonly used in the pheromone literature, in which each molecule is designated according to its distinctive structural features, i.e. stereochemistry and location of the double bond (Z9-, E11-, etc.), length of the hydrocarbon chain (10, 12, 14 or 16 carbon atoms), terminal functional groups (primary alcohol, OH; aldehyde, Ald; acetate, Ac). Thus, 16:Ac, Z7-12:Ac, Z9-14:Ald and Z11-16:OH designated hexadecanyl acetate, (Z)-7-dodecenyl acetate, (Z)-9-tetradecenol and (Z)-11-hexadecenol, respectively.

Electrophysiology. Electroantennograms (EAGs) were recorded from male moths secured in a styrofoam block. The last antennal segments were snipped off and the distal end of the antenna was inserted in the tip of the recording electrode. The indifferent electrode was implanted in the neck of the insect. Both electrodes were glass capillaries filled with Roeder's solution (NaCl: 9 g/l; KCl: 0.2 g/l; glucose: 4.36 g/l). Electrodes were connected to a Neurolog NL 102 D.C. preamplifier through chlorinized silver wires. The signal was filtered (DC to 1 kHz), fed into an oscilloscope, and monitored on a storage display screen.

To stimulate the olfactory receptors, puffs of air (0.5 s; 1.5 l/min) were blown on the antenna through a glass cartridge containing 0.5 µg of the test compound deposited on a piece of filter paper. The cartridges were used for several experiments and were kept refrigerated at -30°C in glass tubes sealed with Parafilm be-

tween test series. Since all the species were not available at the same time of year, different sets of cartridges were used.

Statistical analysis. Raw EAG data (in mV) were corrected to eliminate the differences of absolute sensitivity between individuals or species. All responses of an individual to a given compound were divided by its mean response to the thirty compounds. Measurements were replicated on at least three different animals. Means of the corrected data and their confidence intervals were computed and displayed in a table giving the average response in corrected EAG values of each species to each molecule.

These data were subjected to a Factorial Correspondence Analysis (FCA; Benzécri, 1980). This multiparametric methodology using χ^2 allows one to look upon the field of molecules and the field of insects symmetrically. Positive or negative correlations between chemical structures and insect species can be found by comparing their coordinates on each factorial axis (φ_1 to φ_n). Absolute contribution (AC) of an item (either a molecule or an insect species) to a given factor indicates the level of contri-

bution of this item to the factor. Relative contribution ($RC = \cos^2(\varphi_n)$) of an item reflects the level of explanation of this item by the factor φ_n . In short, high AC and RC indicate that the position of the item is well explained by the investigated factor. We used a 16/32 bits Hewlett-Packard model 9836 microcomputer of 512 kbytes of central memory and a program rewritten by us in BASIC from a Fortran Anacor software (Lebart *et al.*, 1979).

Results

General characteristics of EAG response profiles

The EAG responses of a male moth to the thirty pheromone compounds allowed us to draw its EAG response profile. The profiles of males belonging to the same species were similar, so that the mean response profile for a given species could be calculated. Conversely, EAG response profiles of different species exhibited important differences (Fig. 1) and were species specific. Each EAG response profile

Fig. 1. EAG response profiles of twenty-four Hadeninae palaearctic species to thirty common components of sex pheromone of Noctuidae. The diameters of the circles are proportional to the EAG mean corrected values.

revealed high sensitivity of the olfactory receptors for a few compounds. Most of the profiles were dominated by a key compound (Priesner et al., 1975) that elicited the EAGs with the highest amplitudes. Close analogues of the key compound also produced significant responses, but E stereoisomers generally did not.

Analysis of EAG response profiles

Hada Billberg. The key compound of *Hada nana* was Z9-14:Ac. Moreover, the EAG response profile (Fig. 1) revealed good responses to Z9-12:Ac, Z7-14:Ac and Z11-14:Ac.

Polia Ochsenheimer. Z9-14:Ald was the key compound of *Polia bombycina*, followed by the corresponding acetate (Z9-14:Ac) and alcohol (Z9-14:OH). Another aldehyde, (Z11-16:Ald, and two dodecenyl acetates (Z7-12:Ac and Z9-12:Ac) elicited significant EAGs. The single individual of *P. nebulosa* that was tested showed maximal sensitivity to E11-14:Ac, an unusual structure in Hadeninae on account of the E stereochemistry of its double bond.

Mamestra Ochsenheimer. Z11-16:Ac appeared to be the key compound in four *Mamestra* species, *M. brassicae*, *M. suasa*, *M. oleracea*, and the single specimen of *M. contigua* studied (Fig. 1). This is in accord with the reports on the composition of their sex pheromone secretions. Z11-16:Ac is the most abundant constituent of the pheromone secretion of *M. brassicae* (Descoins et al., 1978; Struble et al., 1980; Farine et al., 1981; Attygale et al., 1987), *M. suasa* (Töth et al., 1986; Frérot et al., 1988) and *M. oleracea* (Descoins et al., 1978). In EAG screening, *M. oleracea* showed greater sensitivity than *M. brassicae* and *M. suasa* to Z11-16:OH, a component of the female secretion (Descoins et al., 1978). *M. suasa* and *M. brassicae* had very similar EAG response profiles with two, almost equal, peaks of sensitivity for Z11-16:Ac and Z9-14:Ac. *M. suasa* could be distinguished from *M. brassicae* by its stronger responses to aldehydes in general and Z11-16:Ald in particular. Z11-16:Ald is a minor component of the sex pheromone blend of *M. suasa* (Töth et al., 1986; Frérot et al., 1988).

Z9-14:Ac was the key compound of *Mamestra biren*, the major component of the female pheromone (Frérot et al., 1987). *M. biren*

responded also to Z9-12:Ac, but poorly to Z11-16:Ac, unlike the former four *Mamestra* species. High amplitude EAGs in response to tetradecenyl acetates (Z9-14:Ac and Z11-14:Ac) were also observed in *M. pisi* (Doré & Renou, 1985) whose pheromone is a mixture of Z9-14:Ac and Z11-14:Ac (Renou et al., 1981). Specific receptor cells for Z9-14:Ac and Z11-14:Ac have been found in the trichoid sensilla of the antennae of male *M. pisi* (Priesner, 1980). *M. thalassina* was characterized by its sensitivity to aldehydes. Its key compound was Z11-16:Ald. Z11-16:Ald could be the key compound for *M. w. latinum* as shown by the responses of the single tested specimen of this species.

M. persicariae differed from the other *Mamestra* species by its responses to a tetradecenyl acetate with a double bond in an unusual position, Z7-14:Ac. A few other acetates were active: Z7-12:Ac, Z9-14:Ac, Z5-12:Ac and Z11-14:Ac. So *M. persicariae* could be characterized by its general sensitivity to C14 and C12 acetates and by its specific key compound.

Tholera Hübner. *T. decimalis* exhibited a lack of sensitivity to the thirty compounds screened (Fig. 1). Consequently, the chemical structure of its pheromone is very probably different from that of other Hadeninae. This was corroborated by high amplitude EAGs (over 3 mV) in response to the trienic hydrocarbon Z,Z,Z-(3,6,9)-heneicosatriene instead of less than 1 mV in other Hadeninae. Polyenic hydrocarbons are common in Catocalinae sex pheromones (Renou et al., 1988). The presence of the same chemical structures in the sex pheromone of *T. decimalis*, if confirmed by analysis of the secretion, would be puzzling in relation to taxonomy.

Egira Duponchel. High sensitivity to tetradecenyl acetates and specially to Z9-14:Ac was detected in *E. conspicularis* (Doré & Renou, 1985; Renou, unpublished data). Unfortunately, not enough males were caught to draw the response profile of this species.

Orthosia Ochsenheimer. Z9-14:Ac was the key compound of three species: *O. gothica*, *O. cruda* and *O. miniosa* whose EAG response profiles looked very similar (Fig. 1). Although Z9-14:Ac was also very active in *O. gracilis*, the key compound of this fourth species was the hexadecene analogue: Z11-16:Ac. Z11-16:Ac could also be the key compound of *O. populeti*

since it elicited the strongest EAGs in a screening carried out on one male of this species.

Orthosia incerta and *O. stabilis* both exhibited high antennal sensitivity to aldehydes. Their response profiles appeared very similar, except for an inversion of their respective key compounds. Z11–16:Ald and Z9–14:Ald both were very active on the antennae of *O. incerta* but Z11–14:Ald dominated in *O. incerta*, while Z11–16:Ald was the key compound of *O. stabilis*. Besides aldehydes, both species showed good responses to Z7–12:Ac

O. munda showed a specific response profile with maximum sensitivity to Z11–16:OH (key-compound) and other hexadecenyl derivatives (Z11–16:Ald and Z11–16:Ac). The EAG response profile revealed a second peak of activity for related tetradecenes, Z9–14:Ald and Z9–14:OH.

Mythimna Ochsenheimer. The species of *Mythimna* showed a great sensitivity to aldehydes: nine species out of eleven had an aldehyde as key compound (Fig. 1). Z11–16:Ald was the key compound of *M. pudorina*, *M. straminea*, *M. pallens*, *M. obsoleta*, *M. conigera*, *M. albipuncta* and *M. comma*. The EAG response profiles of these species were very similar. Z9–14:Ald was the key compound of *M. ferrago*. The two males of *M. albus* also exhibited great sensitivity to Z9–14:Ald, their key compound. *M. impura* was the only species whose key compound was not an aldehyde but the acetate Z11–16:Ac. Z11–16:Ac is also the key compound of *M. unipuncta* (Doré & Renou, 1985).

Multidimensional analysis

A global analysis of the twenty-four EAG response profiles was achieved by Factorial Correspondence Analysis (FCA). The first two factors φ_1 and φ_2 summed up 56.9% of the total inertia. The factorial map φ_1 – φ_2 (Fig. 2) led to a division of the Hadeninae according to their antennal sensitivity into three groups.

Group 1 comprised six species which projected negatively on φ_1 . All six were well explained by φ_1 (RCs from 0.53 to 0.78 except for *M. persicariae*, RC = 0.28). The most involved molecules on φ_1 that were positively correlated with the species of group 1 were Z9–14:Ac (AC = 17.11) and a dodecenyl acetate, Z9–12:Ac (AC = 9.09). Group 1 collected species with high sensitivity to tetradecenyl acetates.

Z9–14:Ac was the key compound of five out of six species and Z7–14:Ac the key compound of *M. persicariae*. Preliminary single hair recordings in *O. gothica* and in *O. cruda* (Renou, unpublished data) revealed the presence of Z9–12:Ac-sensitive neurones in some trichoid sensilla. There is no report in the literature of pheromone activity by Z9–12:Ac with any of the group 1 species. In view of its EAG activity the behavioural role of this compound should be investigated. Furthermore, the species belonging to group 1 were negatively correlated with C14 and C16 aldehydes.

Group 2 comprised five species including three *Mamestra*. They projected positively on φ_2 , had low contributions to φ_1 (Σ ACs = 4.71) but had high contributions to φ_2 (Σ ACs = 32.96). They were positively correlated with two prevalent molecules on φ_2 : Z11–16:Ac (AC = 33.78) and Z11–16:OH (AC = 14.93). Group 2 included species whose key compound is Z11–16:Ac and which showed high sensitivity to hexadecenyl derivatives. Their median position on φ_1 reflects their non-participation to the opposition between group 1, associated with tetradecenyl derivatives, and group 3, associated with aldehydes. Group 2 species exhibited high sensitivity to Z9–14:Ac and responded more to hexadecenals than the species of group 1 (Fig. 1). In return, their positions on φ_2 reflect a relative opposition to short chain pheromones, tetradecenyl derivatives (Z9–14:Ald, AC = 18.71), or dodecenyl derivatives (Z7–12:Ac, AC = 7.69).

Group 3 comprised eight species belonging to the *Mythimna* genus and two *Orthosia* species. Their positive coordinates on φ_1 and their strong contributions to φ_1 (AC = 12.11 for *M. obsoleta*) revealed a positive correlation with aldehydes (Z11–16:Ald, AC = 26.17; Z9–14:Ald, AC = 19.28; Z11–14:Ald, AC = 8.04). Group 3 formed an elongated cloud parallel to the φ_2 axis that reflected the existence of a continuum between species more specific to hexadecenyl derivatives (*M. pudorina*, *M. pallens*, *M. thalassina*, etc.) and species more specific to tetradecenyl derivatives (*M. ferrago*, *O. stabilis*, etc.). In spite of its unusual strong sensitivity to an alcohol, *O. munda* was not isolated from group 3 by the FCA, which indicated that it has common features with this group.

There were three isolated species. *Tholera*

Fig. 2. FCA of the EAG responses of the males of twenty-four species of Hadeninae to thirty pheromone compounds. Main factorial map ϕ_1 – ϕ_2 . Prevalent molecules on both factors are underlined. λ = eigenvalues of the factors; τ = per cent inertia of each factor.

decimalis had poor contributions to ϕ_1 and to ϕ_2 , and was well explained by inferior factors of low reliability ($\Sigma \text{RCs } \phi_5$ to $\phi_n = 0.652$); its central position on factorial map ϕ_1 – ϕ_2 was the consequence of its lack of sensitivity to all the molecules. The two other isolated species, *Polia bombycina* and *Orthosia incerta*, shared Z9–14:Ald as a key compound but were not included in group 3 by the FCA. Both species were well explained by ϕ_2 (RCs = 0.707 and 0.507 respectively) and they were positively correlated with Z9–14:Ald and Z7–12:Ac. By contrast, they were negatively correlated to C16 and their EAG response profiles confirmed that they were less sensitive to C16 than the other species of group 3 (Fig. 1). In the molecule field, Z7–12:Ac appeared to be positively correlated with Z9–14:Ald, whilst its isomer Z9–12:Ac was correlated with Z9–14:Ac.

Inferior rank factors ϕ_3 and ϕ_4 summarized 16.3% of the total system inertia. Factor ϕ_3 was dominated by *M. persicariae* (CA = 46.7), positively correlated with Z7–14:Ac (CA = 19.21). *M. persicariae* was the only Hadeninae whose key compound was Z7–14:Ac. Factor ϕ_4 showed a greater sensitivity of some Hadeninae (*O. munda*: CA = 22.84; *P. bombycina* and *O. incerta*) to alcohols (Z9–14:OH : CA = 11.86; Z9–16:OH : CA = 11.32; Z9–14:OH and Z9–12:OH). Although their response profiles were alike (Fig. 1), *O. incerta* and *O. stabilis* were separated by the FCA on ϕ_4 , due to greater sensitivity of *O. incerta* to Z9–14:OH. Positive correlation with Z7–12:Ac (CA = 10.71) and opposition to alcohols in *M. obsoleta* explained the position of this species relatively to *M. ferrago*. Factors ϕ_5 to ϕ_n summarized only 26.7% of the total inertia.

Discussion

EAGs result from the summated activity of specialist, narrowly tuned receptor cells (Schneider, 1962), belonging to a small number of classes defined by individual response spectra. The EAG amplitude depends on the presence and on the number of specialist receptor cells for a compound on the antenna (Mayer & Mankin, 1985). EAG response profiles in *Mamestra brassicae* and in *M. suasa* revealed two peaks for Z9-14:Ac and Z11-16:Ac. Sensilla trichodea of the males of both species have been shown (Töth *et al.*, 1986; Renou, unpublished data) to house a receptor cell for Z11-16:Ac and a receptor cell for Z9-14:Ac. Receptor cells for Z9-14:Ald and Z11-16:Ald have been found in the sensilla of male *O. incerta* (Renou, unpublished data) in which aldehydes elicited EAGs of high amplitude. Thus, although being a global recording method, EAG is useful for making preliminary investigations of the olfactory system of moths in relation to pheromones.

Sex pheromones of moths are usually mixtures including a major component and several minor components. Although the major component can be attractive to the male by itself, the complete behavioural sequence or the full competitiveness with females are rarely seen with single component stimuli. Furthermore, minor components contribute to the specificity of the pheromone blend. EAG response profiles are dominated by the strong responses to the key compound which is usually the major component of the female secretion. Close analogues of EAG-active compounds may produce strong antennal responses that could be misinterpreted in the absence of behavioural data. However, this does not affect the result of a comparative study as differences in the responses to analogues directly reflect differences of the nature and/or the abundance of receptor cell types and thus give information on the olfactory system under study. Minor components often elicit much weaker EAG responses that can be easily underestimated. With a comparative approach, secondary differences in EAG response profiles in species sharing the same key compound might reflect differences of minor components as exemplified in the three Z11-16:Ac-sensitive *Mamestra*.

Although absent from the female secretion of

M. brassicae and *M. suasa*, Z9-14:Ac elicited EAGs of high amplitude in males of the two species. Single cell recordings have demonstrated that the antennae bear specific receptor cells for Z9-14:Ac and that these cells are as numerous as the receptor neurons for Z11-16:Ac, the major component of the female secretion (Töth *et al.*, 1986; Renou, unpublished data). Z9-14:Ac has been identified as the major component of the pheromone of *M. biren* (Frérot *et al.*, 1988) and it acts as an attractant for males of several Hadeninae species (Arn *et al.*, 1986). Furthermore, Z9-14:Ac reduces the attraction of male *M. brassicae* (Struble *et al.*, 1980) and *M. suasa* (Töth *et al.*, 1986) to synthetic pheromone blend sources. High EAG sensitivity to molecules not present in the female secretion is not limited to moths of the Hadeninae subfamily but might be a general phenomenon. Specific receptor cells for Z7-12:OH, a male behaviour inhibitor, have been found in a Plusiinae, *Trichoplusia ni* Hübner (O'Connell *et al.*, 1983). Disparlure is the sex attractant of the gypsy moth, *Lymantria dispar* L., and of the nun moth, *Lymantria monacha* L., which occur sympatrically in Europe. Sensilla trichodea of the gypsy moth house a receptor cell for (+)disparlure and a receptor cell for (-)disparlure (Hansen, 1984). Pheromone gland extracts from female gypsy moths stimulate only the (+)disparlure cells while both cells are stimulated by the pheromone extracts from the nun moth. Female gypsy moths produce only (+)disparlure but female nun moths emit also (-)disparlure that does not modify the responses of male nun moths but that inhibits attraction to (+)disparlure of male gypsy moths under field conditions. In Hadeninae, as in other Lepidoptera, the presence of specific olfactory neurones enables male moths to detect heterospecific females and to distinguish them from their conspecific females. This reinforces reproductive isolation by pheromones in sympatric species.

Key compounds were not specific to a genus and the same molecule could be the key compound of several species belonging to different genera. EAG profiles of species belonging to the same genus showed important differences. Five different key compounds were found in the *Orthosia*. All these *Orthosia* species were trapped in the same place and their flight periods overlapped so that pheromone specificity might

be an important factor of reproductive isolation between them. The genus *Mamestra* has been revised by Berio (1985) who split it into four genera. Thus they are probably not monophyletic and this could explain the diversity of their EAG response profiles. In contrast, the *Mythimna* were correlated with chemicals having the same function, aldehydes, and they were caught in different biotopes. With the exception of *Mythimna*, there is no simple correlation between genera and chemical structure of pheromones. The necessity for sympatric species to avoid competition for communication channels and to reinforce their reproductive isolation may result in the evolution of different pheromone reception systems in closely related species. Variations of sensitivity inside a genus are useful for species characterization as illustrated in *Mythimna*, a very homogeneous genus with regard to the appearance and the biology of its members despite its subdivision in subgenera. Besides their common sensitivity to aldehydes, there were many more differences in the EAG response profiles between close species of the *Mythimna* subgenus than between remote ones. This was emphasized by the FCA on the main factorial map. Pairs of closely related species (*M. impura* and *M. pallens*, or *M. ferrago* and *M. albipuncta*) occupied remote positions on the factorial map and had different key compounds. Conversely, two distant species such as *M. albipuncta* and *M. pallens* were close to each other on the map as they showed more similar EAG profiles and shared the same key compounds. *M. obsoleta*, the only species of the *Leucania* Ochsenheimer subgenus tested, was removed from the *Mythimna* of the *Mythimna* subgenus by the FCA. Z11-16:Ald was the key compound of *M. obsoleta* as it was for some species of the *Mythimna* Ochsenheimer subgenus, but there could be a convergence of the pheromone structure in the two subgenera.

A limited number of molecules were found to be EAG-active in the investigated sample of Hadeninae species. Pheromone communication channels of Hadeninae use a reduced number of chemicals. Furthermore, the EAG-active molecules presented an unity of chemical structure, and their biosynthesis probably involves only a few biosynthetic elementary steps, mainly a succession of desaturations and chain shortenings (Roelofs & Bjostad, 1984). They were linear alkyl chains of fourteen or sixteen carbon

atoms. Shorter chain lengths (C10 or C12) were less active. All the active molecules were monoenes of Z stereochemistry. The two saturated compounds were not effective stimuli. Although they were produced in significant amounts by the females in many species of Noctuidae, they are often not active in field trapping experiments. However, dodecyl acetate has been proven to modulate the close range search behaviour of male *Trichoplusia ni* (L.) (Bjostad *et al.*, 1980) and to increase the responses of the two types of receptor cells when added to their respective key compounds, Z7-12:Ac and Z7-12:OH (O'Connell, 1985). Thus, the contribution of saturated compounds to pheromone communication might be very variable from one species to another. Double bond positions were commonly located on carbon 9 in the C14 and on carbon 11 in the C16. Although an exception could be found in *M. persicariae*, double bond position and stereochemistry appeared to be stable traits of active molecules. Acetates and aldehydes were the most commonly active molecules. Alcohols were rarely effective stimuli, excepted in *O. munda*. Similar conclusions have been drawn from an analysis of the list of known sex attractants and pheromone components of Hadeninae of the world wide fauna (Renou *et al.*, 1986). Hexadecenyl and tetradecenyl acetates are the most common attractants for male Hadeninae as for the majority of the triline subfamilies of the Noctuidae. Sex attractants with shorter chains (decenyl and dodecenyl acetates) are rare in the Hadeninae, unlike in the Noctuinae and in a quadrigene subfamily, the Plusiinae. Tetradecenals and hexadecenals are EAG-effective compounds in new world species of *Leucania* Ochsenheimer and *Pseudaletia* Franclemont, two genera closely related to the *Mythimna* (Renou *et al.*, 1986). Aldehyde attractants are common only in Hadeninae and Heliothinae.

The Hadenine genera were originally separated in several subfamilies by early authors. However, Hampson (1905) brought them all together into a single triline subfamily defined by hairy eyes and unspined tibiae. Franclemont & Todd (1983) further recognized three tribes. The Glottulini comprised only *Xanthopastis* Hübner, a genus regarded as being not closely related to the other Hadeninae (Kitching, 1984). The Eriopygini included the genera *Orthodes* Guenée, *Tricholieta* Grote and others. The

Hadenini contained the remaining genera, including all the species we investigated in this paper. High EAG responses to Z11-16:Ald were recorded in an *Eriopyga* from Guadeloupe (Renou, unpublished data) but data on sex pheromone chemistry in Eriopygini are lacking. The EAG key compound of the neotropical species *Xanthopastis timais* Cramer is Z11-16:Ald (Renou *et al.*, 1986) and this aldehyde has been found in the female pheromone secretion (Lalanne-Cassou, unpublished data). Thus, sensitivity to aldehydes could have evolved independently in several lineages of Hadeninae. Catocalinae and some other quadrifines respond to polyenic hydrocarbons and to their epoxydes (Renou *et al.*, 1988). The position of *Tholera decimalis*, whose antennal sensitivity is, to our knowledge, unique in Hadeninae but is common in Catocalinae and Geometridae, should be reinvestigated. Its sensitivity to alcene pheromones might indicate that the *Tholera* genus is at an early point in the Hadeninae line of evolution or that it is an isolated lineage having undergone parallel evolution with the Catocalinae. Single trichoid sensilla recordings are needed to determine the cell receptor types present on the antenna. Such recordings, coupled with detailed behavioural data on the role of pheromone compounds, will contribute to a better understanding of the evolution of pheromone reception in moths.

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References

- Arn, H., Töth, M. & Priesner, E. (1986) *List of Sex Pheromones of Lepidoptera and Related Attractants*. OILB-SROP, Paris.
- Attygalle, A.B., Herrig, M., Vostrowsky, O. & Bestmann, H.J. (1987) Technique for injecting intact glands for analysis of sex pheromones of Lepidoptera by capillary gas chromatography: re-investigation of pheromone complex of *Mamestra brassicae*. *Journal of Chemical Ecology*, **13**, 1299–1310.
- Benzécri, J.-P. (1980) *L'Analyse des Données. II. L'Analyse des Correspondances*. Dunot, Paris.
- Berio, E. (1985) Noctuidae. *Fauna d'Italia. Lepidoptera*. Edizioni Calderini, Bologna.
- Bjostad, L.B., Gaston, L.K., Mistrot-Pope, M., Kuenen, L.P.S. & Vetter, R.S. (1980) Dodecyl acetate, a second pheromone component of the cabbage looper, *Trichoplusia ni*. *Journal of Chemical Ecology*, **6**, 727–734.
- Descoins, C., Priesner, E., Gallois, M., Arn, H. & Martin, G. (1978) Sur la sécrétion phéromonale des femelles vierges de *Mamestra brassicae* L. et de *Mamestra oleracea* L. (Lépidoptères, Noctuidae, Hadeninae). *Comptes Rendus de l'Académie des Sciences de Paris*, **286**, 77–80.
- Doré, J.-C. & Renou, M. (1985) Analyse multivariée des relations entre 9 molécules phéromonales et 11 espèces de lépidoptères Noctuidae. *Acta Oecologica, Oecologia Applicata*, **6**, 269–284.
- Farine, J.-P., Frérot, B. & Isart, J. (1981) Facteurs d'isolement chimique dans la sécrétion phéromonale de deux Noctuelles Hadeninae: *Mamestra brassicae* (L.) et *Pseudaletia unipuncta* (Haw.). *Comptes Rendus de l'Académie des Sciences de Paris*, **292**, 101–104.
- Franclemont, J.C. & Todd, E.L. (1983) Noctuidae. *Check List of the Lepidoptera of America North of Mexico* (ed. by R. W. Hodges), pp. 120–159. E.W. Classey and the Wedge Entomological Research Foundation, London.
- Frérot, B., Gallois, M., Malosse, C. & Bues, R. (1987) Identification de la phéromone sexuelle de *Mamestra biren* (Goeze) ex *M. glauca* (Hb.). *Comptes Rendus de l'Académie des Sciences de Paris*, **304**, 139–142.
- Frérot, B., Lalanne-Cassou, B., Gallois, M., Malosse, C., Cain, A.-H. & Bues, R. (1988) Etudes physico-chimiques de la sécrétion phéromonale de *Mamestra suasa* (D. et S.), Lépidoptère, Noctuidae, Hadeninae. *Comptes Rendus de l'Académie des Sciences de Paris*, **307**, 785–788.
- Hampson, G.F. (1905) *Catalogue of the Lepidoptera Phalaenae in the collection of the British Museum. 5. Hadeninae*. London.
- Hansen, K. (1984) Discrimination and production of disparlure enantiomers by the gypsy moth and the nun moth. *Physiological Entomology*, **9**, 9–18.
- Kitching, I.J. (1984) An historical review of the higher classification of the Noctuidae (Lepidoptera). *Bulletin of the British Museum (Natural History)*, **49**, 153–224.

- Lebart, L., Morineau, A. & Fénélon, J.P. (1979) *Traitement des Données Statistiques*. Dunod, Paris.
- Leraut, P. (1980) *Liste Systématique et Synonymique des Lépidoptères de France, Belgique et Corse*. Alexanor, Paris.
- Mayer, M.S. & Mankin, R.W. (1985) Neurobiology of pheromone perception. *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 9. *Behaviour* (ed. by G. A. Kerkut and L. I. Gilbert), pp. 95–144. Pergamon Press, Oxford.
- O'Connell, R.J. (1985) Responses to pheromone blends in insect olfactory receptor neurons. *Journal of Comparative Physiology, A*, **156**, 747–761.
- O'Connell, R.J., Grant, A.J., Mayer, M.S. & Mankin, R.W. (1983) Morphological correlates of differences in pheromone sensitivity in insect sensilla. *Science*, **220**, 1408–1410.
- Priesner, E. (1980) Sex attractant system in *Polia pisi* L. (Lepidoptera: Noctuidae). *Zeitschrift für Naturforschung*, **35c**, 990–994.
- Priesner, E., Jacobson, M. & Bestmann, H.J. (1975) Structure–response relationships in Noctuid sex pheromone reception. *Zeitschrift für Naturforschung*, **30c**, 283–293.
- Renou, M., Lalanne-Cassou, B., Frérot, B., Gallois, M. & Descoins, C. (1981) Composition de la sécrétion phéromonale émise par les femelles vierges de *Mamestra (Polia) pisi* (L.) (Lépidoptère, Noctuidae, Hadeninae). *Comptes Rendus de l'Académie des Sciences de Paris*, **292**, 1117–1120.
- Renou, M., Lalanne-Cassou, B. & Le Duchat d'Aubigny, J. (1986) Etude électroantennographique de la sensibilité antennaire des mâles de 61 espèces de Noctuidae de Guadeloupe (Lepidoptera). *Annales de la Société Entomologique de France*, **22**, 339–352.
- Renou, M., Lalanne-Cassou, B., Michelot, D., Gordon, G. & Doré, J.-C. (1988) Multivariate analysis of the correlation between Noctuidae subfamilies and the chemical structure of their sex pheromones or male attractants. *Journal of Chemical Ecology*, **14**, 1187–1215.
- Renou, M., Lalanne-Cassou, B., Doré, J.-C. & Milat, M.-L. (1988) Electroantennographic analysis of sex pheromone specificity in neotropical Catoxalinae (Lepidoptera: Noctuidae): a multivariate approach. *Journal of Insect Physiology*, **34**, 481–488.
- Roelofs, W.L. (1984) Electroantennogram assays: rapid and convenient screening procedures for pheromones. *Techniques in Pheromone Research* (ed. by H. E. Hummel and T. A. Miller), pp. 131–159. Springer, Berlin.
- Roelofs, W.L. & Bjostad, L. (1984) Biosynthesis of lepidopteran pheromones. *Bioorganic Chemistry*, **12**, 279–298.
- Schneider, D. (1962) Electrophysiological investigation on the olfactory specificity of sexual attracting substances in different species of moths. *Journal of Insect Physiology*, **8**, 15–30.
- Steck, W., Underhill, E.W. & Chisholm, M.D. (1982) Structure activity relationships in sex attractants for North American noctuid moths. *Journal of Chemical Ecology*, **8**, 731–754.
- Struble, D.L., Arn, H., Buser, H.R., Städler, E. & Freuler, J. (1980) Identification of 4 sex pheromone components isolated from calling females of *Mamestra brassicae*. *Zeitschrift für Naturforschung*, **35c**, 45–48.
- Töth, M., Szocs, G., Löfstedt, C., Hansson, B.S. & Subchev, M. (1986) Sex pheromone components of *Mamestra suasa*: chemical analysis, electrophysiological activity and field tests in two European countries. *Entomologia Experimentalis et Applicata*, **42**, 291–299.

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Appendix

List of Hadeninae species

Hada nana Hufnagel (offspring of females caught in June; Col du Glandon, Savoie).

Polia bombycina Hufnagel (offspring of five females caught in July; Font Pédrouse, Pyrénées Orientales).

Mamestra brassicae L. (laboratory reared, strain from Avignon, Vaucluse).

Mamestra persicariae L. (La Roche Guyon, Val d'Oise and Brouëssy, Yvelines; July).

Mamestra thalassina Hufnagel (offspring of females, June; Col du Glandon, Savoie).

Mamestra suasa Denis & Schiffermüller (laboratory reared strain from Avignon, Vaucluse).

Mamestra oleracea L. (offspring of mated females; Brouëssy, Yvelines).

Mamestra biren Goëze (= *glaucia* Hübner) (offspring of females caught in June; Col du Glandon, Savoie).

Tholera decimalis Poda (Brouëssy, Yvelines; September).

Orthosia cruda Denis & Schiffermüller (Brouëssy; March).

Orthosia miniosa Denis & Schiffermüller (Brouëssy; April).

Orthosia gracilis Denis & Schiffermüller (Brouëssy; April–May).

Orthosia stabilis Denis & Schiffermüller (Brouëssy; March–April).

Orthosia incerta Hufnagel (Brouëssy; March–April).

Orthosia munda Denis & Schiffermüller (Brouëssy; April).

Orthosia gothica L. (Brouëssy, March).

Mythimna conigera Denis & Schiffermüller (Brouëssy and Echevis, Drome; July).

Mythimna ferrago Fabricius (Brouëssy and La Roche Guyon, Val d'Oise; July).

Mythimna albipuncta Denis & Schiffermüller (Brouëssy; August–September).

Mythimna pudorina Denis & Schiffermüller (La Roche Guyon, Val d'Oise; July).

Mythimna straminea Treitschke (Buno-Bonneveau, Essonne; July).

Mythimna impura Hübner (Brouëssy; July).

Mythimna pallens L. (Brouëssy; June–July and September).

Mythimna obsoleta Hübner (Buno-Bonneveau, Essonne; July).

Further data were gathered on eight species where low numbers of replicates or uncomplete screenings did not allow us to do a quantitative analysis of EAG response-profiles: *Mamestra contigua* Denis & Schiffermüller, *M. pisi* L., *Mamestra w-latinum* Hufnagel; *Mythimna unipuncta* Haworth, *M. l-album* L. and *M. comma* L.; *Egira conspicularis* L.; *Orthosia populeti* Fabricius; *Polia nebulosa* Hufnagel.