Odour detection in the desert locust, *Schistocerca gregaria*: antennal structure and function

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Title and subtitle Odour detection in the desert locust, Schistocerca gregaria: antennal structure and function

Abstract

The desert locust, *Schistocerca gregaria*, is a polymorphic insect that occurs in two morphological and physiological distinct phases, solitary and gregarious, depending on population density. The two phases are reversible at any developmental stage. The gregarious phase produces and uses stage-specific pheromones, that keep the swarm together.

In my thesis, I investigated the functional morphology of olfactory receptor neurones on the desert locust antennae that are responsible for the detection of aggregation pheromones. First I characterized the different antennal receptor types using electron microscopic methods. Four structurally different types could be distinguished, three bearing olfactory features: sensilla basiconica, s. trichodea, and s. coeloconica, and one belonging to a mechano-/contact-chemoreceptor type, s. chaetica. The distribution of olfactory sensilla was different between the phases, with solitary phase individuals having significantly more sensilla than the gregarious phase locusts.

I then investigated the physiological characteristics of receptor neurones present in the various olfactory sensillum types by electrophysiological techniques, stimulating them with synthetic compounds of behavioural significance to the locust. Olfactory receptor neurones housed in s. basiconica were found to be excited in a dose-dependent manner by aggregation pheromone compounds, produced both by nymphal stages and mature adults. Receptor neurones housed in s. coeloconica were found to be excited by a general plant odour and nymphal-produced aggregation pheromones, while the adult-produced pheromones inhibited their activity. Receptor neurones in s. trichodea were excited only by a possible sex pheromone compound of *S. gregaria*. Receptor neurone sensitivity was found to be higher in solitary phase locusts than in gregarious individuals.

These studies show that the desert locust is endowed with a specific and sensitive olfactory system for monitoring its environment. The results presented lay a firm foundation for future short- and long-term studies of semiochemicals that modulate desert locust behaviour.

Key words Schistocerca gregaria , olfaction, insect antennae, chemoreceptors, receptor neuron, electrophysiology, aggregation pheromone, SEM, TEM.

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Cover picture:
The desert locust, *Schistocerca gregaria* 5th instars of gregarious (black and yellow), and solitary (green) phase.

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A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (in press, submitted or in ms).

To Grace and our children

Contents

Odour detection in the desert locust, *Schistocerca gregaria*: antennal structure and function

This thesis is based on five papers which are referred to by their Roman numerals as listed on page 7.

General introduction

Insects are among the most successful organisms in the animal Kingdom. Their capacity to survive and reproduce depends greatly on their ability to identify and respond selectively to cues from a heterogeneous environment. They can identify conspecifics and mates, differentiate between hosts, both plant and animal, and distinguish between many microclimatic factors such as variation in humidity, temperature and airflow. All these factors are attributed to the complexity of their sensory systems that facilitate both simple and complex behaviours.

The insect is, however, isolated from its external surroundings by a cuticular structure that is relatively impermeable to chemical sensation. But insects can perceive signals from their environment since the cuticle has been modified at certain points to give rise to sensory detectors for external stimuli. Many sensory organs are housed in hair-like structures, known as sensilla, that protrude from the cuticle. These structures participate in the detection of stimuli that can be categorised as mechanical, thermal or chemical. Receptor neurones associated with these structures convey messages from outside of the insect body to the central nervous system (CNS), where signals are integrated. The fully integrated signal can subsequently participate, together with internal stimuli, in the moulding of appropriate behaviours such as posture, movement, feeding and behaviours associated with mating and oviposition.

Information regarding the chemical environment of an animal is collected by the olfactory and gustatory systems. In land-living animals, the difference between the systems is the phase in which the molecules occur. The gustatory system detects molecules dissolved in liquid or waxes, while the olfactory system detects molecules in gaseous phase. In order to understand how chemical signals are detected and analysed by the olfactory system, and how these signals ultimately affect insect behaviour, it is important to study the anatomical and functional organization of the olfactory pathway involved in odour processing. This has been the main aim of this thesis.

The morphological features of antennal sensilla and olfactory receptor neurones associated with them were investigated by scanning (SEM) and transmission (TEM) electron microscopy and by activity dependent staining of neurones, whereas the

physiological characteristics of olfactory receptor neurones were determined using electrophysiological methods.

Pheromone communication in insects

Insects show an intensive use of chemicals in communication, both within and between species. These odours, termed semiochemicals, are produced for many purposes. Those used for communication within the species are called pheromones. Since their first isolation in the 1950s, pheromones have been described as "substances that are secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction, e.g., a definite behaviour or development process" (Karlson and Lüscher, 1959).

Pheromones can be categorised into those that release a specific behaviour (e.g., sex or releaser pheromones), and those that cause long-term, irreversible physiological changes (e.g., aggregation or primer pheromones). Sex pheromones are further categorised into those that cause sex attraction at a distance (long-range pheromones) and those involved in close range courtship (courtship pheromones). Production and release of long-range sex attractant pheromones in insects are often restricted to one sex, in most cases the female. In moths, the released volatile pheromones stimulate a characteristic behaviour in males within range of the odour plume. An aroused recipient raises the antennae, orients towards the source and walks or flies upwind, often in a zig-zag track, until the source is reached (Birch and Haynes, 1982).

Courtship pheromones are deployed immediately prior to mating. This type of pheromone may be simply a high concentration of the attractant pheromone, but in many insect species are 'aphrodisiac' chemicals produced by the males in large quantities from their abdominal hairpencils. The effect of this pheromone is to prevent the female from escaping prior to copulation, and is possibly used in species recognition and mate evaluation (Gullan and Cranston, 1994).

In contrast, crowding or aggregation pheromones, which in most cases are produced and responded to by both sexes, cause conspecific insects to crowd around the source of the pheromone. Aggregation may lead to increased likelihood of mating, augmented security from predation, maximum utilisation of scarce food resources and overcoming of host resistance as well as cohesion of social insects (Birch and Haynes, 1982).

Other forms of pheromones used by insects are maturation pheromones that influence physiological processes that lead to development; oviposition stimulation and/or deterring pheromones, that female insects use in deciding the substrates on which to lay eggs. In addition, trail-marking pheromones are used to mark paths particularly to food

sources and nests and alarm pheromones are characteristic of most social insects (ants, bees, wasps, termites, etc) (Birch and Haynes, 1982).

The desert locust, *Schistocerca gregaria* (Forskål)

Locusts are orthopteran insects. The name is derived from the Greek words (*orthos*-straight, *pteron*-wing), hence 'straight winged' referring to the position of the wings when the insect is at rest. Orthopterans are hemimetabolous insects that undergo a number of stages in development, slowly changing their morphological and physiological characteristics between the instars with no pupal stage before reaching adulthood.

Locusts differ from other orthopteran species in that they occur in two distinctive phases, solitary and gregarious. The dividing line between the phases is not always sharply defined and under certain circumstances the transition from one phase to the other can be interrupted or even reversed by changes in environmental factors. When fully developed, the two forms are so different in their colours, habits, physiology and ecology such that in the past they were regarded as belonging to two completely different species (Preston-Mafhan, 1990).

References to the locust appear in very early historical records: a locust swarm was one of the plagues of Egypt described in the Old Testament. Despite intensive field and laboratory research undertaken so far, locust swarms are still major disasters in agricultural areas, and the exact mechanisms responsible for initiation and maintenance of the locust swarm are not yet fully understood.

Considerable data exist that outline the behaviour and ecology of locusts (Steedman, 1988; Chapman, 1990; Preston-Mafhan, 1990). The desert locust, *Schistocerca gregaria*, is the species of most economic importance and is also the species most sensitive to changes in population density. Phase transformation from the solitarious to the gregarious phase and vice versa occurs rapidly and frequently. During recession periods, when individuals are subject to low densities, their life history resembles that of any other grasshopper, and their habitats are limited to the recession distribution areas in Africa and Asia covering over 16 million km^2. During plagues, when most individuals occur in dense bands in the nymphal stage and in swarms in the adult stage, the invasion area extends beyond the recession area to include the whole of North, West, and East Africa, the Mediteranean region, and all of Middle East countries to over about 29 million km^2 (Fig. 1) (Steedman, 1988).

Four main factors contribute to the desert locust status as a major agricultural pest insect: i) the food intake per individual; as nymphs and actively migrating young adults eat about their own weight of fresh vegetation each day; ii) the range of food plants and parts eaten; the desert locusts, being polyphagous insects, feed on a wide range of plants;

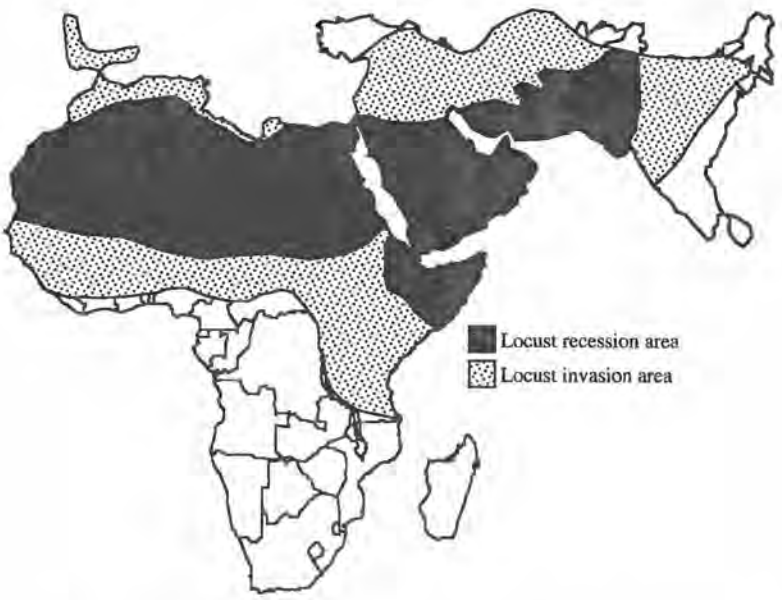

Figure 1. Distribution of the desert locust, *Schistocerca gregaria*. During recession periods when individuals are subject to low densities, the locust inhabits a broad belt of arid and semi-arid land which stretches from the Atlantic Ocean to N.W. India and covers over 16 million km^2. During plagues, when most individuals occur in dense bands in the nymphal stage and in swarms in the adult stage, the invasion area extends southwards and north-eastwards, beyond the recession area, to over about 29 million km^2. (after Steedman, 1988, modified).

iii) the frequency of occurrence of high-density populations and iv) the mobility of populations. The greatest recorded crop losses occur when young migrating swarms of immature adults reach cultivated areas.

Diurnal insects such as butterflies and locusts are often thought to depend greatly on visual stimuli, and their antennae have been assumed to be less important as sensory organs. However, in the desert locust, the importance of antennal olfactory receptor neurones in relation to the aggregation behaviour has been demonstrated in studies where different sensory appendages were incapacitated (Mordue, 1977; Gillett, 1983; Heifetz, *et al.*, 1996). When the antennae of locusts reared in a group were removed, they lost their aggregation behaviour and developed characteristics typical of solitary phase individuals. Manipulations affecting other sensory modalities such as visual or tactile receptors did not alter their behaviour.

Among locusts, *S. gregaria* is the only species whose pheromone systems have been studied in some detail. It has been shown that different life stages produce and respond behaviourally to stage-specific aggregation pheromone compounds. Mixed sexes

of nymphal stages (2nd to 5th instars) produce a set of short chain aldehydes (6 to 9 carbon chain lengths) and corresponding acids as aggregation pheromones (Torto *et al.*, 1996). Mature adult males (about three weeks after final moult), produce a set of phenolic compounds that aggregate both sexes of young and old adults (Torto *et al.*, 1994; Njagi *et al.*, 1996). So far, only one compound, (*E,Z*)-2,6-nonadienal, identified from the solitary female volatiles has been proposed as a sex pheromone of *S. gregaria*.

The aim of my study of the desert locust was to characterise, morphologically and physiologically, the olfactory receptor neurones responsible for the detection of behaviourally active odours and the central projection pathway of these neurones. A comparative characterisation of antennal sensillum distribution and olfactory receptor neurone responses was made between the solitary and gregarious phase locusts.

Chemosensors

Detection of chemical stimuli from the environment is performed by specific receptor neurones housed in sensory organs (sensilla) located mainly on the insect antennae, mouthparts, ovipositor and the tarsi (Blaney and Simmonds, 1990). Both contact chemoreceptors and olfactory chemoreceptors are present. These receptor neurones trap chemical molecules which are transferred to a site of recognition and where the interaction between molecule and receptor site leads to nerve impulse generation.

For effective trapping of chemical cues, chemoreceptors are strategically located in certain regions of the insect body. Thus many contact chemoreceptors are found mostly on the mouthparts, ovipositor and tarsi, to assist with identification of suitable feeding and oviposition sites. The antennae, which are often forward-directed, prominent and easily movable to sample a large volume of air for pheromones and other odours, are endowed mainly with olfactory receptors. Additionally, mechano-, as well as thermo- and hygroreceptors may be present.

All cells constituting a sensillum are of epidermal origin. A single epidermal "mother cell" undergoes division when a new sensillum is being formed (Hansen, 1978). A second division yields four cells, three of which become concentrically arranged around the innermost cell (Fig. 2). This inner cell develops into one or several bipolar neurones that send an axon to the CNS and a dendrite into the peripheral cuticular part of the sensillum (Blaney and Chapman, 1969; Steinbrecht, 1969).

The remaining cells, the so-called accessory or auxiliary cells, form a series of sheaths and sinuses around the neurones (Keil and Steinbrecht, 1984; Steinbrecht, 1987). The innermost sheath cell, the thecogen cell, wraps round the neurone cell bodies and ensheathes the proximal region of each of the dendrites separately. It encloses the ciliary sinus (the inner dendritic lymph), and during the formation of the sensillum it

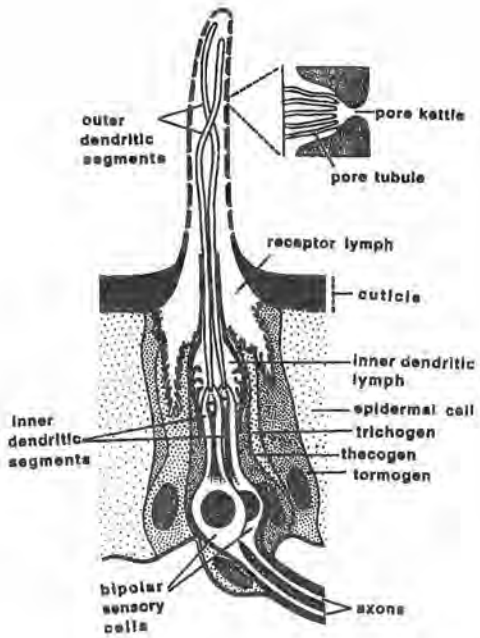

Figure 2. Longitudinal-section of an olfactory sensillum. The odour molecules enter the sensillum through the pore-kettle and are transported through the pore tubules and receptor lymph to the receptor sites on the outer dendritic segments. A receptor potential is induced in the dendrites by odours and propagated down the dendrites towards the cell body. The summed effect of the receptor potentials triggers a release of action potentials that are transmitted to the central nervous system via the axons. The cell bodies are surrounded by three auxiliary cells: the thecogen, trichogen and tormogen cells, which regulate the composition of the receptor lymph.

secretes the dendritic sheath (scolopale) which encloses all or part of the distal dendrites. The middle cell, the trichogen cell, secretes the cuticular hair-like part of the sensillum, and the outer sheath cell, the tormogen cell, secretes the basal socket of the sensillum. The auxiliary cells connect the receptor neurones to the epidermis so that the epithelial organization is maintained.

The capacity of the olfactory system to distinguish different odours depends on the response characteristics of olfactory receptor neurones, which are exposed to the environment and are responsible for the initial recognition of odours. Olfactory receptor neurones vary in specificity, but most of them are tuned to certain odours. In male moths for example, specialised receptor neurones detect individual components of the sex pheromones emitted by the female.

Perireceptor and receptor events

The sensory process of a receptor neurone (RN) as shown in Fig. 2 is divided into a dendritic outer segment, which is a transformed cilium, surrounded by the outer receptor lymph. The inner dendritic segment is surrounded by enveloping auxiliary cells. The

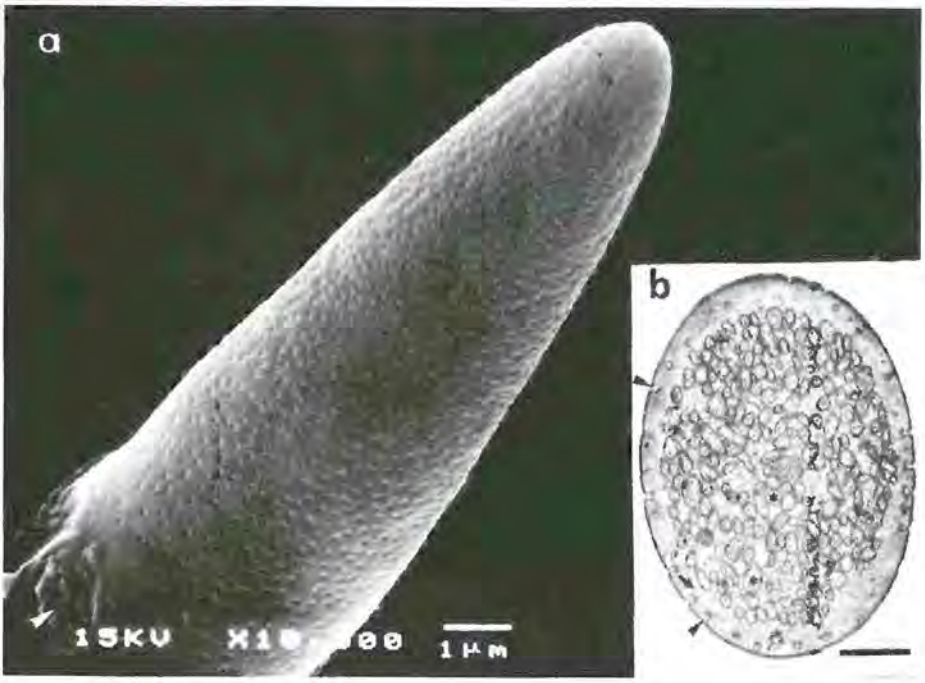

Figure 3a, b. Sensillum basiconicum on the locust antenna. Scanning electron micrograph (a) displaying massive cuticular pore system on the surface and a moulting channel (*arrow head*) at the sensillum base. A tranverse section (b) of the sensillum by use of a transmission electron microscope shows massive dendritic branchings (*asterisk*), and numerous cuticular pores (*arrow head*), which are continuous with pore tubules internally (*arrow*). *Bar*: b, 500 nm.

stimulus-transduction process of the olfactory receptor neurones has been shown to take place at the membrane of the outer dendritic segment (Zack-Strausfeld and Kaissling, 1986).

The wall of olfactory sensilla is normally perforated by a great number of pores(Steinbrecht, 1984). Passage of odour molecules through the hair wall is aided by pore-tubules in single-walled multiporous sensilla basiconica (Figs 2, 3) and the spoke-channel system of double-walled multiporous sensilla coeloconica (Steinbrecht, 1969). The mechanism by which odour molecules reach the receptor sites on the receptor

neurone dendrite inside the sensillum is not fully elucidated. Earlier hypotheses claimed that odour molecules reached the dendritic surface by diffusion through the pore tubules. These tubules were thought to reach all the way through the sensillum lymph to the dendritic surface. In this model, the odorant-binding proteins (OBP), present in the receptor lymph, were mainly thought to be involved in the inactivation of stimuli (Kaissling, 1974 Kanauijia and Kaissling, 1985). However, today the widely accepted theory is that transport of the odour molecule is actually mediated by the OBPs. The odour molecule enters the sensillum through the pore and pore tubules and is released into the sensillum lymph. There it is bound by an OBP molecule and is transported to the receptor sites on the dendritic surface (Vogt and Riddiford, 1981; Vogt, 1987). How the molecule interacts with the receptor site is also under debate. Either the OBP releases the stimulus molecule into the receptor site, or the odour molecule stays bound to the OBP and the whole complex interacts with the membrane receptor.

When the odour molecule interacts with a receptor site, a chain of biochemical events is induced (Kaissling, 1986). The binding of the ligand to the receptor site causes the release of inositol phospate 3 through a G-protein mediated process. The chain-reaction ends with the opening of ion channels and the formation of a receptor potential. The receptor potential is then conducted through the dendrite proximally towards the receptor neurone cell body. Near the cell body, the receptor potential, after reaching a threshold, triggers an action potential which is subsequently propagated along the axon to the CNS. When a strong stimulus is applied to a receptor neurone, numerous action potentials can be triggered for several seconds after stimulation. Each neurone in a sensillum usually has a characteristic action potential amplitude that is normally used, in electrophysiological experiments, to identify the different neurones.

After the odour molecule has interacted with the receptor site, it has to be deactivated and removed from the receptor site to allow new molecules to be detected. Recent data show that also in this step the OBP might be involved. By occurring in two oxidative states (Ziegelberger, *et al.* 1990), the protein can act as a mediator of the stimulus in one state, and a "hider" of the stimulus in the other. In this way, the fast deactivation required by the system can be achieved. The odour molecule is subsequently enzymatically degraded (Vogt and Riddiford, 1981).

Into the brain

From the antennal sensillum, the olfactory receptor neurone sends its axon towards the primary olfactory centre of the brain, the antennal lobe or deutocerebrum. The neurones are primary sensory cells and their axons reach the CNS without any intervening synapses. The receptor neurone axons are grouped together to form the antennal nerve.

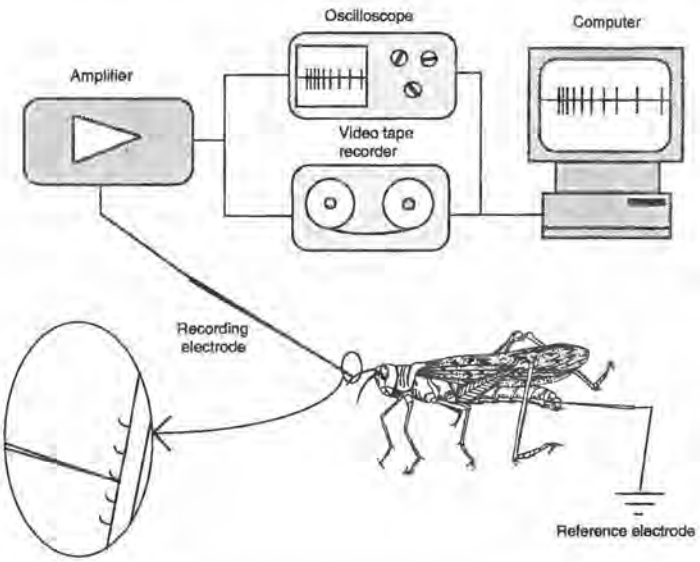

Figure 4. Schematic view of an electrophysiological recording from a sensillum on the antenna of a locust, using a single-sensillum technique. A tungsten electrode sharpened to a very fine tip is inserted at the base of a sensillum and placed in contact with the receptor neurones through the rereceptor lymph. A reference electrode is inserted into the abdomen of the locust. When the receptor neurones are stimulated by a pheromone, the action potentials elicited are picked up by the recording electrode. The signals are amplified, monitored on an oscilloscope and stored in a video tape recorder and a computer for analysis.

At the point of entry into the antennal lobe, the axons are organized into bundles, the number of which may vary with species. Within the lobe, the axons of olfactory receptor neurones project into rounded structures known as glomeruli (Ernst and Boeckh, 1983).

The moth antennal lobe usually contains about 50 glomeruli, while the locust lobe consists of about 1,000 small glomerular structures. In most insects, each receptor neurone axon invades a single glomerulus with its axonal branches (Serby and Chobor, 1992; Hansson, 1995). It has been shown that receptor neurones of identical specificity target a single glomerulus, thus bestowing a functional identity on the structure (Hansson *et al.* 1992; Hansson, 1997). In rare examples receptor neurones do, however, branch and invade several glomeruli (II).

In moths utilising distance sex pheromone, there is a strong sexual dimorphism in the antennal lobe morphology. In male moths, a greatly enlarged complex of glomeruli occur just at the entrance of the antennal nerve into antennal lobe, whereas no such structures are found in females. This male-specific macroglomerular complex (MGC) is the target for pheromone-detecting sensory neurones present on the male antenna

Figure 5. Set up used for extracellular recordings in insects. Photo: Peter Anderson

(Hansson, *et al.*, 1992; Ochieng', *et al.* 1995). The signals from the antenna are synaptically transferred to higher order neurones for integration.

Electrophysiological methods

Electrophysiology is the study of electrical properties of living cells and how information instigated by chemical and electrical changes in these cells is conveyed within the nervous system. Signals initiated in the peripheral receptor neurones and response characteristics transmitted to the CNS can be recorded by electrophysiological methods. On the insect antenna, two types of measurements can be made: electroantennogram (EAG) and single-sensillum recordings. The EAG technique measures the total responses of all antennal receptor cells to particular stimuli, while single-sensillum techniques allow for registration of nervous responses of single olfactory receptor neurones.

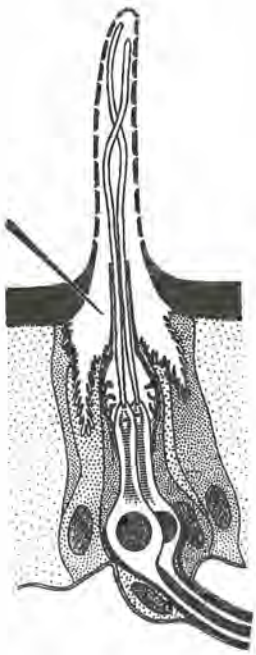

Figure 6. Close-up of the electrophysiological method illustrated in Fig. 4. The tip of the finely-sharpened tungsten electrode is inserted through the cuticle into the receptor lymph close to the dendrites.

In recording EAGs, an antenna is excised and placed between two glass capillaries filled with saline solution. One end of the antenna is placed in the recording electrode and the other end is grounded with an indifferent electrode. Whole animal preparations can be used when the antenna is very small or a long lasting preparation is desired, and in this case the indifferent electrode is positioned in contact with the haemolymph near the base of the antenna, while the recording electrode is put in contact with the excised antennal tip. The recording electrode is connected to a high impedance amplifier and detects the electrical responses when a biologically active compound (such as a pheromone component) is blown over the antenna. An electroantennographic response (measured in mV) is registered as a direct current (DC) deflection over the antenna. The amplified electrical signal can be viewed on an oscilloscope and processed in a computer (Fig. 3).

The electroantennogram technique has been used extensively in pheromone identification studies as a quick method of bioassaying compounds for activity. For example, the antennal responses of a male moth to the natural sex pheromone obtained from conspecific females can be compared with responses to synthetic pheromone components or mixtures. Clean air is blown continuously over the antenna at a constant

Figure 7. Preparation for single cell recording. The locust is restrained inside a holder with the antennae sticking out. The antenna is placed against a stage to allow for penetration of the cuticle with an electrode when viewed under a stereo microscope (× 320 magnification).

rate and the samples to be tested are introduced into the air stream and the EAG response is observed. Different compounds usually elicit EAG responses with different amplitudes.

The EAG method has also been used in combination with gas chromatographic (GC) techniques in identification of pheromone gland extracts from female moths (Arn *et al.*, 1975). The outlet of the GC is split: 50% of the effluent is passed onto the flame ionization detector (FID), and 50% is released into an airstream flushed over the antennal preparation. When a substance with biological activity gives a peak on the FID, it will simultaneously elicit a response from the electroantennographic detector (EAD). This makes it possible to pinpoint a pheromone component very quickly.

Specific information regarding the function of single receptor neurones is obtained by recordings from individual sensilla on the antenna (single-sensillum recording). In this case, action potentials elicited in the receptor neurones present in a sensillum are registered as AC-component, while receptor potentials can be recorded as the DC-component of the signal. The first quantitative evaluation of responses from single olfactory receptor neurones was made by Boeckh (1962), using tungsten microelectrodes (Hubel, 1957) to penetrate the base of the sensillum wall and make electrical contact with receptor neurones. In that manner nerve impulses could be recorded extracellularly, and the impulse frequency could be correlated to the intensity of the stimulants. Both excitation and inhibition were seen in response to odorants. The main advantage of this

method is that it allows for recordings from very short sensilla. However, depending on the sensillum density and distribution on the antennae, it may not be possible to know the type of sensillum one is recording from with this technique.

When recordings are made from long sensilla (e.g. long sensilla trichodea on moth antennae), the tip-recording technique introduced by Kaissling (1974), is more suitable and more commonly used. The hair tip is cut with microscopic knives, and a glass microelectrode filled with saline, is slid over the cut tip of the sensillum. This technique again allows recording of both nerve impulses, AC and DC receptor potentials of the neurones present in a particular sensillum. Another advantage of the tip-cutting method is that it makes it possible to stain the neurones being recorded from by adding a dye into the electrolyte. Thus axonal projections to the brain can be visualised. The disadvantage with this method is that it is only applicable in antennae with long enough sensilla to allow the knife to operate.

Depending on the number of sensory neurones present in a sensillum, extracellular recordings may display nerve impulses of one or several units that can be distinguished by different action potential amplitudes. However, when action potentials of more than three cells are present, the size difference is often not reliable for distinguishing responses of particular cells. In such cases a selective adaptation method is employed (Kaissling, 1979), in which a cell is selectively adapted by stimulation with the 'key' compound for that cell. A second stimulus, given immediately afterwards, will elicit no or minimal response of the adapted cell. Here the response elicited by the second stimulus is assumed to emanate from neighbouring cells.

Summary of results

Morphological characteristics and distribution of antennal sensilla (Paper I)

Antennal olfactory sensilla are vital in the detection of different behaviourally relevant odours in the desert locust. The antennae are filiform and there is no morphological sexual dimorphism. Morphological features of the antennal surface were investigated by scanning (SEM) and transmission (TEM) electron microscopic methods. Four morphological sensillum types could be identified by size and shape, three of them having features typical of olfactory sensilla (Altner and Prillinger, 1980; Zacharuk, 1980). These were recognized as sensilla basiconica, s.trichodea, and s. coeloconica. The fourth sensillum type is of a bimodal contact-chemoreceptive and mechanoreceptive nature, s. chaetica (Blaney and Chapman, 1969).

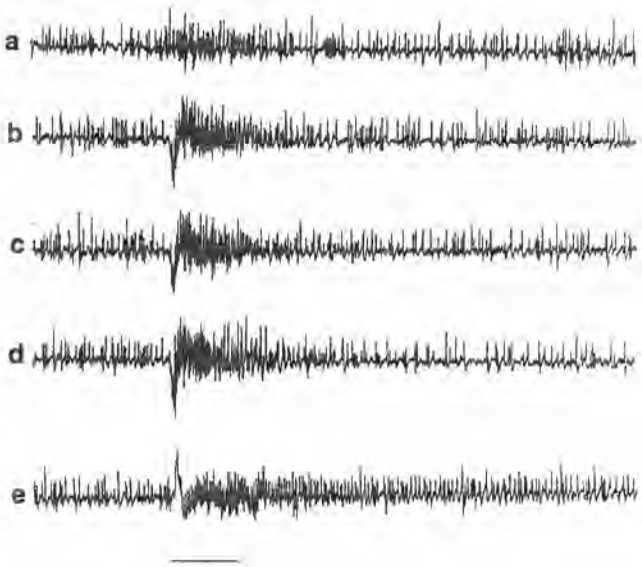

Figure 8. Action potentials elicited by stimulation of receptor neurones in sensillum basiconicum of adult *Schistocerca gregaria*. Stimulation with 100µg of a general plant odour, *E*-2-hexenal elicited a phasic response (a). Stimulations with 100µg each of aggregation pheromone components, benzaldehyde (b), phenylacetonitrile (c), and guaiacol (d), elicited phasic responses preceded by a depolarization of the receptor potential. Stimulation with an organic acid, butyric acid (e), elicited a tonic response preceded by a receptor potential hyperpolarization. Stimulus duration (1 s) is indicated by the horizontal bar.

Sensillum number and antennal length increased proportionally with the developmental stage, reaching a maximum after the final moult. Solitary phase locusts were found to possess relatively higher numbers of olfactory sensilla than the gregarious individuals. This finding was remarkable, given the fact that it has been shown that distribution of food sources and environmental conditions (such as localised warm spots and perches) affect phase shift in *S. gregaria* (Bouaïchi *et al.*, 1996). These authors demonstrated that provision of multiple resource sites promoted dispersion of solitary locusts and inhibited aggregation behaviour, whereas provision of only a single site encouraged gregariousness. Thus, forced to deal with wide choices of consumables appears to promote the development of richly endowed olfactory appendages in the locusts.

Schistocerca gregaria, though a diurnal insect, is indeed equipped with olfactory sensory structures similar to those of night active moths. These results confirm the importance of antennal chemoreceptors in modulating locust behaviours (Mordue, 1977; Gillett, 1983; Heifetz *et al.*, 1996).

Antennal lobe projection patterns of olfactory receptor neurones (Paper II)

The antennal lobe morphology of *S. gregaria* was found to be clearly different from that of moths. The primary olfactory centre contains about 1,000 glomerular structures concentrically arranged around a central fibre core. Activity-dependent receptor neuron staining revealed that the receptor neurone axons projected into several olfactory lobe glomeruli, a feature quite different from that of moths, but similar to that of crustaceans (Schmidt and Ache, 1992).

Physiological characteristics of adult receptor neurones (Papers II and III)

In *Schistocerca gregaria*, single sensillum recordings were for the first time made using behaviourally relevant stimuli (II). Receptor neurones present in sensilla basiconica were found to respond to stimulations with aggregation pheromone compounds with increased action potential frequency. Receptor neurones present in sensilla coeloconica were excited by plant odours and organic acids, but their spontaneous activity was inhibited by aggregation pheromone compounds.

A more detailed physiological study, revealed a clear separation of odour detection by receptor neurones present in morphologically identifiable sensilla (III). Aggregation pheromones, both nymphal- and adult produced, were found to excite neurones present in sensilla basiconica. It was also demonstrated that the sensitivity of receptor neurones present in s. basiconica to aggregation pheromone compounds were significantly higher in solitary than in gregarious phase locusts. Receptor neurones present in s. coeloconica were confirmed to be excited by green leaf odours and by a mixture of nymphal-produced aggregation pheromones, whereas adult-produced pheromones inhibited the activities of these neurones.

For the first time, single-sensillum recordings were unambiguously made from neurones present in sensilla trichodea. It was established that these neurones were excited only by a potential sex pheromone compound, (*E,Z*)-2, 6-nonadienal. As found in most insect species, RNs associated with sensilla trichodea are often tuned to sex pheromone detection. Aggregation pheromone compounds and plant odours did not affect the neurones associated with this sensillum type.

Receptor neurone specificities (Paper IV)

The specificity of sensory receptor neurones residing in the same sensillum can be investigated by a selective adaptation method (Payne and Dickens, 1976). During

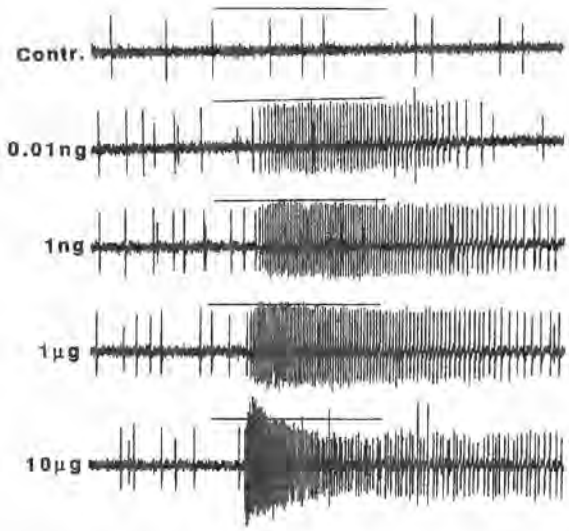

Figure 9. Action potentials elicited by a receptor neurone in sensillum coeloconicum to different doses, 0.01ng-10µg of a plant odour, *E*-2-hexenal. No response elicited to control stimulus (10µl liquid paraffin on filter paper). Stimulus duration (1 s) is indicated by the horizontal bar above each trace.

adaptation, the adapting stimulus reduces subsequent effectiveness and perceived intensity of the same (self-adaptation) or another (cross-adaptation) test stimulus. This reduction in perceived intensity is generally interpreted as evidence that the stimuli are processed by the same receptor sites on the dendritic membrane. If cross- adaptation does not occur between the test and adapting stimuli, it is assumed that the compounds are processed by separate receptor channels.

To investigate receptor specificities for compounds of different significance to *S. gregaria*, we performed cross- and self-adaptation experiments using young adult locusts known to produce no aggregation pheromones but can detect and respond behaviourally to nymphal- and adult produced pheromones (Torto *et al.*, 1994; Njagi *et al.*, 1996). We used representative compounds from nymphal aggregation pheromones (nonanoic acid), adult aggregation pheromones, phenylacetonitrile (PAN), sex pheromone [(*E,Z*)-2,6-nonadienal], and a green leaf odour [(*E*)-2-hexenal]. Self-adaptation experiments showed that stimulation with PAN was least adapting (23% reduction), and (*E,Z*)-2,6-nonadienal elicited the highest adaptation (42% reduction). Self adaptations with *E*-2-hexenal and nonanoic acid reduced their responses by 33 and 31% respectively.

Cross-adaptation experiments revealed that when *E*-2-hexenal and nonanoic acid responses were adapted with PAN, their effects were slightly enhanced, whereas cross-adapting responses of PAN with both compounds resulted in reductions close to its self-

adaptation value. This suggests that both *E*-2-hexenal and nonanoic acid are detected by receptors different from those detecting PAN. Cross-adapting the response of (*E,Z*)-2,6-nonadienal with PAN resulted in a reduction that was less than the self-adaptation values, whereas cross-adapting PAN with (*E,Z*)-2,6-nonadienal resulted in a reduction similar to PAN's self-adaptation value. This assymetry suggests that these two compounds are detected only partly by different receptor assemblies.

From these experiments, we concluded that at least three specific receptor assemblies detecting the tested semiochemicals are present on *S. gregaria* antennae: one type detecting plant odour and nymphal pheromones; a second type detecting a potential sex pheromone, (*E,Z*)-2,6-nonadienal, whose effect is not affected much by cross-adapting with either both aggregation pheromones or the plant odour; and a third receptor assembly that detects the adult-produced pheromone (PAN), but whose responsiveness is equally adapted by all other compounds.

Pheromone detection by nymphal stages (Paper V)

Pheromone communication in *Schistocerca gregaria* is stage-specific, i.e., each developmental stage has been shown to produce a set of chemical substances that only members of their respective stages respond to (Obeng-Ofori *et al.*, 1993; Torto *et al.*, 1994; 1996; Njagi *et al.*, 1996). Pheromones associated with egg froth has been shown to attract gravid locusts to a common egg-field (Saini *et al.*, 1995). Nymphal stages (2-5 instars) produce pheromones that cause aggregation of these stages, and at the same time retard maturation of young adult locusts (Torto *et al.*, 1996). Sexually mature males produce aggregation pheromones that keep adult members together and accelerate maturation of young adults (Torto *et al.* 1994). Sexually mature solitary females have been shown to produce a potential sex pheromone compound (Njagi and Torto, in press).

In a morphological study (I), it was shown that antennal olfactory receptor neurone numbers increase in each developmental stage, reaching a maximum at the final moult. Since nymphal aggregation pheromones affect the aggregation behaviour of all nymphal stages (except the 1st instars), we investigated the response sensitivities of receptor neurones present in antennal sensilla basiconica of 2-5th nymphal stages. It is known that these receptor neurones are responsible for the detection of aggregation pheromones (II and III). In addition, we tested the responses of adult-produced pheromones and a general plant odour, *E*-2-hexenal.

Results from this study show that, even though responses to some individual nymphal-produced compounds differed between different nymphal stages, responses to a blend of nymphal pheromones were similar in all the stages. Surprisingly, responses to major components of adult-produced aggregation pheromones occurred at lower threshold values than responses to nymphal pheromones.

Concluding remarks

It is clear that locusts have evolved to use a multitude of chemical substances allowing intraspecific communication. However, we have only begun to understand how the chemical substances interact with receptor organs. In my thesis, I could show that in *S. gregaria*, the distribution and sensitivity of olfactory receptor neurones is correlated with behavioural changes during development. Not only is there an increase in sensillum numbers with developmental stage that is higher in the solitary than in the gregarious phase locusts, but this change is also accompanied by a parallel increase in sensitivity of olfactory receptor neurones. The solitary phase individuals seem better endowed to detect semiochemicals from their surroundings than their gregarious counterparts. Furthermore, I demonstrated that odours of different behavioural significance to the locust are conveyed to the brain through different channels, as receptor neurones in specific sensillum types were found to respond only to a given odour modality. The fact that central projections of receptor neurone axons were shown to target several antennal lobe glomerular structures, is suggestive that integration of olfactory information is made in the higher order centres.

These results provide a firm foundation upon which short- and long-term behavioural studies on semiochemicals of importance to locust life will be undertaken. Short and long term exposure assays to specific semiochemicals will determine whether these chemicals play part in locust development such as affecting duration of nymphal stages or maturation of adult insects.

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Fine structure and distribution of antennal sensilla of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae)

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Abstract. Fine structure and distribution of different types of antennal sensilla in three nymphal stages and in adults of both solitary-reared (solitarious) and crowd-reared (gregarious) phases of the desert locust, *Schistocerca gregaria*, were investigated by scanning and transmission electron microscopy. Four types of sensilla were identified: sensilla basiconica, s.trichodea, s. coeloconica, and s. chaetica. Sensilla basiconica contain up to 50 sensory neurons, and each one displays massive dendritic branching. The sensillar wall is penetrated by a large number of pores. In contrast, sensilla trichodea contain one-to-three sensory neurons that branch to 5 or 6 dendrites in the sensillar lumen, and the sensillum wall is penetrated by relatively few pores. The sensilla coeloconica are situated in spherical cuticular pits on the antennal surface. The s. coeloconica are of two types: one type contains 1-3 sensory neurons with double-sensillar walls penetrated by slit-like pores, while the second type contains four sensory neurons with nonporous double sensillar walls. The sensilla chaetica have a flexible socket and contain four sensory neurons that send unbranched dendrites to the terminal pore; they have a thick, nonporous sensillum wall. A fifth sensory neuron of the s. chaeticum terminates in a tubular body at the base of the hair. Sensilla basiconica and coeloconica are normally distributed on the entire antennal flagellum, with a maximum number in the middle segments, whereas s. trichodea have three maximum points on the 5th, 10th, and 14th flagellar segments. Sensilla chaetica are, however, most abundant on the terminal segment. Solitarious phase locusts have relatively more olfactory sensilla (s. basiconica and s. coeloconica) than gregarious phase locusts. The differences in sensillar numbers is more evident in adults than in nymphs. These results suggest that differences in odor mediated behaviours of nymphs and adults, as well as between the phases of *S. gregaria*, may be due to differences at the sensory input level.

Keywords: Olfaction - Chemoreceptors - Aggregation pheromone - SEM - TEM - Insect antennae

Introduction

In the desert locust, *Schistocerca gregaria* (Forskål) (Orthptera: Acrididae), olfactory input plays an important role in moulding both development and behaviour. The influence of odours in mediating locust behaviour has been known since the 1920s (Uvarov, 1921; Norris, 1954, 1964; Loher, 1961; Gillet, 1975). *S. gregaria* occur in two phases, solitarious and gregarious, depending on whether nymphs, fledglings, or mature adults are raised in isolation or in groups. The two phases differ in morphology, physiology, and behaviour, and several experiments (see reviews by Loher, 1990; Byers, 1991; Pener, 1991) have implicated semiochemicals produced by the locusts as modulating changes between the phases. Obeng-Ofori *et al.* (1993; 1994) demonstrated in olfactometric bioassays that crowded nymphs and adults were capable of choosing and aggregating in an airstream permeated by odours from conspecific groups. Moreover, nymphs showed indifference to odours from adult locusts, and similarly adults were not attracted to nymphal odours.

In various antennectomy experiments, the antennal olfactory receptor neurons of the desert locust have been implicated as the site of detection of gregarization pheromones. Mordue (Luntz) (1977), demonstrated that removing antennae from crowded *S. gregaria* in the third instars resulted in fifth instars with green colour typical of the solitarious phase, whereas removal of tarsae or injury to other body parts did not elicit similar effects. Mordue (Luntz) concluded that removal of the antennae from crowded insects may simulate the solitarious condition of the locusts. The detection sites of maturation-accelerating pheromones of *S. gregaria* was shown by Loher (1961) as the antennae, since removal of antennae prevented the vibration reactions consisting of antennal waving and lateral movements of the hind legs caused when mature adults or their extracts were held in front of immature locusts (Loher, 1958). Removal of palpi from immature males did not prevent the vibration response when the antennae were left intact. More recently, Saini *et al.* (1995), demonstrated that gravid *S. gregaria* were attracted to and relatively preferred to oviposit in sand contaminated by volatiles from froth of egg pods. The locusts were shown to probe the sand with their antennae prior to ovipositing.

These investigations show that aggregation pheromones of *S. gregaria* are detected by antennal olfactory receptor neurons. However, no examination of the chemosensory system of the desert locust antennae has been reported. In this study, we have investigated chemosensory sensillar types and their distribution on the antennae in different developmental stages of both solitarious and gregarious phases of *S. gregaria* as a structural base for further physiological and behavioural studies of semiochemicals involved in gregarization and phase transformation of this species.

Materials and methods

Insects

Egg pods of solitarious *S. gregaria* were supplied from a colony maintained at the International Centre of Insect Physiology and Ecology (Nairobi, Kenya), and gregarious phase nymphs and adults were obtained from Blades Biological (Edenbridge, UK). Gregarious locusts were bred under crowded conditions (50-100 per cage) in perspex cages (40 × 60 × 70 cm) in a ventilated, temperature controlled (32 ± 2^{0}C day: 26 ± 2^{0}C night) room, and maintained on a 12:12 L:D and 45% relative humidity. Fresh wheat shoots and wheat bran were provided daily. Solitary locusts were bred in a separate room but under similar conditions. Solitarious hoppers were isolated after hatching into 1-litre cylindrical Perspex jars, the walls of which were covered with paper to eliminate visual input. Antennae of 1st, 3rd, and 5th instars as well as adults of both phases 2-5 d after the final moult were used for morphological investigations.

Scanning electron microscopy (SEM)

Antennae for SEM were fixed in 70% ethanol for 1-3 days before dehydration in a graded series of ethanol concentrations followed by either critical point or air drying. All antennae were mounted on aluminium stubs with the ventral surface upwards and sputter coated with gold/palladium (40:60) in a Polaron E 5400 high resolution sputter. Specimens were examined in a JEOL T 330 scanning electron microscope operated at 15 kV. The sensillar types were identified and counted on the microscope screen . Ventral surface sensilla were counted on all the segments of 1st instars and the adults, whereas for the 3rd and 5th instars, the ventral sensilla on segments 2, 8, and 14 were counted, with segment 1 being the most distal. A total of 12 antennae was investigated for each locust stage.

Transmission electron microscopy (TEM)

For TEM, antennae were cut into pieces of 2-4 segments each and fixed in 3% glutaraldehyde in 0.15M cacodylate buffer overnight at 4^{0}C. The specimens were rinsed in the buffer three times for 10 minutes each, post-fixed in 1% osmium tetroxide for 2 hours at room temperature, rinsed in buffer again, and dehydrated in an ethanol series. The fixed antennae were then embedded in Epon and polymerized at 60^{0}C. Ultrathin

sections were cut with a diamond knife on a LKB ultratome and stained with uranyl acetate and lead citrate in a LKB ultrostainer. The sections were studied in a JEOL 1200EX transmission electron microscope.

Distribution of sensilla on the antennae

In the present study, we have adopted a nomenclature proposed for the insect chemosensilla by Altner (1977). The distribution of the different sensillar types was examined in the 1st, 3rd, and 5th instars as well as in adult locusts. Because sensillar distributions were found to be identical in both sexes, data were pooled for each age group. In the 1st instars and adults, the sensillar population of the ventral surface of each antennal segment was counted, whereas in the 3rd and 5th instars, three diagnostic segments were chosen for quantification and comparison with the corresponding segments in the adults. We chose to count sensilla only on one surface as it has been shown in other acridids that no major differences exist between the dorsal and ventral antennal surfaces (Abushama, 1968; Bland, 1982).

Table 1. Mean antennal length and number of flagellar segments in different stages of the gregarious phase of *S. gregaria*; n = 10 antennae for each stage.

	Antennal length (mm)	Number of flagellar segments
1st instar	3	12
2nd instar	3.8	16
3rd instar	5	19
4th instar	7.5	21
5th instar	11	23
Adult	14	24

To complete the picture of changes in sensillar type, proportions, and distribution during development, we investigated the sensillar populations of the 2nd, 8th, and 14th segments in the 3rd and 5th stages of both phases and compared the sensillar density with that in the same segments in adults. Differences between the phases in sensillum numbers along the entire length of the antenna in 1st instar and adult locusts were tested statistically by the Wilcoxon signed rank test. Sensillar differences between stages and phases (Fig. 7) were tested by an ANOVA followed by a Fisher's PLSD test. Sensillar

numbers were initially log-transformed (ln x + 1) to equalize variances between the samples. All statistical calculations were performed in Statview version 4.51 for Macintosh.

Results

Fine structure

The antennae in both sexes of *S. gregaria* are filiform. The antennal length and the number of segments increase through the nymphal stages reaching a maximum of 13 to 14 mm and 24 segments in adults (Table 1). In both sexes, the antenna has an even diameter throughout its length. The antennal surface is covered by cuticular plates that also surround the sensillum (Figs 1a, b). Four different types of surface sensilla are present: sensilla basiconica, s. trichodea, s. coeloconica, and s.chaetica (Figs 1a, b). Identical sensillar types were observed in both sexes, all stages, and both phases.

Sensilla basiconica. The basiconic sensillum is the most abundant type, especially in the adults (Table 2). The hairs are set in shallow depressions of the antennal cuticle (Fig. 1). The sensillum length varies between 9 and 11 μm, the basal diameter is about 4 μm, and the sensillum lacks a basal socket. Each s. basiconicum is innervated by 20-50 sensory neurons present below the hair base and surrounded by the enveloping cells (Fig. 2b). The outer dendritic segments send dendrites that branch profusely close to the base of the sensillum. More than 200 dendritic branches occur distally in the hair; the branch diameter varies between 0.05 μm and 0.3 μm (Fig. 2c). The thinner processes contain only a single microtubule, whereas up to five microtubules are present within the larger dendritic branches. The hair's surface is perforated by between 20 and 30 pores μm^{-2} (Fig. 2a), and the pores have a diameter of about 50 nm leading to pore tubules (Fig. 2c).

Sensilla trichodea. The s. trichodea are 8-10 μm long but are more slender and have a smaller basal diameter, about 3 μm, compared with the s. basiconica (Fig. 3a). The s. trichodeum has 1-3 sensory neurons that send unbranched dendritic outer segments into the hair where they divide into 5 or 6 branches with a diameter of 50-100 nm each. Smaller dendrites contain single microtubules whereas larger ones contain 2 or 3 microtubules. The hair wall has a moderate density of pores between 10 and 12 μm^{-2}, that are connected internally with pore tubules (Fig. 3).

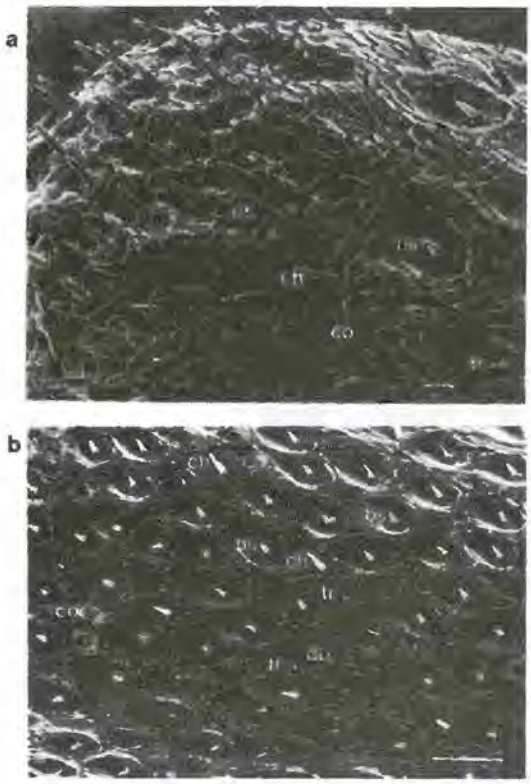

Fig. 1a, b. Different sensillar types on the terminal segment (1st segment) (**a**) and the 6th segment (**b**) on the adult *S. gregaria* antenna. The tip of the terminal segment is endowed with more sensilla chaetica than any other sensillar type, while segments proximal to segment 1 are dominated by olfactory sensilla. Sensillum basiconicum (bs), s. coeloconicum (co), s. trichodeum (tr), and s. chaeticum (ch). The antennal surface is coveredby cuticular plates (*arrow heads*). *Bar*: a, 10 µm; b, 50 µm.

Sensilla coeloconica. The s. coeloconica are short and housed in spherical cuticular pits with an opening of about 5 µm in diameter (Figs 4a, b). Two types of coeloconic sensilla were observed: one has a double wall that is penetrated by radial pores, 1-3 unbranched sensory neurons, and longitudinal ridges (Figs 4b, c), and the second has a nonporous wall and contains four sensory neurons (Fig. 4d). The dendritic outer segments are 100-200 nm in diameter, unbranched, and each contain more than 10 microtubules (Figs 4c, d).

Sensilla chaetica. The s. chaetica are 13-16 µm long with a basal diameter of about 4 µm. The sensillum has a flexible socket, suggestive of a mechanoreceptor (Fig 5b). The s. chaeticum contains six sensory neurons, five of which send unbranched dendrites to a terminal pore (Fig. 5c), whereas one terminates in a tubular body at the base of the sensillum (Fig. 5d). The dendrites are surrounded by a dendritic sheath and bathed in receptor lymph (Fig. 5c). The sensillar surface has longitudinal, cuticular grooves and a terminal pore at the tip of the sensillum. There are no pores on the remainder of the sensillar wall (Figs. 5a - c).

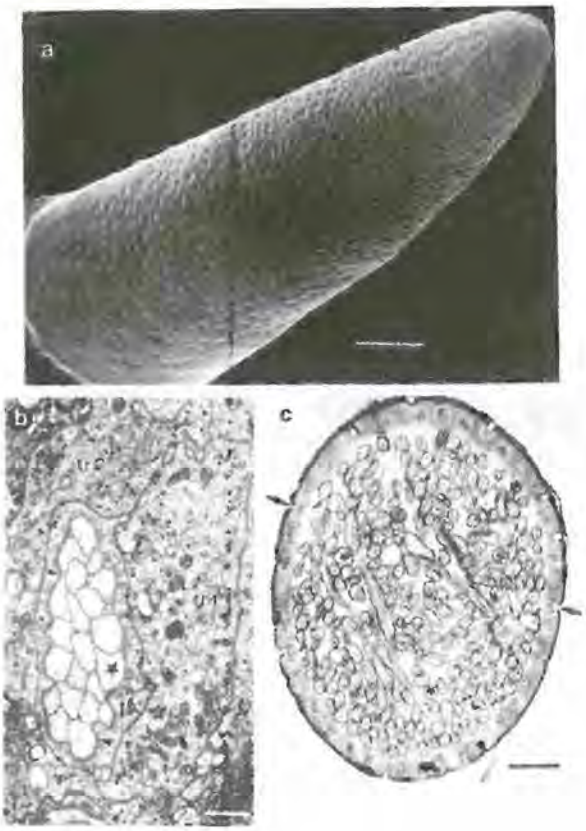

Fig. 2a-c. Sensillum basiconicum. Scanning electron micrograph (**a**) displaying massive cuticular pore system. A transverse section beneath the cuticle (**b**) displays inner dendritic segments (*asterisk*) surrounded by supporting cells: one thecogen (th) and two trichogen cells (tr1 and tr2). A tranverse section from the middle of s. basiconicum (**c**) shows massive dendritic branchings (*asterisk*), and numerous cuticular pores (*arrows*), which are continuous with pore tubules internally (*arrow head*). a × 15 000; *Bar*: a, 1 µm; b and c, 500 nm.

Distribution of sensilla on the antennal surface

The distribution of sensilla on the ventral surface of antennae of 1st instars was similar in both gregarious and solitarious phases for the olfactory sensillar types. There were, however, significantly more chaetic sensilla in solitarious than in gregarious nymphs ($p=0.0033$) (Fig. 6). Sensilla chaetica were present in highest number on the terminal segment, but the number declined substantially in segment 2 whereafter it remained at a constant level to the base of the antenna.

When sensillar distributions were determined for adult locusts, it became clear that solitarious and gregarious phases have a pronounced difference in the number of olfactory sensilla. The solitarious phase possessed significantly more basiconic

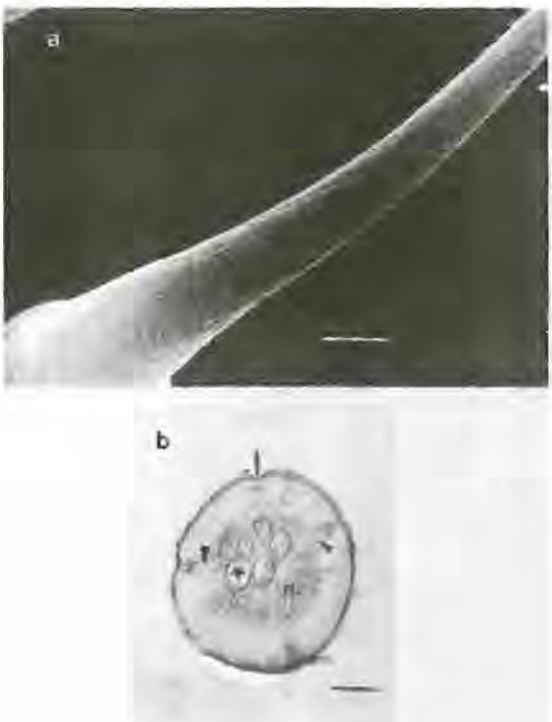

Fig. 3a, b. Sensillum trichodeum. Scanning micrograph (**a**) displaying relatively fewer cuticular pores as compared with s. basiconicum. Transverse section from the middle of s. trichodeum (**b**) displays outer dendritic segments (*asterisk*) bathed in a receptor lymph (rl), and pore tubules (*arrow heads*), connecting the dendrites with the cuticular pore system (*arrow*). **a** × 15 000 *Bar*: a, 1 µm; b, 200 nm.

($p<0.0001$), coeloconic ($p<0.0001$), and trichoid ($p=0.0040$) sensilla (Fig. 6), whereas the chaetic sensilla were found in equal numbers in the two phases. The different sensillar types occurred in maximum numbers at different locations along the length of the antenna. Sensilla basiconica were found in highest numbers on segments 9-15; the numbers of s. coeloconica peaked on segments 8-14; the distribution of s. trichodea was found to be trimodal with local maxima on segments 5, 10, and 14. The contact-chemoreceptive s. chaetica occurred most densely at the terminal antennal segment. The maxima were found to be the same in the two locust phases (Fig. 6).

Sensillar distributions in the 2nd segment of the 3rd and 5th instars revealed no difference in sensillar numbers between the phases. On the 8th and 14th segments, differences, especially in coeloconic and basiconic sensilla, were found in all the stages, as well as between the phases. A role reversal could be observed in the 3rd instar where the gregarious phase had more sensilla of all types on segment 8 and of basiconic and trichoid sensilla on segment 14 (Fig. 7).

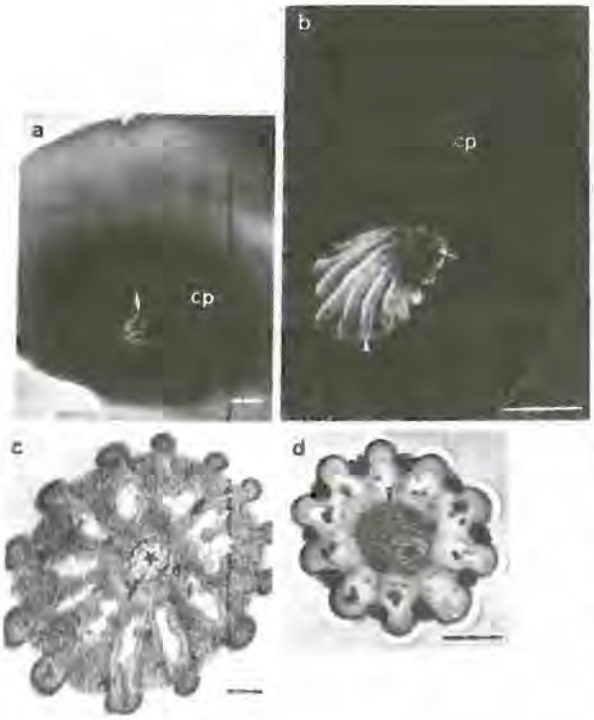

Fig. 4a-d. Sensilla coeloconica. Scanning micrographs (**a**, **b**) displaying sensilla coeloconica situated in the cuticular pit (cp); a sensillum with a beaded tip (*arrow*) (**a**), and a sensillum displaying longitudinal ridges (*arrow head*), and a terminal moulting pore (*arrow*) (**b**). Transverse sections through a double-walled sensillum shaft with one (**c**) and four outer dendritic segments (*asterisk*) (**d**). The sensillum with a single outer dendritic segment has slit-like spoke channels, (*arrows*), between the longitudinal ridges and the dendrite is bathed in a receptor lymph (rl). The outer dendritic segments (*asterisk*) of a nonporous sensillum (**d**) are tightly enclosed by a dendritic sheath (*arrow head*). In this case an electron dense pre-cipitate is found externally (*arrow*).a × 7 500; b × 15000; *Bar*: a, b, and d, 1 µm; c, 200 nm.

When the average number of sensilla on the entire ventral surface was compared between the 1st instar and adult, coeloconic sensilla were found to increase by a factor of 7.2 and 10.2 for gregarious and solitarious locusts, respectively. For s. basiconica, the total number increased by a factor of 12.9 in gregarious locusts and 15 in solitarious locusts. There was no major differences in s. trichodea and s. chaetica between 1st instars and adults (Table 2).

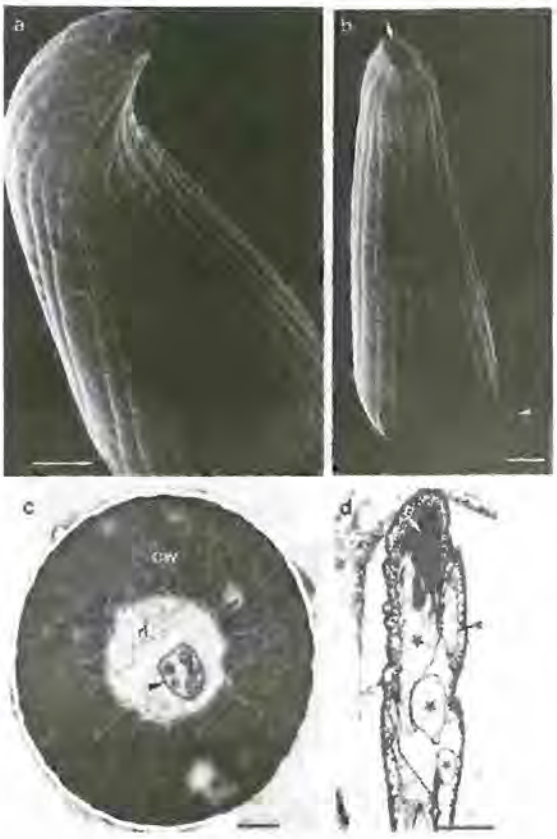

Fig. 5a-d. Sensilla chaetica. Scanning micrographs (**a**, **b**) displaying nonporous longitudinal ridges with a terminal pore (*arrow*), and a flexible socket (*arrow head*), at the sensillum base (**b**). A transverse section (**c**) from the middle of the sensillum shows a thick, nonporous cuti-cular wall (cw). Five outer dendritic segments (*asterisk*) are enclosed by a dendritic sheath (*arrow head*) within the receptor lymph (rl). A longitudinal section (**d**) through the sensillum base shows inner dendritic segments (*asterisk*) enclosed by a dendritic sheath and one of the neurons terminating in a tubular structure (*arrow*). a × 15 000; b × 10 000; *Bar*: a, b, and d, 1 µm; c, 500 nm.

Discussion

Characteristics and types of antennal sensilla

The external morphology and distribution of sensilla on the antennae of *S. gregaria* conform in most respects to sensillar types found in other acridid species (Slifer *et al.*, 1959; Abushama, 1968; Greenwood and Chapman, 1984). We have found similar sensillar types as described for *Locusta migratoria* L. (Altner *et al.* 1981; Ameismeier, 1987) and *Hypochlora alba* (Dodge) (Bland, 1982), although the nomenclature of sensillar types used for these species is different.

The inner structures of basiconic and trichoid sensilla of the desert locust conform well to what has been reported in other locust species. Sensilla basiconica have a thin

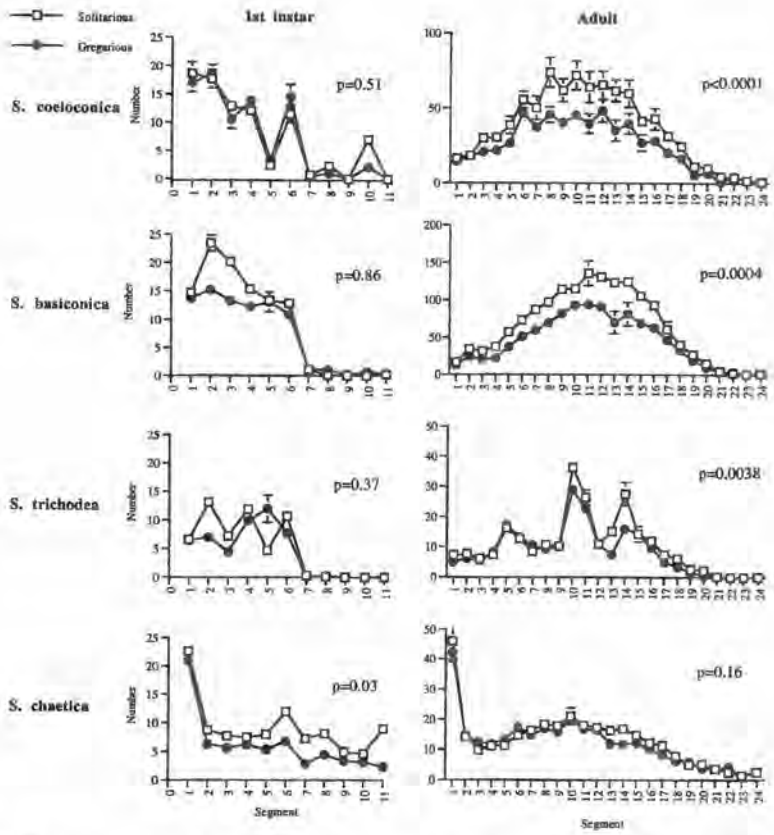

Fig. 6. The mean number of sensillar types distributed on the ventral surface along the antenna of 1st instar and adult *S. gregaria*. Segment 1 being the most distal. For each stage and phase, 12 antennae were investigated. Vertical bars indicate the standard error; p-values indicate the significance level of differences between the curves for solitarious and gregarious locusts in each diagram. Where no bars are visible, they are covered by the symbol. Statistical significance levels were calculated using the Wilcoxon signed rank test.

wall, a multitude of wall pores, and several branched, outer dendritic segments; s. trichodea have a thicker wall, fewer sensory neurons, and wall pores (Keil and Steinbrecht, 1984). The structural features of s. basiconica and s. trichodea are typical of olfactory receptors in general, as described by Altner and Prillinger (1980) and Zacharuk (1980). In *L. migratoria*, the antennae have two types of single-walled wall-pore sensilla that differ from one another in peg diameter, density of pores, number of sensory neurons, branching of dendritic segments, and number of enveloping cells (Ameismeier, 1987). The sensilla basiconica described here correspond to single-walled wall-pore type A described in *L. migratoria*, whereas s. trichodea correspond to type B (Ameismeier, 1987). Receptor neurons present in s. basiconica have been shown to respond to

stimulation with aggregation pheromone compounds of *S. gregaria* (Hansson *et al.*, 1996).

The two types of sensilla coeloconica we found in *S. gregaria* correspond to the sensillar types found in other insects. Functional properties have been ascribed to the two sensillum types using both morphological and physiological techniques. In *L. migratoria*, s. coeloconica with cuticular pore channels were shown to be chemosensitive and one with a nonporous wall responds to hygro- and thermostimulation (Boeckh, 1967; Steinbrecht, 1969; Kafka, 1971; Altner *et al*, 1981). A morphologically similar sensillum has also been studied on the antennae of *Periplaneta americana* that possesses thermo-, hygro-, and chemoreceptors (Altner *et al.*, 1977). In *S. gregaria*, some coeloconic sensilla that respond to chemostimulation have been found (Hansson *et al.*, 1996). However, detailed physiological investigations remain to be done to show whether the s. coeloconica with a nonporous wall in *S. gregaria* respond to hygro- and thermostimulation.

Sensilla chaetica probably function as taste/mechanoreceptors. This can be deduced from their strategic location found mainly on the terminal antennal segment and from their structural features: being mounted on a flexible socket, possessing a thick, nonporous cuticular wall, and an apical pore. There is, however, no evidence from the present morphological investigation to indicate that the apical pores of these sensilla are capable of being opened and closed as those of gustatory sensilla on the maxillary palps (Blaney and Chapman, 1969). We can only conclude this from their morphological similarities. A physiological study should be performed to prove the contact chemoreceptive properties of these hairs. Saini *et al.* (1995), showed that gravid *S. gregaria* probe the sand with their antennae prior to ovipositing in a common egg laying site. Electroantennogram recordings confirmed the presence of antennal receptors responsive to odours from egg froth. It has also been shown that the action of maturation-accelerating pheromones was enhanced when immature locusts were allowed to touch either mature males or other sources containing pheromones (Norris, 1954; Amerasinghe, 1978). Mahamat *et al.* (1993), however, confirmed accelerated activity of maturation pheromones without involving contacts, suggesting that olfactory, rather than contact chemical cues may be playing a significant role in adult maturation.

Phase- and stage-specific differences in sensillum numbers

The peripheral olfactory apparatus of the desert locust changes dramatically during development. It has been suggested that because gregarious locusts act as a unit, with the behaviour of individuals being influenced by the behaviour of others closeby, the gregarious individuals require a less sensitive olfactory system which results in smaller numbers of chemoreceptors (Chapman, 1982).

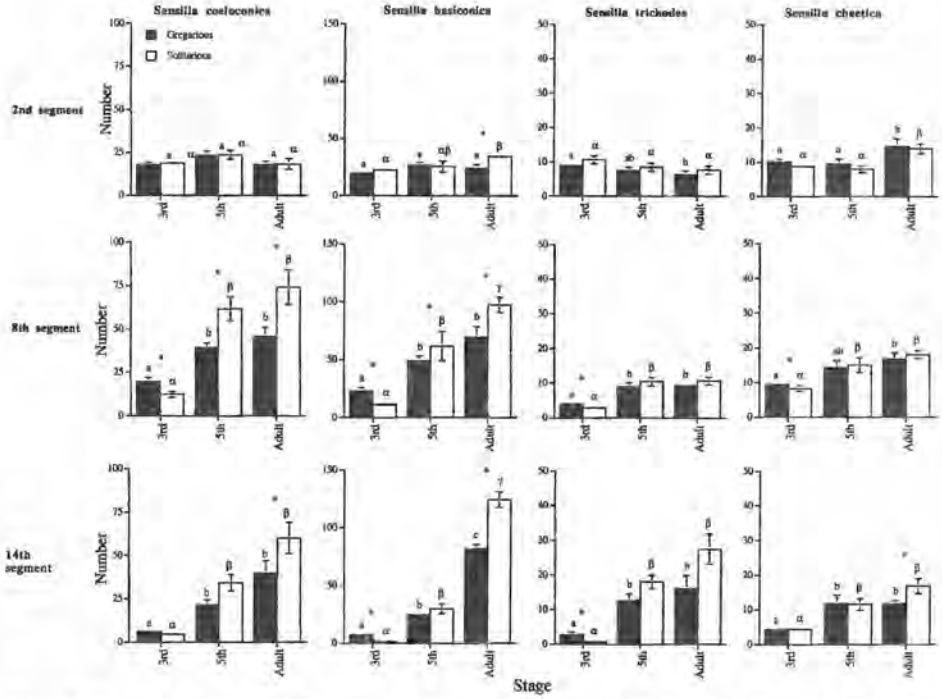

Fig. 7. The mean number of sensillar types distributed on segments 2, 8, and 14 of 3rd, 5th, and adult stages, respectively, of *S. gregaria*. Vertical bars indicate the standard error. Different letters above bars of one phase within one diagram indicate that these values are significantly different ($p<0.05$) when tested by an ANOVA followed by Fisher's PLSD test. An asterisk above a pair of bars indicate that these two values are statistically different as described above.

The type of phase shift encountered in desert locusts is very rare. A clear selective advantage must be gained for such a system to evolve. As we understand it today, phase-shifts are triggered by the fine-scale distribution of resources (Bouaïchi *et al.*, 1996). These authors demonstrated that access to multiple resource sites such as food, perches, and warm spots, dispersed solitary locusts and inhibited gregarization both in the laboratory and in semi-field situation. In contrast, provision of only a single resource site promoted gregarization behaviour. The concentration of locust-produced odours is much higher in a swarm than what the solitarious insects will encounter during their eremitic life in the desert. Solitarious individuals, on the other hand, living in sparsely populated environments, have to detect conspecifics from a distance both for swarming and for mating. In accordance with Chapman (1982), we propose that it is these differences in social environment that exerts the selective pressures responsible for the phase differences observed in the peripheral olfactory system. Differences in food location is, with the clumped vs. non-clumped resource theory (Bouaïchi *et al.*, 1996), more obscure. Are odours emitted by large, scarce resources easier to detect than those

emanating from many small ones? The olfactory background to resource location in the two phases remains to be studied.

Starting from the same numbers at 1st instar, the chemoreceptor sensilla increase more in solitary than in gregarious phase as the locusts approach adulthood, whereas the taste/mechanoreception system does not change. The clearest trend between the different phases was observed in the 5th instar and in adults where more olfactory sensilla were present in the solitary phase. A reversal of the general pattern of higher sensillum numbers in solitary individuals was found in the 3rd instars. Here, all three olfactory sensillum types on segment 8, and basiconic and trichoid sensilla on segment 14,

Table 2. Mean number of sensilla on the ventral surface of antennae of 1st intar and adult *S. gregaria*; n = 12 antennae for each stage.

Sensillar type	Gregarious		Order of magnitude	Solitarious		Order of magnitude
	1st	Adult		1st	Adult	
Coeloconicum	81.5	592.5	7.3	81.3	872.6	10.7
Basiconicum	82.3	1,059.6	12.9	101.6	1,529.2	15.1
Trichodeum	48.0	206.1	4.3	54.6	248.8	4.6
Chaeticum	66.9	294.6	4.4	99.9	315.3	3.2

occurred in higher numbers in the gregarious phase. The behavioural significance of this observation is unknown.

In *L. migratoria*, the distal segments are fully differentiated at the time of hatching and no new sensilla are added to them at subsequent moults (Chapman and Greenwood, 1986). Sensillum differentiation proceeds from distal to proximal along the antenna and more distal segments attain full development at each moult. Our results show that a similar developmental pattern applies to *S. gregaria*, since the terminal segments bears equivalent numbers of sensilla in all stages, whereas additional chemosensilla occur only in the median segments in the later instars (Figs 6 and 7).

We found no differences between the sensillar arrays in male and female *S. gregaria*, a result similar to that in *L. migratoria* (Greenwood and Chapman, 1984). However, in *H. alba*, a monophagous grasshopper, adult males were shown to possess 50 to 80% more sensilla than females (Bland, 1982). Furthermore, we counted sensilla only on the ventral antennal surface, and this may account for the low overall numbers compared to *L. migratoria* (Greenwood and Chapman, 1984), even though the general trend in distribution was similar in the two locust species.

The locust olfactory system offers a rare opportunity to study both development and plasticity in a sensory system. Extensive changes occur in the peripheral olfactory system during phase-shifts and growth. Are these changes parallelled by changes also in the functional characteristics of antennal receptor neurons? Are the olfactory centers of the central nervous system affected to further increase sensitivity in solitarious individuals? These are questions that need to be answered for us to gain a more complete knowledge of olfactory structure and function in the desert locust.

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ORIGINAL PAPER

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Physiological responses and central nervous projections of antennal olfactory receptor neurons in the adult desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae)

Accepted: 10 February 1996

Abstract Olfactory receptor neurons present in two morphological sensillum types on the male *Schistocerca gregaria* antenna were for the first time investigated physiologically when stimulated with behaviourally relevant odours. Neurons present in trichoid/basiconic sensilla showed clear excitatory responses to compounds present in the male-produced aggregation pheromone and also to a plant produced compound. Sensilla could be categorised physiologically according to the responses of their receptor neurons to the tested stimuli. Also receptor neurons present in sensilla coeloconica responded to aggregation pheromone components, but always in an inhibitory fashion. These neurons could, however, be excited by a plant produced compound and by some acids present in the nymphal odour. The antennal lobe of the male *S. gregaria* was observed to contain about 1000 very small glomerular structures. Single receptor neurons were stained from the antenna to the antennal lobe using a cobalt lysine technique. These stainings revealed a multi glomerular axonal branching pattern of antennal receptor neurons.

Key words Antennal lobe projection · Single sensillum · Locust · Receptor neuron · Electrophysiology

Abbreviations *AN* antennal nerve · *AL* antennal lobe · *RN* receptor neuron

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Introduction

Physiological characteristics of olfactory receptor neurons involved in pheromone detection have been intensely studied in moths and to some extent in beetles (for reviews see, Hansson 1995; Masson and Mustaparta 1990). These investigations have revealed an extremely specific and sensitive olfactory apparatus in the attracted sex, usually the male. Neural responses have been recorded to very low numbers of pheromone molecules (Kaissling and Priesner 1970). Pheromone detection in Orthoptera has, however, been neglected, mainly due to the lack of unambiguously identified, behaviourally active pheromone compounds. Early single sensillum investigations on locusts have been performed on *Locusta migratoria*. In the first investigation by Boeckh (1967), a few plant produced odours were tested, and responses to these were recorded from sensilla coeloconica. The same type of sensillum was also studied by Kafka (1970), who challenged it with 370 different compounds. Phenolic compounds, like the ones now identified as being part of the aggregation pheromone of *Schistocerca gregaria* (Forskål), were, however, not included. The highest responses were obtained to acids containing four-to-six carbon atoms and to some so called green leaf volatiles like (*E*)-2-hexenal and (*Z*)-3-hexenol. Responses from receptor neurons present in other types of sensilla on the locust antenna have not been investigated, and no single sensillum recordings whatsoever have been performed on the *S. gregaria* antenna.

Migratory locusts appear in two different phases depending on the density of the population, solitary or gregarious (Uvarov 1921). Aggregation behaviour, connected with morphological and physiological changes, is induced by optical and tactile cues (Ellis 1951) and also to a large extent by olfactory stimuli (for review, see Byers 1991). Under favourable climatic conditions and resurgent food supplies, the locust populations can

increase in density resulting in olfactory-dependent behavioural, physiological and morphological changes as the young hoppers develop to adults (Bennett 1975; Waloff and Green 1975).

During development and sexual maturation, numerous pheromones have been shown to affect locust behaviour (Byers 1991). One of the earliest examples of pheromonal activity discovered for any insects was the maturation pheromone of *S. gregaria* (Norris 1954). Since then, several locust pheromones have been reported, involved in numerous processes including maturation, oviposition, mate identification, stimulation and recently aggregation (Whitman 1990). Locusts are also known to use odour cues to detect suitable food plants from a distance (Blaney and Simmons 1990).

The existence of an aggregation pheromone in the air surrounding the animals and in their faeces has been shown independently in several studies (Fuzeau-Braesch et al. 1988; Gillett 1975; Obeng-Ofori et al. 1993, 1994; Torto et al. 1994). A variety of substances which might be part of the aggregation pheromones have been suggested, but some contradictions persist on their effect on behavioural and/or morphological and physiological differentiation (Nolte et al. 1973; Nolte 1976; Fuzeau-Braesch et al. 1988). Recent behavioural experiments showed that there are differences in the effect of aggregation pheromones between the different developmental stages (Obeng-Ofori et al. 1993, 1994). The composition of male-emitted odours has been analysed, showing that the major component is phenylacetonitrile (Luber et al. 1993; Obeng-Ofori et al. 1994; Torto et al. 1994). Minor components were identified as guaiacol, phenol, benzaldehyde, veratrol and anisole. All these components were also shown to release a gross physiological response from the male antenna (Obeng-Ofori et al. 1994; Torto et al. 1994). This adult male odour attracts both young and old adult females and males (Obeng-Ofori et al. 1993, 1994; Torto et al. 1994).

A morphological investigation of the antennal sensilla in *S. gregaria* (Ochieng', Hallberg and Hansson, unpubl.) showed that three basic types of olfactory sensilla are present: Sensilla trichodea containing three RNs, S. basiconica containing 30 to 40 RNs and s. coeloconica with one-to-four RNs. The s. trichodea and the s. basiconica displayed a very similar external morphology, while the s. coeloconica were easily distinguished also in the light microscope. The structure of chemoreceptors in locusts have earlier been investigated in *L. migratoria* (e.g., Ameismeier 1987) and have been reviewed by Blaney and Simmons (1990).

The primary olfactory centre of the locust, the antennal lobe (AL), displays considerable differences when compared to the well-known patterns from the AL of moths or cockroaches. Instead of having a moderate number of glomeruli, ≈50, the locust AL possesses about one thousand very small glomerular structures (Ernst et al. 1977). In moths, the central nervous projection pattern of pheromone detecting RNs have been studied (Hansson et al. 1992, 1995; Ochieng' et al. 1995). Olfactory RNs in these species always display monoglomerular projection patterns in the AL. Male moths display an enlarged glomerular array in the antennal lobe, the macroglomerular complex (MGC). Pheromone-detecting RNs all project to the MGC, while non-pheromone-detecting RNs project to ordinary glomeruli. A functional separation between antennal lobe projections of different physiological types of pheromone-detecting RNs has been observed in several male moths (Hansson et al. 1992; Ochieng' et al. 1995). In *L. migratoria*, Golgi stainings have indicated that RN axons may target multiple glomeruli in the antennal lobe (Ernst et al. 1977).

In order to investigate the function and axonal target areas of olfactory RNs involved in detection of aggregation pheromone and host plant odours in *S. gregaria*, electrophysiological recordings were performed at the single sensillum level on male antennae. To reveal the central nervous projection patterns of single RNs, cobalt staining techniques were utilised.

Materials and methods

Insects

Gregarious male and female *S. gregaria* adults and fifth instar larvae were purchased from Blades Biological, Cowden Kent, United Kingdom. The locusts were kept in 70 × 60 × 40 cm perspecs cages at 30°C, 45%, RH, 12 h light: 12 h dark cycle. The locusts were fed fresh lettuce, carrots, wheat bran and green wheat daily. To decrease infection of protozoans, sulfanilamide (Sigma Chemical Co., St Louis, USA) was added to the wheat bran at a 0.1% concentration.

Electrophysiological techniques

For electrophysiological recordings, the insect was placed in a plastic tube so that only the head protruded from one end. The head was immobilised with dental wax (Surgident, Miles Inc., USA) and the antenna was fixed with wax in a desirable position. A piece of moistened tissue paper was inserted behind the animal to keep it in place, and an Ag/AgCl wire was inserted into the abdomen to serve as a ground connection. The whole preparation was put into an alligator clip, making contact with the silver wire. The alligator clip was positioned on a metal rod, which in turn was fixed in a Leitz micro manipulator in the experimental setup.

Due to the hidden localisation and the short dimensions of the olfactory sensilla, recordings were made with the penetration technique (Hubel 1957). Tungsten wire electrodes (Ø0.1 mm) (Clarks) were sharpened electrolytically. The electrode was connected to a Syntech AC/DC UN-05 high impedance amplifier (Syntech, P.O. Box 1547, NL-1200 Hilversum, The Netherlands), and mounted in a second Leitz micro manipulator. The signal was observed on a Philips oscilloscope and recorded on a Tascam 4-Track 234 Syncaset. The amplifier was also connected to a loudspeaker to provide an indication of the quality of the contact.

The recorded responses were transferred from the tapes to a Compaq Prolinea 4/66 computer by means of a Syntech analog/digital

interface with Autospike for Windows V1.2 software (Syntech). This software was also used for data analysis.

The response acquired at a stimulation was calculated as the difference between the number of spikes during 1 s after the stimulation and the number of spikes during 1 s before stimulation, minus the response elicited by the blank. Response strengths were indicated by 0(no response), – (clear inhibition), + ($\geq$ 50% of response maximum of the neuron [RM], < 80% RM), + + ($\geq$ 80% RM, < 90% RM) or + + + ($\geq$ 90% RM).

Chemicals

The 20 different compounds tested and their abbreviations used in the text are listed in Fig. 1. They were all obtained from Sigma Chemical Co. and were at least 98% pure. Compounds 1 to 6 were used to make up the aggregation pheromone mixture (7). The other compounds are other locust derived odours (compounds 8–11), two plant odours (compounds 12–13), three acids that are present in the nymphal odour (16–18) and four acids of unknown behavioural significance (14, 15, 19, 20) (Torto and Njagi personal communication).

All the compounds were diluted in paraffin oil, except compound 5 in hexane, compound 10 in ethanol and compound 11 in ether, in decadic steps corresponding to stimulus cartridges containing from 1 to 1000 µg. Each dilution was applied to a piece of filter paper (5 × 15 mm). The filter paper was inserted into a Pasteur pipette. The air from the pipette atmosphere was injected during 200 ms at a flow of 5 ml/s using a stimulation apparatus (Syntech) to control airflow and stimulus duration precisely. The antenna was continuously flushed with a charcoal-filtered and humidified air stream (0.5 m/s) that was delivered through a glass tube (i.d. 8 mm) ending 10 mm in front of the preparation. The stimuli were injected from the pipette into the air stream 150 mm upstream of the antenna.

In the screening experiments, the filter paper was loaded with 100 µg of each compound, and a blank cartridge was loaded with solvent only. In the dose-response test, 1, 10, 100 and 1000 µg loadings were utilised.

Compounds were presented to the antenna in a random order. Lower concentrations were tested before higher concentrations to avoid adaptation of the neurons. Adaptation with high amounts of a stimulating substance was used to establish if all neurons in a sensillum were stimulated by the same substance.

Morphological techniques

A classical cobalt-lysine technique was used, in order to observe the projection of receptor neurons into the CNS (Obermayer and Strausfeld 1980). A 0.5 *M* solution of cobalt-lysine (2.38 g cobalt chloride hexahydrate and 5 g L-lysine for 20 ml of solution, pH 7.2–7.4) was used.

The locust was prepared for single sensillum recording. The electrode used was a glass electrode filled with the cobalt-lysine solution. The electrode was inserted at the base of the sensillum at a similar location as the tungsten electrode in the physiological investigations. When a response to one of the tested pheromonal compounds was obtained, the electrode was left in contact with the receptor for two hours. During this time, the cell was stimulated with 0.2 s puffs of the stimulating odour, with a frequency of 0.25 Hz to facilitate diffusion of the marker into the neuron.

After staining, the locust, still in the plastic tube, was transferred to a covered Petri dish containing a piece of moist tissue paper. The dish was placed at 4°C for 48 h for post-filling. The head was then excised, and the brain was dissected out in an insect Ringer solution (in m*M*: 150 NaCl, 3 KCl, 2 $CaCl_2$ 9 TES (N-tris- [hydroxymethyl] -methyl-2-aminoethane-sulfonic acid), 25 sucrose, pH 6.9). To silver intensify the preparations, a modification of Timm's procedure (Timm 1958) was used. This procedure demands pretreatment of the brain with a weak solution of ammonium sulphide, followed by treatment with colloidally-carried $AgNO_3$ and hydroquinone (Obermayer and Strausfeld 1980).

For observation, the brain was dehydrated in alcohol steps and finally cleared in methyl salicylate. At this stage it was possible to establish whether a cell had been stained or not. The brain was embedded in epoxy resin (Durcupan, Fluka), and cut into 10 µm sections. The sections were counter stained with a 1:1 mixture of 1% solutions of azur and methylene blue. The stained neurons were reconstructed with the help of a microscope with a camera lucida attachment.

Results

Physiological characteristics of antennal olfactory receptor neurons

Receptor neurons present in two distinguishable sensillum types were contacted during the experiments. The trichoid and basiconic sensilla could not be distinguished on morphological grounds, and are thus treated together (t/b sensilla). Most contacts with these sensillum types were, however, most likely with basiconic sensilla, as a large number of action potential amplitudes were observed, indicating the presence of many (> 10) receptor neurons, which is typical for the basiconic type. Coeloconic sensilla could be unambiguously identified already in the light microscope of the electrophysiology setup.

Out of 142 sensilla investigated in a first experimental series, 87 contained RNs responding to one or more of the compounds tested. The stimuli consisted of synthetic, locust produced odours and a single host plant produced volatile (Fig. 1, compounds 1–12). All the sensilla containing RNs responding to this set of stimuli were of the trichoid/basiconic (t/b) type. To investigate the response characteristics of RNs present in the s. coeloconica, a second series of experiments was performed, where the stimulus spectrum was expanded to include compounds shown to be stimulating receptor neurons present in s. coeloconica on the *L. migratoria* antenna (Kafka 1970) (Fig. 1).

In the t/b sensilla contacted, several RNs were usually stimulated, and a great variance in spike amplitudes prevented an unambiguous identification of single RNs (Fig. 2). Therefore, the response obtained from the entire RN ensemble of the sensillum was measured, i.e., all action potentials evoked by the stimulus in a t/b sensillum were counted. Out of the 87 responses obtained from RNs in t/b sensilla, 78 were of adequate quality to allow an evaluation of the response. The spontaneous activity of the entire receptor neuron ensemble of a t/b sensillum typically ranged between 10–30 Hz. Among the RN ensembles contacted, narrowly and widely tuned ones could be discerned (Fig. 3). The responses observed were assigned to 15 different categories depending on their specificities (Table 1). Sensilla containing RN groups responding

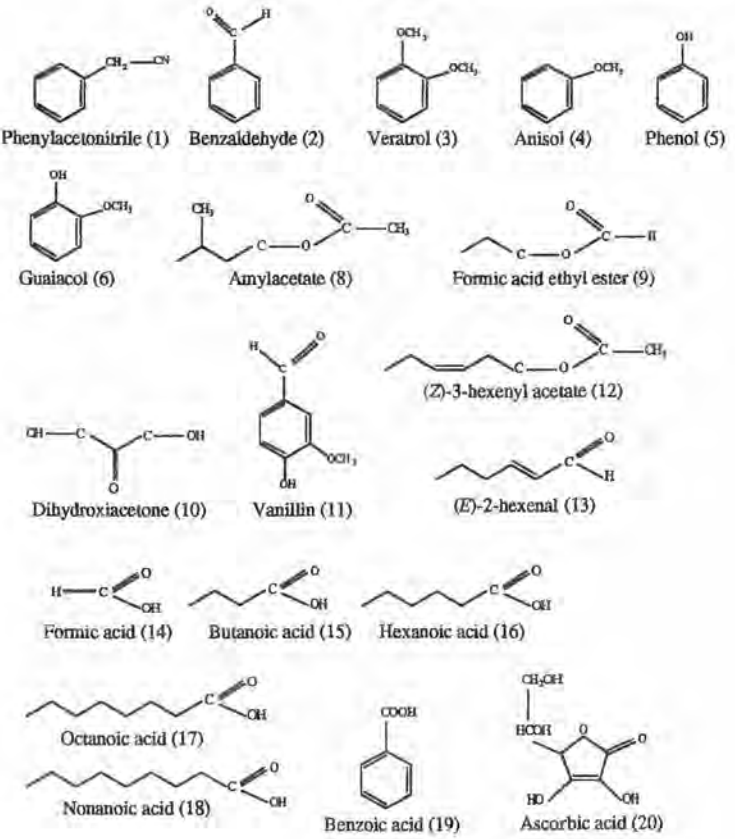

Fig. 1 Chemical compounds used as odour stimuli in the investigation. Blend (7) in text indicates the pheromone blend (phenylacetonitrile (1) 80%, benzaldehyde (2) 5%, veratrol (3) 5%, anisol (4) 4%, phenol (5) 3%, guaiacol (6) 3%)

Fig. 2 Example of a response obtained from a multitude of receptor neurons present in what is most likely a basiconic sensillum. The sensillum was stimulated with compound 1 (phenylacetonitrile)

strongly to a single component were assigned the number of the component, followed by an S, so that a sensillum containing RNs specifically responding to compound 4 would be of the type 4S. Sensilla containing RNs responding to a number of compounds were assigned the number of the compounds stimulating, e.g., 1, 3, 5, 6.

By adaptation with high amounts of a stimulating substance it was possible to abolish the spontaneous activity of all the neurons present in a sensillum. Subsequent stimulations by compounds evoking a clear response when not preceded by adaptation did not result in a response.

Forty-five out of the tested sensilla contained RNs responding to components of the pheromone blend as identified by Torto et al. (1994) (Table 1). Specifically responding RN ensembles were found for all the single components (Fig. 3A, C–E, Table 1), but in almost half of the sensilla, more widely tuned RN groups responded to compounds 1, 3, 5, and 6. In two contacts with an RN group responding to compound 1, inhibitions were observed when the same group was stimulated with compound 11 and with compound

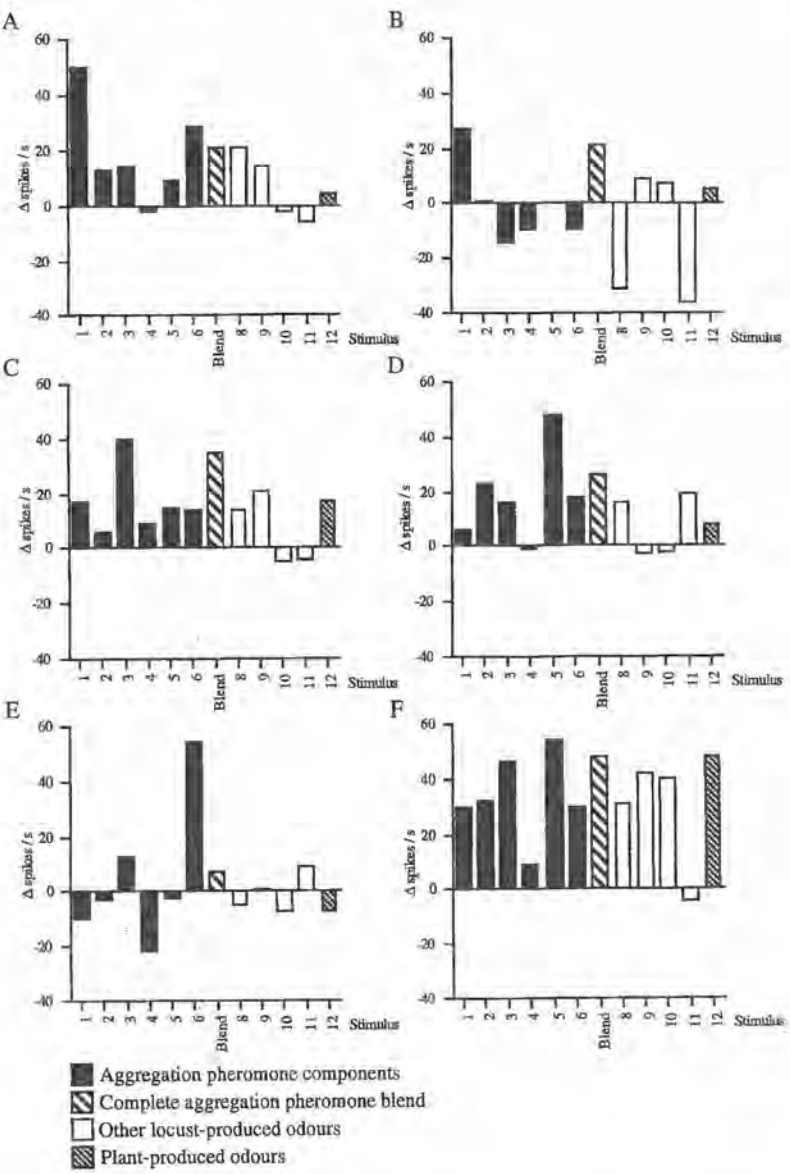

Fig. 3A–F Response spectra of receptor neuron ensembles responding to compounds involved in aggregation behaviour, present in six individual trichoid/basiconic (t/b) sensilla. The response to pheromone components are shown as *black bars*, the response to the pheromone blend is shown as a *hatched bar*, the response to other locust produced odours is shows as *white bars* and the response to the plant produced odour is shown as a *darker, hatched bar*. **A** Compound 1 (phenylacetonitrile) specific. **B** Compound 1 excited and compound 8 (amylacetate) and compound 11 (vanillin) inhibited. **C** Compound 3 (veratrol) specific. **D** Compound 5 (phenol) specific. **E** Compound 6 (guaiacol) specific. **F** A generalist

8 (Fig. 3B). Excitatory responses were also obtained to locust derived odours not present in the pheromone blend (compound 8–11). In 10 sensilla, RNs specifically tuned to a host plant odour (12) were present (Fig. 4). Eleven RN groups showed a very broad response to almost all the compounds tested (Fig. 3F, Table 1). Out of all the coeloconic sensilla tested in this first experiment series, none contained RNs displaying a clear response, even though very high quality contacts were established.

RN ensembles present in eleven t/b sensilla were tested for their dose-response characteristics when challenged with the chemicals earlier tested in the screening experiments. Three type (12S) (Fig. 5C–D), 6 type (1, 3, 5, 6)

Table 1 Response characteristics of the different response types obtained from trichoid/basiconic sensilla on the male *S. gregaria* antenna. *Numbers in left column* indicate compound (see Fig. 1), where 7 indicates the full blend (see legend Fig. 1). *Numbers on top* indicate response type, while *horizontal numbers at bottom* indicate number of response type contacted. Response strength is indicated by 0 (no response), – (clear inhibition), + (≥ 50% of response maximum [RM], < 80% RM), + + (≥ 80% RM, < 90% RM) or + + + (≥ 90% RM). The response was quantified from receptor neurons present in 78 individual sensilla

Compounds	1S	2S	3S	5S	6S	3,4	1,8,11	1,3,5,6	8S	2,10	2,12	3,11	3,6,12	12S	Generalist
1	+ + +	0	+	0	0	0	+ + +	+ + +	+	0	0	0	0	0	+ + +
2	0	+	0	0	0	0	0	0	0	+	+ +	0	0	0	+ + +
3	+	0	+	+	+	+ +	+	+ +	+	0	0	+	+ +	0	+ + +
4	0	0	0	0	0	+ +	0	0	0	0	0	0	0	0	+ + +
5	+	0	+	+	+	+	+	+ +	+	0	0	0	0	0	+ + +
6	+	0	0	0	+	0	+ +	+ +	+	0	0	0	+ +	0	+ + +
7	+	+	+	+	+	+	+ +	+	+	0	+	+	+ +	+	+ + +
8	0	+	0	0	0	0		0	+	0	0	0	0	0	+ + +
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ + +
10	0	0	0	0	0	0	0	0	0	+	0	0	0	0	+ + +
11	0	0	0	0	0	0	–	0	0	0	0	+	0	0	+ + +
12	0	0	0	0	0	0	0	0	0	0	+ +	0	+ +	+ +	+ + +
Total	4	1	5	4	10	1	2	22	3	1	1	2	1	10	11

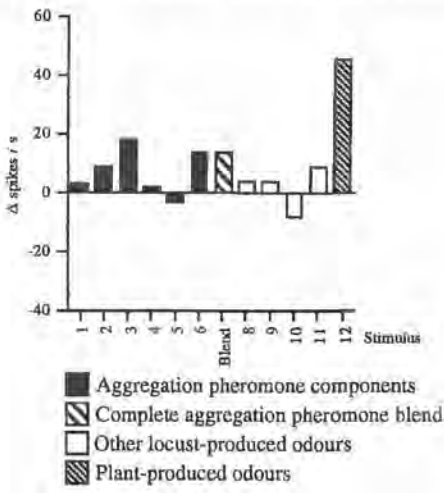

Fig. 4 Response spectrum of a receptor neuron ensemble responding to a host-plant-produced odour. The neurons were present in a t/b sensillum

(Fig. 5A–B), 1 type (5S), 1 type (2, 3, 5) and one type (5, 6) were investigated. The response to the key stimuli was clearly dose dependent. Threshold values were found to range from below 1 µg to 10 µg for the key stimuli.

To investigate the response characteristics of RNs present in s. coeloconica, the stimulus spectrum was extended to include another green leaf volatile, (13) and also some odours of unknown significance to the adult locust, but in earlier studies shown to activate RNs present in *Locusta migratoria* coeloconic sensilla (14–20) (Kafka 1970). The concentration of the compounds used in the screening was also increased one decadic step. Single RNs could be identified in the contacts from s. coeloconica, and different types of RNs were distinguished. One type showed a strong excitation when stimulated with (*E*)-2-hexenal (13) and was also stimulated by different acids (14–20), while showing no response whatsoever to the aggregation pheromone components (Figs. 6B and 7A–B). This type displayed a very long latency. A second type almost exclusively displayed inhibitory responses. Inhibition was evoked by guaiacol (6), veratrol (3) and the pheromone blend (Figs. 6A and 7C–D). A third RN type was inhibited by veratrol (3) and the blend, but was excited by ascorbic acid (Fig. 7E).

Antennal lobe projection patterns of antennal olfactory receptor neurons

More than one thousand small glomeruli make up the bulk of the desert locust antennal lobe. These glomeruli

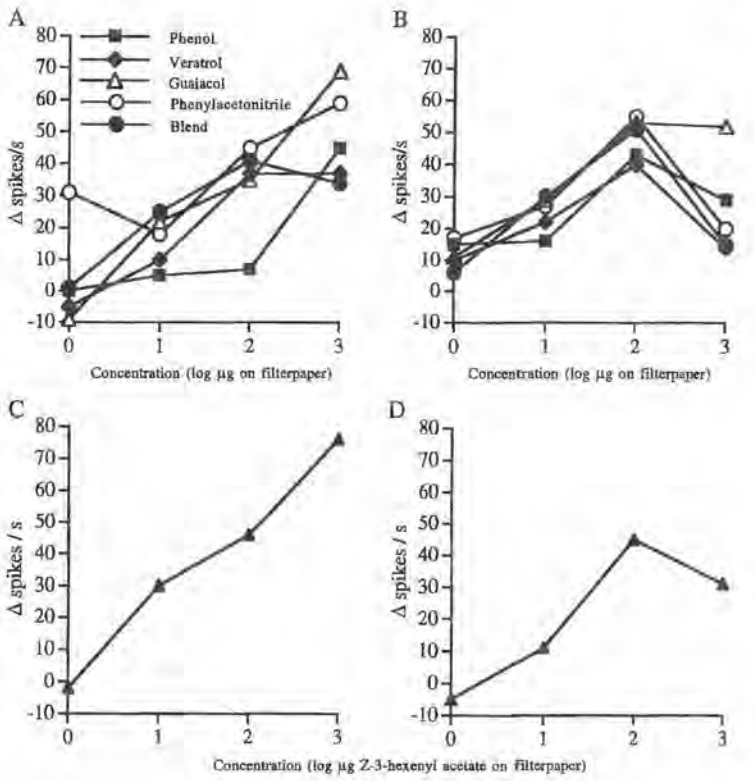

Fig. 5A–D Dose-response curves from receptor neuron ensembles present in two physiological t/b sensillum types on the male *S. gregaria* antenna. **A, B** Two sensilla containing RNs responding to four aggregation pheromone components (1, 3, 5, 6) and to the pheromone blend. **C, D** Two sensilla containing a RN specifically tuned to the host plant produced odour (*Z*)-3-hexenyl acetate (12)

surround a coarse inner core (Fig. 8A). The cell bodies of antennal lobe interneurons lie together in a large, frontal cluster. The RNs stained displayed a very complex structure. After entering the antennal lobe through the antennal nerve, the RNs branched extensively, and targeted several of the small glomeruli present in the antennal lobe. Some RNs projected straight into the lobe and branched (Fig. 8B–C), while others sent their axon along the perimeter of the lobe, to enter it from a more lateral or medial position (Fig. 8D). All RNs stained displayed a multi glomerular innervation pattern. The fine axonal arborizations found in each glomerulus were of a very small diameter, which made it very hard to stain them, and to visualise them after staining.

Discussion

The desert locust, *S. gregaria*, clearly possesses a sensitive and selective olfactory detection system for semiochemicals involved both in species aggregation and host plant location. Antennal olfactory RNs, present in sensillum structures, are tuned to different locust produced odours and/or to plant produced odours.

The pheromone detecting apparatus has been intensely studied in other insect orders, and especially in the Lepidoptera. There it has been shown how single RNs can have a sensitivity high enough to detect low numbers of molecules of substances involved in the intraspecific sex communication. The main part of the male moth olfactory apparatus is often devoted to the detection of the female produced sex pheromone. In female moths, RNs just as specific and sensitive as the male's have been found. Usually, these neurons are tuned to plant produced odours, and serve a function in host plant location (Hansson 1995).

The antennal sensilla of the migratory locust, *Locusta migratoria*, have been studied ultra-structurally. Sensilla specialised for the detection of different types of stimuli were observed: Olfactory, taste, thermo-, hygro- and mechanical stimuli. Several different types of potential olfactory sensilla were reported. These fall into three categories: sensilla trichodea,

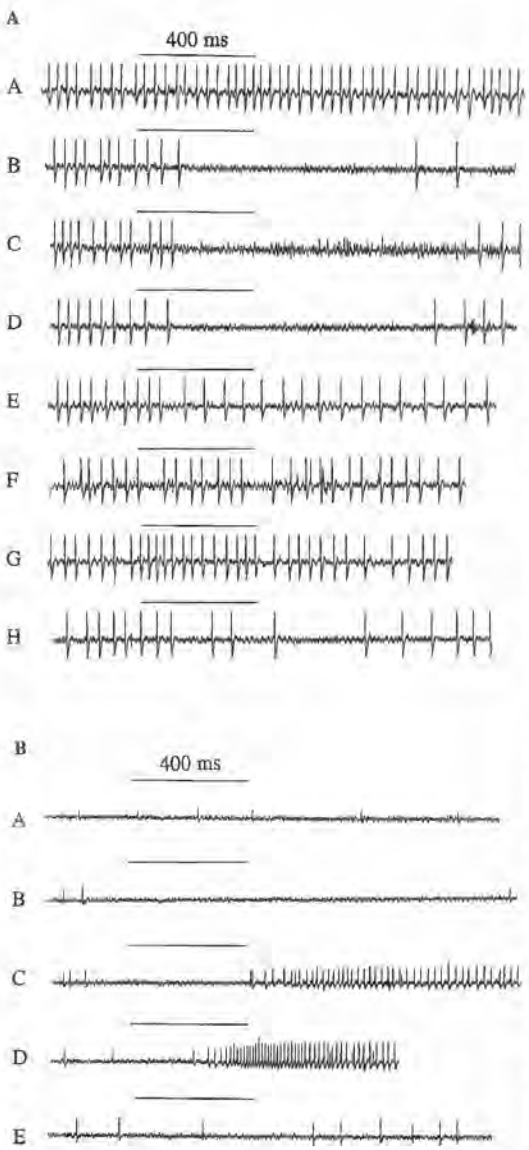

Fig. 6A, B Typical responses from two contacts with sensilla coeloconica. In this sensillum type, single RNs could be unambiguously identified by their action potential amplitude. **A** A RN with high spontaneous activity (*A*) which was inhibited by the complete pheromone mixture (*B*), by a partial mixture (*C*) and by veratrol (*D*). The neuron was also slightly inhibited by butanoic acid (*H*), whereas guaiacol (*E*), (*E*) -2-hexenal (*F*) and benzoic acid (*G*) elicited no or very low responses. **B** A RN with very low spontaneous activity (*A*), showing no response to the pheromone blend (*B*) or to benzoic acid (*E*), while being strongly excited by (*E*) -2-hexanal (*C*) and by butanoic acid (*D*). The response to all other tested compounds (veratrol, guaiacol and the remaining acids) did not differ from the blank

s. basiconica and s. coeloconica (Ameismeier 1987). In an investigation of *S. gregaria* antennal olfactory sensilla, the same types of structures were found (Ochieng, Hallberg and Hansson, unpubl.). The inner structure of the s. basiconica and trichodea adhered very well to what has been reported both in moths and locusts, with the s. basiconica displaying a thin wall, a multitude of wall pores and several branched outer dendritic segments and the s. trichodea with a thicker wall, fewer pores and 2–3 unbranched dendritic segments. The s. coeloconica also displayed a structure very similar to what has been reported from other insect types, with a double sensillum wall with large pores enclosing a low number of unbranched RNs.

In the recordings performed on RNs present in trichoid/basiconic sensilla, single RNs could not be discerned due to the large number of RNs present and to variation in the action potential amplitude elicited in a neuron. The responses recorded from RN ensembles present in the t/b sensilla were, however, clearly diverse, and in several sensilla, all the RNs seemed to be activated by a single stimulus set, as one stimulus could adapt the activity in all the neurons. This fact parallels what has earlier been observed in olfactory sensilla on the sawfly, *Neodiprion sertifer*, antenna, where 10–11 RNs present in the same sensillum were shown to be tuned to the same pheromone component (Hansson et al. 1991).

The responses recorded from olfactory RNs in the desert locust could be arranged into different categories. From the results it was evident that detection of pheromone compounds involved in aggregation behaviour is very important for the male locust. More than half of the contacted sensilla, where a response was recorded, contained RN ensembles responding to one or more of the compounds involved in the elicitation of the aggregation behaviour. Different degrees of specificity were observed among these RN groups. It has to be kept in mind that the compounds that are part of the aggregation pheromone mixture are all quite similar, with a phenol ring as basic structure. The specific detection of different phenolic substances was, however, often very clear e.g., in the sensillum containing a RN ensemble with neurons specifically stimulated by phenylacetonitrile that could be inhibited by another phenol derived compound, vanillin. These results show that locust olfactory receptors display a selectivity similar to what has been found among moths. Pure structure-activity studies still remain to be performed, to establish the absolute specificity of single RNs. RNs present in another physiological type of t/b sensillum were tuned to a plant produced odour, (*Z*)-3-hexenyl acetate. The sensillum type containing RNs specifically tuned to this compound was the second most common type encountered.

Responses could not be elicited from RNs present in s. coeloconica during the first experiment series, neither by the locust-produced odours nor by (*Z*)-3-hexenyl

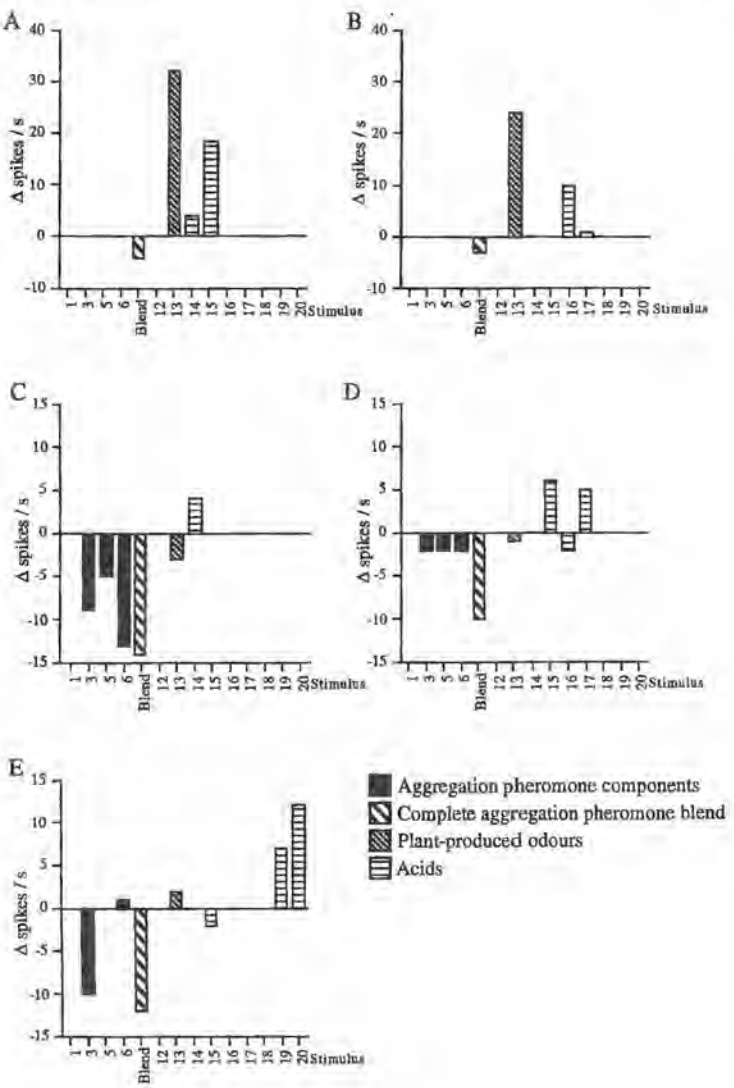

Fig. 7A–E Response spectra of two types of RNs found in sensilla coeloconica. **A–B** Two RNs excited mainly by (*E*)-2-hexenal (13), but to a lesser degree also by some acids (14–16). **C–E** RNs inhibited by several pheromone compounds (3–6) and by the pheromone blend, while being excited by some acids (14–20)

acetate. However, when the concentration was increased for the pheromone compounds and some new stimuli were introduced, responses were indeed obtained. Also from RNs present in s. coeloconica, compounds present in the aggregation pheromone mix elicited responses. These responses were, however, always inhibitory. Also the plant compound (*E*)-2-hexenal elicited an inhibitory response in this RN type. In another RN type, only excitatory responses were observed, and in this case to (*E*)-2-hexenal and to some four and six carbon acids, some of which have recently been found in the odour of 5th instar nymphs (Torto and Njagi, personal communication). The excitatory responses obtained from neurons present in s. coeloconica occurred with a very long latency. This phenomenon might be explained by blocking of the entrance to the sensillum by the recording electrode, thus delaying the entrance of molecules into the sensillum cavity.

The RNs present in the two sensillum categories (t/b and coeloconic) thus showed clearly different response

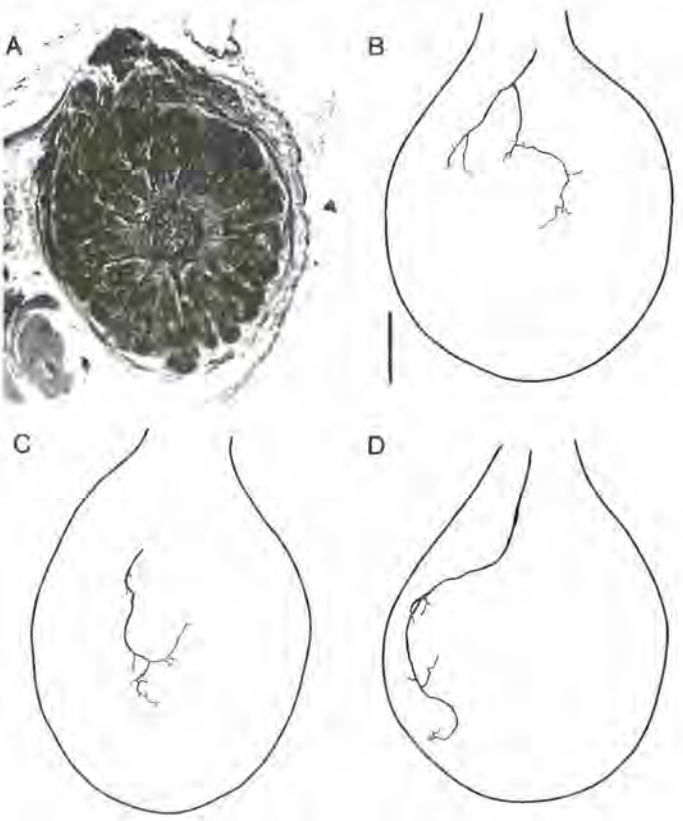

Fig. 8 A An overview staining of the *Schistocerca gregaria* antennal lobe, showing the large number of glomeruli. **B–D** Typical projection patterns observed after staining of single antennal RNs. Note the multiglomerular branching of the axon

characteristics, both to pheromone components and to plant produced compounds. In the t/b type, pheromone components elicited mainly excitatory responses, while in the coeloconic sensillum RNs the same compounds elicited purely inhibitory responses. This arrangement provides the insect with two parallel pathways for information regarding the presence of pheromone and plant odours, and can also allow an interaction between these odour inputs already at the peripheral level.

The projection pattern of single RNs into the antennal lobe, revealed an interesting feature different in the locust as compared to e.g. moths. A single RN branches and targets several different glomerular structures in the lobe, a feature never observed in moths. Recent investigations of the antennal lobe projection patterns of olfactory RNs in the lobster, do however imply multiple glomeruli targeting of single neurons also in this crustacean (Schmidt and Ache 1992). The glomerular array is also very different in the locust as compared to moths. In moths the number of glomeruli is usually around 50 (Rospars 1983, 1988). In the locust, about 1000 glomeruli are present (Ernst et al. 1977). The functional significance of these differences is still unknown. Investigations of axonal terminations in the moth antennal lobe has provided some evidence to the fact that glomeruli have a functional significance, i.e., that RNs of a particular specificity project to a certain glomerulus (Hansson et al. 1992, 1995; Ochieng' et al. 1995). If this would hold true for all insects, locusts should have an almost hundredfold higher diversity of RN types than moths. Now we do, however, observe that in the locust one RN targets several different glomeruli. The antennal lobe functional architecture is thus considerably different. Many glomeruli might represent one physiological input type, or the system might be even more complex so that the same glomerulus receives input from several different RN types. If this is the case, RNs could possibly also have a function similar to that of local interneurons in moths, connecting different glomeruli and serving a "cross-talk" function between these. Future electron

microscopic investigations can provide information regarding out- and input synapses on RNs.

In a parallel study, the morphology and physiology of antennal lobe interneurons were investigated. Responses to the same type of substances were obtained. The morphological data further accentuated antennal lobe architectural differences between locusts and moths (Anton and Hansson, in press).

The locust provides a very interesting research area in the elucidation of insect olfaction. Contrary to the moth, the locust is hemimetabolous, and can be scrutinised through a number of developmental stages. Behavioural data indicate that different pheromones are acting in the different stages (Obeng-Ofori et al. 1993). Future research will be devoted to investigations of the olfactory processing of behaviourally relevant odours in younger stages of the locust, and in individuals of the solitary phase.

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Responses of olfactory receptor neurones to behaviourally important odours in the adult gregarious and solitarious desert locusts, *Schistocerca gregaria*

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Abstract.

Recordings from antennal olfactory receptor neurones in young adult *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) showed that behaviourally important odours are detected by receptor neurones present in morphologically identifiable sensillar types. Both nymphal- and adult-produced aggregation pheromones activate receptor neurones housed in sensilla basiconica. The receptor neurones in this sensillar type in solitary-reared locusts display a higher sensitivity to aggregation pheromones and to some other behaviourally relevant odours than the same type of neurones in gregarious locusts. Receptor neurones present in sensilla coeloconica respond to green leaf odours, organic acids, and nymphal odours but are inhibited by mature adult-produced aggregation pheromones. Receptor neurones housed in sensilla trichodea respond to a possible sex pheromone. No phase differences were found in the response of coeloconic- or trichoid-associated receptor neurones.

Key words. *Schistocerca gregaria*, antenna, single sensillum, receptor neurone, electrophysiology, olfaction, aggregation pheromones.

Introduction

The desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae), is a polymorphic grasshopper that exists in solitary or gregarious phase, depending on population density. In nature, the phases are capable of transforming into one another, and intermediate forms or transients usually are present in a locust swarm (Rainey, 1962). Encounters between isolated individuals take place soon after hatching, when mature adults (about 3 weeks after final moult) meet to mate, as well as when they encounter gregarious groupings during swarming (Roffey & Popov, 1968). During such encounters, the locusts communicate through chemical cues, in addition to visual and tactile stimuli. The chemical cues have been shown to be detected by antennal olfactory receptor neurones (RNs). In antennectomy studies by Mordue (1977), and Heifetz *et al.* (1996), it has been shown that group-reared locusts with their antennae removed lose their gregarization behaviour and develop solitary-phase characteristics even when kept in the company of other locusts, and with visual and tactile stimuli unaffected. Operations on other body parts did not affect behaviour.

Stage-specific and behaviourally active volatiles have been identified from the air surrounding *S. gregaria* and from their faeces (Obeng-Ofori *et al.*, 1993; Torto *et al.*, 1994, 1996; Njagi *et al.* 1996). These studies demonstrated that nymphal stages were attracted only by nymphal-produced odours consisting of short chain aliphatic aldehydes and corresponding acids. Mature, gregarious, adult males were shown to produce phenolic compounds to which only young and mature adults of both sexes responded behaviourally, whereas mature solitary males produced only trace amounts of these compounds, with the key compound phenylacetonitrile (PAN), missing (Njagi *et al.* 1996). Young adults that under natural conditions associate with both nymphs and mature adults, were found not to produce any stage-specific pheromones, but responded behaviourally to both nymphal- and adult-produced odours (Torto *et al.* 1994).

Gravid desert locusts have been shown to be attracted to odours emitted by egg pod froth (Saini *et al.*, 1995). These odours have been identified as acetophenone and veratrole (Rai *et al.*, 1996). (*E,Z*)-2,6-nonadienal, a major constituent of a preferred host plant of *S. gregaria*, *Tribulus terrestris* (Zygophyllaceae), has been identified from solitarious females and may serve as a sex pheromone of this species (Njagi & Torto, in press).

A recent morphological study (Ochieng' *et al.*, in press) revealed three types of sensilla housing olfactory receptors on *S. gregaria* antennae: sensilla basiconica, each housing between 20 and 50 RNs that send branched dendrites into the multiporous sensillar lumen; sensilla trichodea containing from two-to-five neurones, with relatively fewer sensillar wall pores; and sensilla coeloconica containing from one-to-three neurones and having a sensillar wall with slit-like pores. In the same study, the densities

of s. basiconica and s. coeloconica were shown to be higher in solitary-reared locusts than in individuals of the gregarious phase.

Hansson *et al.* (1996) demonstrated that physiological activity could be recorded from single olfactory RNs on the antennae of *S. gregaria*. However, physiological activity was recorded only from gregarious desert locusts, using a limited spectrum of stimuli. With a deeper understanding of the olfactory sensillar types present on the *S. gregaria* antennae (Ochieng' *et al.*, in press), we have undertaken a more detailed investigation of RN specificity and sensitivity. The main aim of the study was to establish whether the differences in gregarization behaviour observed between the stages (nymphs and adults), as well as between the phases (solitarious and gregarious), are discernible at the peripheral olfactory level. Knowledge about the electrophysiological properties of olfactory RNs is important not only to understand the manner in which olfactory information is conveyed to the locust brain but also to help predict behavioural responses to relevant odour stimuli (Boeckh, 1980; Masson, 1984).

Materials and Methods

Insects. Solitarious locust egg pods were obtained from the International Centre of Insect Physiology and Ecology (Nairobi, Kenya), and gregarious nymphs were obtained from Blades Biological (UK). Solitarious nymphs were isolated after hatching into 1-litre perspex jars with perforated lids. The walls of the jars were covered with paper to eliminate visual input. Gregarious locusts were bred under crowded conditions (50-100 per cage) in perspex cages (40 × 60 × 70 cm) in a separate room from solitarious locusts. The rearing rooms were ventilated, temperature controlled (32 ± 2^{0}C day: 26 ± 2^{0}C night), and maintained on a LD 12:12 h photoperiod and 45% relative humidity. The air in the rearing room for the solitary phase was continuously evacuated by a duct system to avoid accumulation of volatiles. Young adult locusts (5-10 d after the final moult) were used in the experiments.

Single sensillum recordings and stimulation. A locust was placed in a plastic tube (15 mm i.d.) with the head exposed and the antennae fixed in place with dental wax. Single-cell recordings were performed using the penetration technique (Hubel 1957). Tungsten wire electrodes (0.1 mm diameter) were sharpened electrolytically to a tip diameter of less than 0.3 µm. The tip of the recording electrode was inserted at the sensillar base by using a Leitz micromanipulator. The recording electrode was connected to a high impedance AC/DC amplifier (Syntech, The Netherlands). The indifferent electrode (Ag/AgCl wire) was inserted into the locust abdomen. The signal was observed

on a Phillips oscilloscope and recorded on a video cassette recorder, SLV-750HF (Sony T1-11, Park Ridge, New Jersey). The amplifier was also connected to a loudspeaker to provide an indication of contact quality. The recorded responses were analysed using a Syntech Autospike version 2.0, Syntech 1995 software.

Test stimuli. The test compounds were obtained from Sigma Co. and were at least 98% pure. They included the following compounds that have been identified from the volatile collections of mature male *S. gregaria* and shown to affect aggregation behaviour in this species: phenylacetonitrile (PAN), benzaldehyde, guaiacol, and phenol; and a blend of these formulated to approximate the composition of volatiles from live locusts in the ratio 100:15:4:4 (Torto *et al.*, 1994; Njagi *et al.* 1996). A 4:9:20:20:14:50:100:16 blend of synthetic hexanal, octanal, nonanal, decanal, hexanoic acid, octanoic acid, nonanoic acid, and decanoic acid, respectively, was used as the nymphal pheromone blend. The ratio represents the relative composition of a volatile collection from fifth instars (Torto *et al.*, 1996). In addition, we tested olfactory responses to the green leaf volatile (*E*)-2-hexenal and to butyric acid, and acetophenone, the last having been identified as an attractant of gravid *S. gregaria* to oviposition sites (Rai *et al.*, 1997). A potential sex pheromone compound, (*E,Z*)-2,6-nonadienal, produced by the solitary female and by a preferred host plant, *T. terrestris*, was also included among the stimuli. All the compounds were diluted in decadic steps in liquid paraffin from 100 µg/µl to 0.1 pg/µl.

Stimulus application. A continuous flow of charcoal-filtered, humidified air was passed over the antennae through a glass tube (8 mm i.d.) at a speed of 0.5 m/s. The outlet of the tube was situated *c.* 15 mm from the antenna. A Pasteur pipette containing a piece of filter paper (10 × 15 mm) loaded with 10-µl aliquotes served as a stimulus source. Two milliliters of the pipette atmosphere was injected during 1 s into the continuous airstream through a small hole (2 mm i.d.) in the wall of the tube, *c.* 150 mm from the antenna, by using a stimulus control device (Syntech). Once successful contact was established with a neurone, stimuli were presented in random order, beginning with the lowest concentration. The interstimulus interval was 30-40 s at lower dosages and up to 2 min at higher concentrations or until the spontaneous activity had returned to normal. A stimulus pipette containing 10 µl of liquid paraffin served as a control.

Response quantification. The response intensity was determined as the difference between the number of action potentials during 1 s before the stimulus onset and the number 1 s after stimulation, minus the response to the control stimulus. In s. basiconica, the response of the entire RN assembly was counted, as single RN responses could not be distinguished (Hansson *et al.*, 1996). The data from RNs present in s. basiconica

Fig. 1. A typical response of a receptor neurone assembly present in a sensillum basiconicum to a stimulation with pheromone compounds. Note the large number of neurones indicated by many different action potential amplitudes. The stimulus in this case was 100µg of guaiacol. Stimulus duration (1 s) is indicated by the horizontal bar.

were square-root transformed and subsequently subjected to a two-way analysis of variance by stimulus and phase followed by a Fisher's PLSD test using SYSTAT (1992).

In one experiment, we screened for olfactory RN responses in 10 immature solitary locusts (7 females and 3 males), using doses of 10-1,000 µg/µl of aggregation pheromone compounds: PAN, benzaldehyde, guaiacol, phenol, and their blend. In addition, we tested (*E*)-2-hexenal and butyric acid. In total, we recorded from 94 RNs: 51 were present in s. coeloconica and 43 in s. basiconica.

In a second experiment, we established dose-response relationships in RNs from 27 immature locusts (6 solitary females, 4 solitary males, 6 gregarious females, and 11 gregarious males). Stimulus doses of 0.1 ng/µl - 1,000 µg/µl were tested on RNs present in s. basiconica and s. trichodea, whereas 1 pg/µl - 1,000 µg/µl were used to stimulate RNs in s. coeloconica. In this experimental series, all compounds listed earlier were tested.

Results

Antennal olfactory RNs present in morphologically identifiable sensilla, responded differentially to the various stimuli tested. Responses of both an excitatory and inhibitory nature were recorded from s. coeloconica and s. trichodea (Figs 3-6), whereas only excitatory responses were recorded from s. basiconica RN assemblies (Figs 1 and 2). Receptor neurones in s. basiconica were excited by aggregation pheromone compounds (both nymphal- and adult-produced); RNs in s. coeloconica were excited by (*E*)-2-hexenal, butyric acid, and the blend of nymphal-produced pheromone compounds, whereas all adult-produced pheromone compounds, except benzaldehyde, inhibited spontaneous activity at high dosages. Receptor neurones present in s. trichodea were

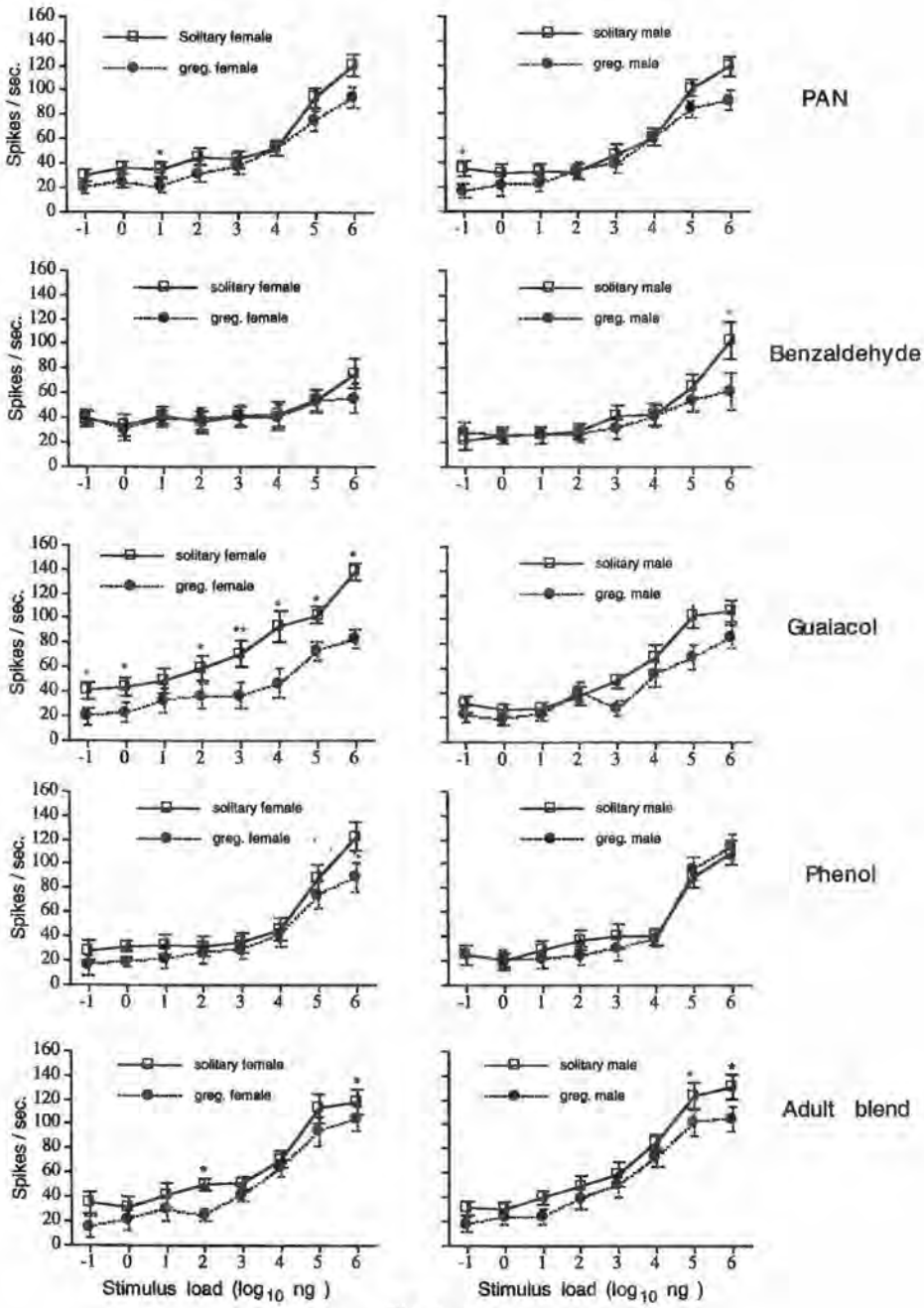
Solitary female
greg. female
solitary male
greg. male
Spikes / sec.
PAN
Benzaldehyde
Guaiacol
Phenol
Adult blend
Stimulus load (log10 ng)

Fig.2a

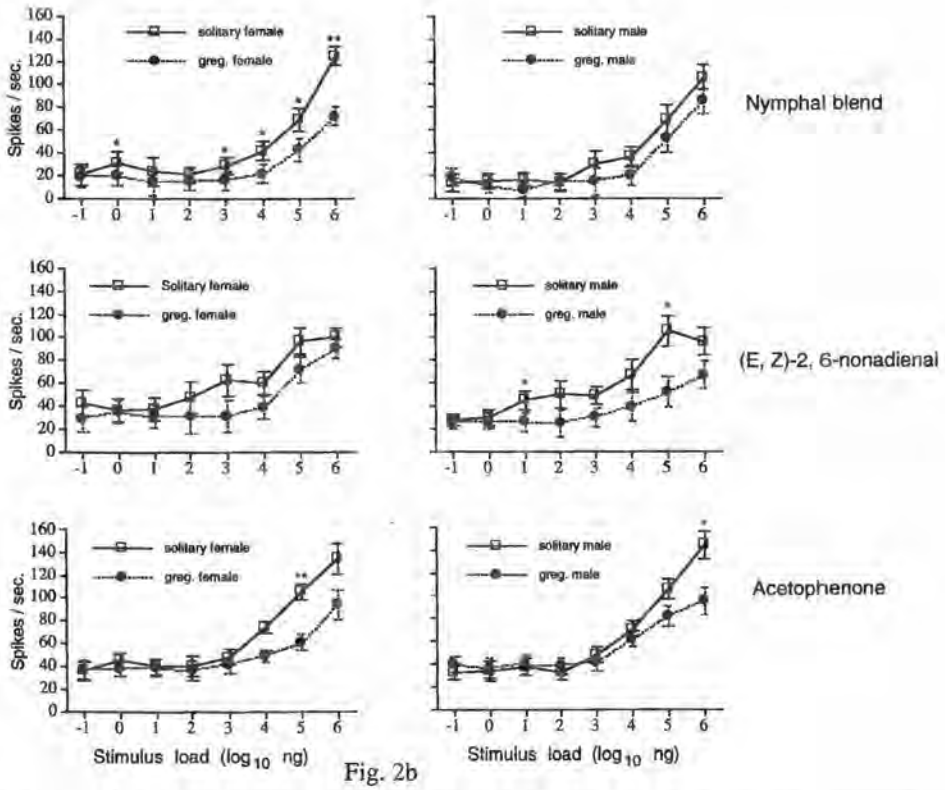

Fig. 2b

Figures 2a-b. Dose-response curves from stimulation of receptor neurone assemblies present in sensilla basiconica. Stimuli are indicated beside each graph. The x-axis indicates the stimulus load in the stimulus source. The y-axis indicates the net number of action potentials (spikes) ([spike # after stimulation - spike # before stimulation] - spike # elicited by control stimulation). Spike frequencies with asterisks at different stimulus loads were significantly different (* $P < 0.05$; ** $P < 0.01$), between phases within each sex (mean ± S.E.; n= 10).

excited by a possible sex pheromone compound (*E,Z*)-2,6-nonadienal, whereas butyric acid caused an inhibition at high dosages (Fig. 5).

Characteristics of RNs present in sensilla basiconica. Contacts made with RNs present in s. basiconica displayed spontaneous activity with action potentials (spikes) of varying amplitudes (Fig. 1). The large number of RNs active prevented an unambiguous identification of single neurones. Therefore the response of the entire neural assembly of single s. basiconica was calculated.

In the first series of experiments, 43 basiconic RN assemblies were contacted. All responded to the adult-produced aggregation pheromone and to its individual components. In the second series of experiments, dose-response relationships were

constructed from 10 replicates. Synthetic pheromone compounds produced by mature male locusts generated a phasic response that was dose dependent (Fig. 2). The response threshold values for PAN, guaiacol, and the adult pheromone blend (Fig. 2a) were relatively low (10-1,000 ng dosage), whereas response thresholds for benzaldehyde and phenol were shifted 2-to-3 decadic concentration steps higher. The nymphal pheromone blend elicited responses in both sexes with a response threshold of *c.* 10 µg (Fig. 2b).

Among the non-aggregation pheromone compounds tested, the potential sex pheromone component (*E,Z*)-2,6-nonadienal and the egg-laying attractant acetophenone elicited action potentials with a response threshold of *c.* 1 µg (Fig. 2b). (*E*)-2-Hexenal and butyric acid evoked responses in RNs in s. basiconica that were low and not dose-dependent.

Response characteristics of RNs present in sensilla coeloconica. The spontaneous activity of RNs present in s. coeloconica could be assigned to two types depending on the the manner the recording electrode was positioned. When the recording electrode was inserted directly into the cuticular pit housing s. coeloconica, contacts were made with RNs that displayed continuous firing but did not respond to any odour stimulations. A second type of RNs was contacted when the recording electrode was inserted at an angle to the cuticular pit. The RNs contacted this way were characterised by a low spontaneous activity but were highly sensitive to some of the tested stimuli (Figs 3 and 4). In most contacts, a single RN was observed, while in a few experiments, a second RN could be distinguished based on spike amplitudes.

In the first series of experiments, 51 RNs present in s. coeloconica were screened for their specificity. Out of these, 19 were excited by (*E*)-2-hexenal and butyric acid and inhibited by the adult aggregation pheromone components. An additional 12 RNs displayed the inhibitory response only, whereas 20 RNs showed spontaneous activity but no response to any of the stimuli tested. All of the non-responding RNs were contacted when the electrode was inserted directly inside the pit.

In the second series of experiments, the excitatory response characteristics of the coeloconic RN type were confirmed by (*E*)-2-hexenal and butyric acid, and also by benzaldehyde and the nymphal pheromone blend (Figs 3 and 4). The RNs responded in a clearly dose-dependent manner (Fig. 4), and were inhibited by higher concentrations of the adult aggregation pheromone compounds (Figs 3 and 4). At stimulus amounts 1 pg-10 µg, responses of these RNs were phasic but changed to phasic-tonic at 100-1,000 µg dosages (Fig. 3). The phasic-tonic responses caused by (*E*)-2-hexenal and butyric acid could be inhibited by subsequent stimulation with aggregation pheromone compounds (Fig. 3). When a second RN was observed, it was always of a smaller amplitude than the one already described, and did not respond to any of the stimuli tested.

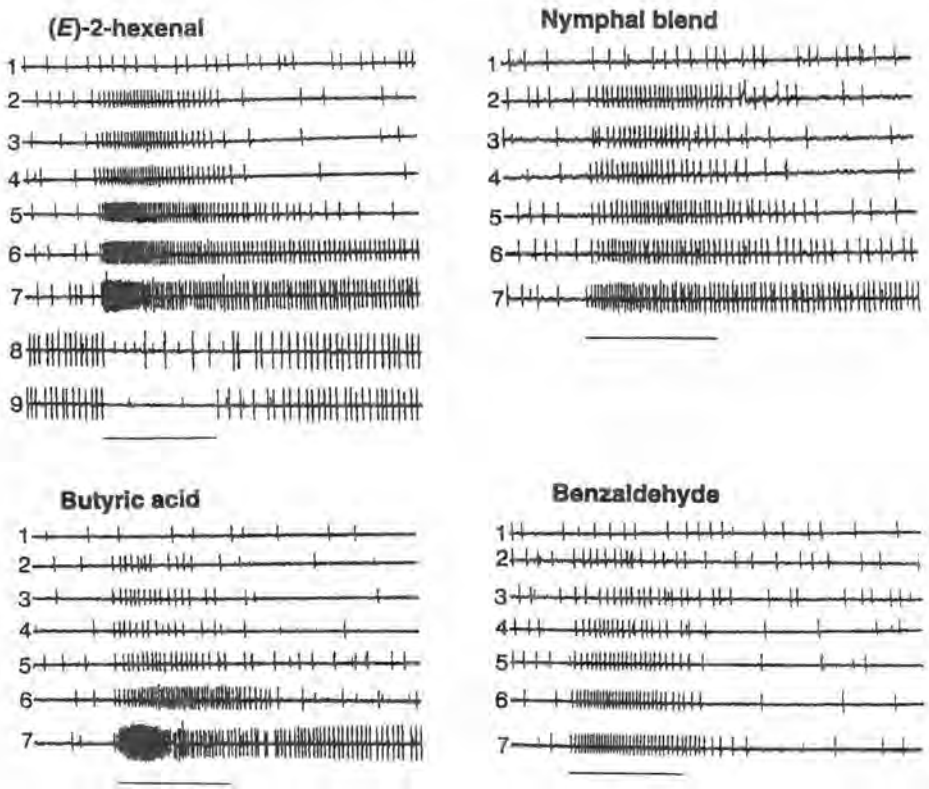

Fig. 3. Response pattern of a receptor neurone present in a sensillum coeloconicum to serial concentrations of (*E*)-2-hexenal, nymphal pheromone blend, butyric acid, and benzaldehyde. Stimulus concentrations in all cases: 1= control, 2 = 1pg, 3 = 1ng, 4 = 1µg, 5 = 10µg, 6 = 100µg, 7 = 1000µg, 8 = 1000µg adult pheromone blend following stimulation with 1000µg (*E*)-2-hexenal, and 9 = 1000µg phenol following stimulation with 1000µg (*E*)-2-hexenal. Horizontal bars below the recordings indicate stimulus duration (1 s).

Response characteristics of RNs present in sensilla trichodea. Receptor neurones present in s. trichodea were only investigated in the second series of experiments. This sensillar type appeared morphologically similar to s. basiconica under the light microscope (× 320 magnification), but the spontaneous activity of the two RNs observed was much lower and clearly different from the background firing of RNs in s. basiconica that was always multi-neuroneal. Furthermore, the physiological specificity of RNs in s. trichodea was different from that of RNs present in both s. basiconica and in s. coeloconica. Nearly all the RNs present in s. trichodea contacted were only excited by (*E,Z*)-2, 6-nonadienal (Figs 5 and 6). Out of 24 s. trichodea RNs contacted, all

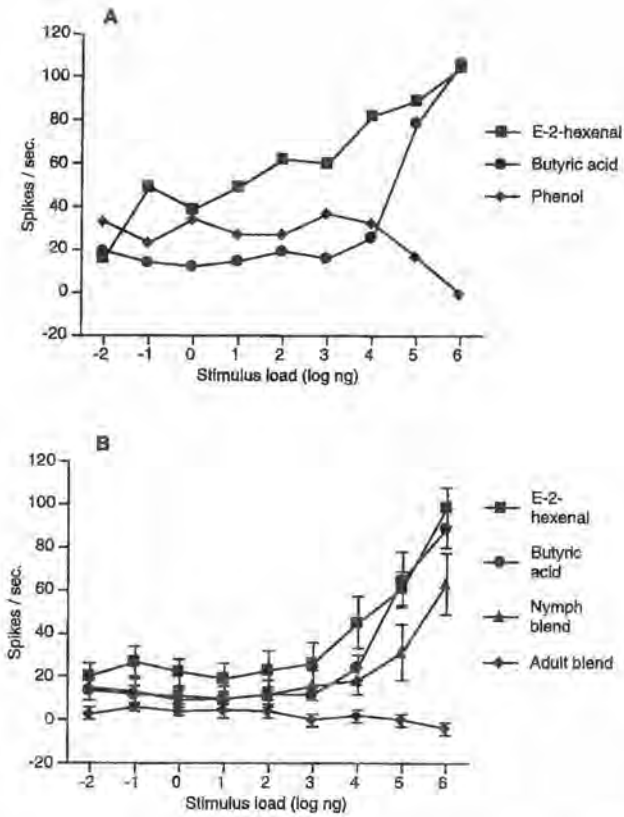

Fig. 4a-b. (a) Dose-response curves for a receptor neurone present in a sensillum coeloconicum. This neurone is very sensitive to (*E*)-2-hexenal at low dosages, to butyric acid at high dosages, but is inhibited by phenol at high dosages. (b) Mean dose-response curves of RNs in s. coeloconica to (*E*)-2-hexenal, butyric acid, nymphal pheromone blend and adult pheromone blend (mean ± S.E.; n= 8).

responded strongly to (*E,Z*)-2,6-nonadienal, especially at the 100-1000 µg/µl dosages. In most of these contacts, an inhibition was observed when the RN was stimulated with butyric acid.

Differences between the phases. Generally, the frequency (# of spikes/s) of action potentials elicited in the RNs present in s. basiconica, more or less irrespective of stimulus, was higher in the solitary than in the gregarious locusts (Fig. 2). Among the aggregation pheromone components, a highly significant difference ($F = 17.144$, $P < 0.001$) in spike frequency was observed between the phases when RNs in female locusts were stimulated with guaiacol. Stimulation with adult pheromone blend yielded a

significant difference in spike frequency in both sexes ($F = 5.914$, $P = 0.028$ in females, and $F = 5.365$, $P = 0.034$ in males). Nymphal pheromone blend stimulation produced spike frequencies that differed significantly between the phases only in females ($F = 10.667$, $P = 0.005$).

Among other compounds tested, (*E,Z*)-2,6-nonadienal elicited significantly higher spike frequencies ($F = 7.686$, $P = 0.014$) in RNs present in the males. For the egg-laying compound acetophenone, RN spike frequencies were significantly different in both sexes ($F = 2.678$, $P = 0.013$ in females and $F = 2.766$, $P = 0.011$ in males).

In the sample of RNs present in s. coeloconica and s. trichodea, no differences in spike frequencies were observed between the locust phases.

Discussion

The immature desert locust, *S. gregaria*, possesses an antennal arsenal of olfactory RNs well adapted for the detection of behaviourally relevant odours. Receptor neurones present in morphologically identifiable sensilla display different physiological specificity. Stimulations with aggregation pheromone compounds generated action potentials from RNs present in s. basiconica, whereas host-plant-derived odours excited s. coeloconica-associated RNs. A potential sex pheromone component was the exclusive stimulant for a RN present in s. trichodea. This association coincides with studies in Lepidoptera (Hansson, 1995), where different sensillar types house RNs of different specificity.

Generally, a higher sensitivity to several of the pheromonal compounds tested was found in olfactory RNs of locusts belonging to the solitary phase. This result is in agreement with data from both electroantennographic and behavioural studies (Njagi *et al.*, 1996). As solitary locusts lead a much more dispersed life than individuals belonging to the gregarious phase, the need for a higher olfactory sensitivity to detect conspecific-produced odours is easy to envisage. The higher RN sensitivity in solitarious locusts will act in conjunction with the fact that this phase also possess a higher number of sensilla, i.e., RNs. A higher sensitivity in combination with a higher number of detecting units (Greenwood and Chapman, 1984; Ochieng' *et al.* in press) will increase the chance of odour detection considerably.

When dose-response characteristics of RNs in s. basiconica were compared between the phases, it was clear that phase differences occurred for some compounds but not for others, and sometimes only in one sex. Female solitary locusts generated higher spike frequencies to stimulations with aggregation pheromone compounds than their gregarious counterparts. There were, however, no significant differences in aggregation pheromone detection between the sexes, even though these odours are produced only by the males (Torto *et al.*, 1994). Acetophenone evoked a significantly higher spike

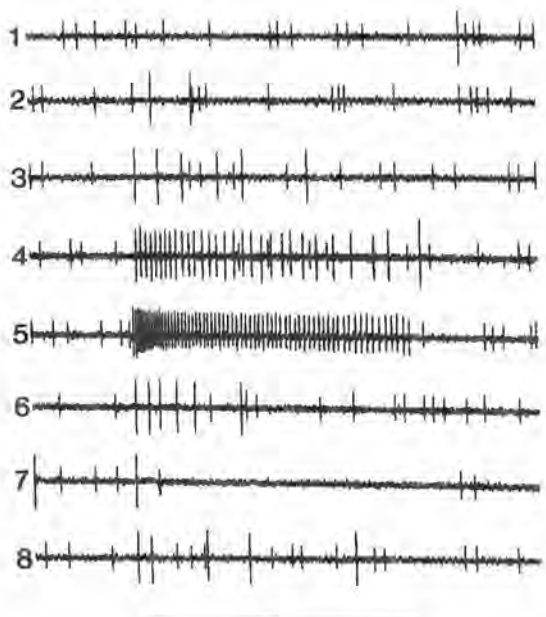

Fig. 5. A typical response pattern of a receptor neurone present in a sensillum trichodeum to stimulations with increasing dosages of (*E,Z*)-2,6-nonadienal: 1= control, 2 = 1µg, 3 = 10µg, 4 = 100µg, 5 = 1000µg, 6 = 1000µg (E)-2-hexenal, 7 = 1000µg butyric acid, and 8 = 1000µg adult blend. Horizontal bars below the recordings indicate stimulus duration (1 s).

frequency in solitary individuals of both sexes. This compound has been identified as an oviposition attractant of gravid *S. gregaria* (Rai *et al.*, 1997). The reason for the male locusts to have a high sensitivity for this compound is obscure. The solitarious male exhibited a higher sensitivity to (*E,Z*)-2,6-nonadienal than any other locusts of either phase or sex. This fact coincides well with the postulated action of this compound as a sex pheromone in the solitary phase (Njagi and Torto, in press). In species where the male is attracted to a female-emitted sex pheromone, a sexual dimorphism in antennal sensitivity often is observed (for review see, Hansson 1995).

In s. coeloconica, we found RNs that were sensitive to (*E*)-2-hexenal and to butyric acid. Pheromone compounds did, at higher dosages, inhibit the spontaneous activities of these neurones, a phenomenon similar to responses in some desert locust antennal lobe neurones (Anton & Hansson, 1996). The behavioural significance of green leaf volatiles and butyric acid to *S. gregaria* remains to be investigated. Haskell *et al.* (1962) showed

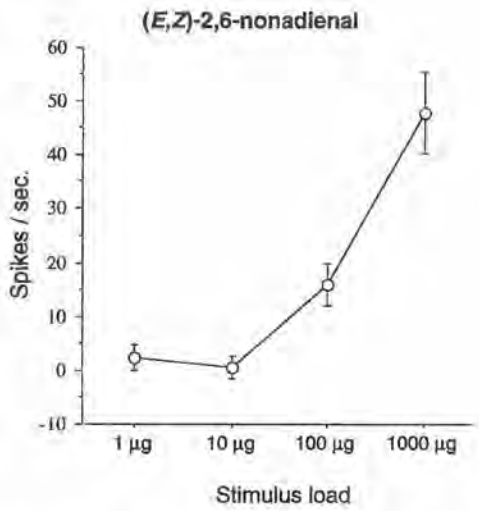

Fig. 6. Dose-response curve (mean ± S.E.) of receptor neurones present in s. trichodea to stimulations with increasing dosages of (*E,Z*)-2,6-nonadienal (mean ± S.E.; n= 9).

that *S. gregaria* nymphs were attracted upwind in response to grass odours in a wind tunnel. Green leaf volatiles have been shown to elicit electrophysiological responses in antennal olfactory RNs of many insect species. In the bark beetles *Dendroctonus frontalis* Zimm., *Ips avulsus* Eichhoff., and *Ips grandicollis* Eichhoff. (Coleoptera: Scolytidae), green leaf volatiles were found to interrupt responses to aggregation pheromones (Dickens *et al.*, 1992). (*E*)-2-Hexenal was shown to attract the carrot fly, *Psila rosae* Fabr. (Diptera: Psilidae) (Guerin *et al.*, 1983). In the female white cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae), green leaf volatiles and butyric acid present on eggs activated a wide range of antennal RNs (Den Otter *et al.*, 1980). It will be of interest to test the behavioural significance of green leaf volatiles and butyric acid when presented alone or together with the aggregation pheromones of *S. gregaria*, as the RNs tuned to these compounds indicate that they are important for survival and/or reproduction.

Receptor neurones present in s. trichodea were affected differently by the tested compounds. These RNs were excited by (*E,Z*)-2,6-nonadienal, a compound produced by mature solitary females. This compound also occurs in a preferred host plant of *S. gregaria* (Njagi and Torto, in press). Receptor neurones present in s. trichodea are in many insect species specialized to detect pheromone components at low concentrations

(Baker, 1989; Masson and Mustaparta, 1990; Hansson, 1995). It is interesting that (*E,Z*)-2,6-nonadienal also was detected by RNs present in s. basiconica of the male solitary locusts to a significantly higher degree than in the gregarious males. This compound has only been identified from mature solitary females. It is possible that solitary locusts use this compound as a sex pheromone. It interacted with olfactory RNs in a similar manner as aggregation pheromone components by exciting RNs present in s. basiconica and inhibiting RNs in s. coeloconica, indicating that it might have an attractive property just like the aggregation pheromones. A sex recognition pheromone has been shown to exist in *S. gregaria*, allowing males to recognise females (Amerasinghe, 1978), and Inayatullah *et al.* (1994) demonstrated that female *S. gregaria* release pheromone(s) that attract conspecific males upwinds.

Our findings support the hypothesis that in *S. gregaria*, as in other insect species, information about host odour quality, aggregation pheromones, and sex pheromones are conveyed to the brain via both labeled-line and accross-fibre mechanisms (Dethier, 1976; Masson & Mustaparta, 1990). Sensilla basiconica were excited mostly by aggregation pheromones; s. coeloconica by plant odours but inhibited by aggregation pheromones whereas s. trichodea were excited exclusively by the sex pheromone. In s. basiconica, the interaction of single pheromone components with single RNs cannot be elucidated by the present data. Further experiments, using selective adaptation, will be performed to establish if different RNs are tuned to detect different compounds. Data from this and an earlier study (Hansson *et al.*, 1996) do, however, indicate that all RNs present in this sensillum type are affected by all pheromonal stimuli.

The locust olfactory system offers a challenging research area where we can study both development and phase differentiation. Because odour input in early locust life stages can alter the morphology and function of the olfactory system in later stages, the mechanisms behind this shift and their action in the central nervous system are future research areas that will be given high priority.

Acknowledgements

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Cross- and self-adaptation of electroantennogram responses to behaviourally relevant odours in the desert locust, *Schistocerca gregaria*

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Abstract: Electroantennogram (EAG) responses were recorded from young adult *Schistocerca gregaria* antennae using behaviourally active synthetic compounds as stimuli. Pheromone compounds elicited significantly higher EAG amplitudes than a green leaf odour. Responses to a component of the nymphal aggregation pheromone were not dose dependent. Receptor adaptation experiments support the results of a previous single sensillum study that demonstrated the presence of at least three functionally different receptor neurones on *S. gregaria* antennae. Cross-adaptations with an aggregation pheromone compound enhanced responses to a green leaf odour and a component of nymphal aggregation pheromone, whereas these compounds reduced responses to the aggregation pheromone compound.

Key Word Index: Locust, aggregation pheromone, electroantennogram, adaptation

INTRODUCTION

Adaptation, defined as the reversible decrease in response after a previous stimulus, is a fundamental property of sensory systems including pheromone receptors on the antennae of insects. This property has been used to determine receptor systems' specificities by selectively adapting different classes of receptor neurones (RNs), and in mating disruption studies of many insect species (Lucas, *et al.*, 1994; Schmitz, *et al.*, 1997; Rumbo and Vickers, 1997). Adaptation is usually specific to olfactory receptor neurons that are stimulated by the adapting compound even when there are multiple receptor cells with different specificities inside an individual sensory structure (Baker and Roelofs, 1976; Payne and Dickens, 1976; Rumbo, 1988).

During adaptation, the adapting stimulus subsequently reduces the effectiveness and perceived intensity of the same (self-adaptation) or another (cross-adaptation) test stimulus. This reduction in perceived intensity is typically interpreted as evidence that the adapting and test stimuli are processed by the same receptor sites. When cross-adaptation does not occur between perceptually similar odours, it is assumed that the compounds are processed by separate receptor channels.

Adaptation of olfactory receptors has been used to explain specific parts of the orientation behaviour of male moths during flight in a pheromone plume to locate a female. The upwind progress of males in some species is arrested during flight in uniform clouds of pheromone in a wind tunnel (Baker *et al.*, 1989), and adaptation or habituation of sensory pathways have been attributed to be the cause. The adaptation effect has been proposed to operate at the peripheral nervous system, while habituation occurs at the central nervous system level (Bartell, 1982). However, in a recent investigation of olfactory adaptation processes in the male moth pheromone detecting system, the hypothesis of adaptation as a mechanism of male arrestment in high concentration pheromone environments did not gain support (Valeur, Hansson & Löfstedt, unpublished).

The desert locust, *Schistocerca gregaria* Forskål (Orthoptera: Acrididae), has been shown to produce and respond to stage-specific semiochemicals affecting the aggregation behaviour. Nymphal stages (second to fifth instars) were found to produce short chain aliphatic aldehydes and corresponding acids as aggregation pheromones (Torto *et al.*, 1996). Additionally, aromatic compounds, guaiacol and phenol, which were found to act synergistically with the acids and aldehydes were detected in faecal volatiles. Mature gregarious adult males were shown to produce a blend of aromatic compounds consisting of phenylacetonitrile (PAN), benzaldehyde, guaiacol, and phenol (Torto *et al.*, 1994). Mature solitary females were recently shown to produce a compound that is also found in a preferred host plant of *S. gregaria*, (*E,Z*)-2,6-nonadienal, (Njagi and Torto, in press). This compound has been proposed as a potential sex pheromone component in the solitarious phase. Young adults were found to produce no pheromones. However, they responded both behaviourally and physiologically to both nymphal- and adult-produced aggregation pheromones (Obeng-Ofori *et al.* 1993; Torto *et al.*, 1994).

In a recent single sensillum study, we demonstrated that aggregation pheromones mainly excited RNs present in sensilla basiconica but inhibited the activities of RNs present both in s. trichodea and s. coeloconica. A common leaf odour from many plants, (*E*)-2-hexenal (Buttery and Ling, 1984), was found to excite mainly RNs present in sensilla coeloconica. A possible sex pheromone compound, (*E,Z*)-2,6-nonadienal was found to excite RNs in both s. trichodea and s. basiconica, whereas it inhibited activities of RNs in s. coeloconica. In each s. basiconicum, about 30 RNs are present, while the other sensilum types on the locust antenna contain between one and four RNs (Ochieng'

et al., in press). In the physiological investigations mentioned above, it was often not possible to tell if a specific compound was detected by a specific neuron, or if several compounds were detected by the same neurones. In order to discern the antennal RN specifity, we here investigated the adaptation properties of compounds with different behavioural importance to *S. gregaria* on olfactory receptors present on the antenna.

MATERIALS AND METHODS

Schistocerca gregaria used in the investigation were obtained from Blades Biologicals (UK) as fifth instars and maintained in crowded conditions as described by Hansson *et al.* (1996). Electroantennograms (EAGs) were recorded from antennae of immature adults, 3 to 5 days after the final moult. The antenna was cut from the head capsule and the base placed in a drop of locust saline (Hoyle, 1952). The scape and pedicel of the antenna were excised, and the basal end of the flagellum was inserted into the recording electrode (Ag-AgCl in a glass micropipette containing locust saline). The recording electrode was connected to an AC/DC UN-05 high impedance amplifier (Syntech, The Netherlands). The antennal terminal segment was also excised and the exposed end inserted into the indifferent electrode.

Representative compounds of different behavioural significance to the locusts were used as stimuli: phenylacetonitrile (PAN), a major aggregation pheromone compound produced by mature *S. gregaria* males; a possible *S. gregaria* sex pheromone (*E,Z*)-2,6-nonadienal, a component of *S. gregaria* nymphal aggregation pheromones (nonanoic acid), and a green leaf odour (*E*)-2-hexenal. Each compound was prepared as a serial dilution in dichloromethane (HPLC grade) in increasing concentrations from 1ng to 100 µg/µl. From each stimulus dosage, a 10 µl aliquote was applied on a filter paper (10 × 15 mm) inserted into the barrel of a Pasteur pipette. For adaptation experiments, all stimuli were used at 100 µg/µl dosage. Ten microliters of the solvent was used as control in the dose-response experiments.

A continuous flow of charcoal-filtered, humidified air was passed over the antenna through a glass tube (8 mm i.d.) at a speed of 0.5 m/s. The outlet of the tube was situated about 15 mm from the antenna. Two milliliters of the Pasteur pipette atmosphere was injected during 1 s into the continuous airstream through a small hole (2 mm i.d.) into the wall of the tube, about 150 mm from the antenna, using a stimulus control device (Syntech).

To elucidate receptor neuron specificity, the differential adaptation technique was used (Payne and Dickens, 1976). Each preparation was subjected to adaptation-stimulation with pairs of the compounds. For each pair, the preparation was first exposed to the test stimulus, and after 1 min the adapting compound was injected into the

airstream three consecutive times through a second hole in the wall of the tube about 140 mm from the antenna, using a second stimulus control device. Within milliseconds of the conditioning stimulation, the preparation was re-stimulated with the test compound (Fig. 1). Both self adaptation (adapting and test compound the same) and cross adaptation (compounds different) rates were established. Electroantennogram responses were viewed on a Tectronix oscilloscope and recorded on a computer using a Syntech EAG version 5 program (Syntech, The Netherlands). Each antenna was used for one series of stimuli only.

The EAG amplitude was taken as a measure of the relative number of responding receptor neurons. The presence or absence of response, or the degree of response to the test and adapting compound was a measure of the relative interaction of the compounds with the same receptors. For example, if two compounds stimulated the same RNs, effects of the adapting compound would eliminate the response to the test compound. If the two compounds were detected by totally separate RN populations, a minimal effect of the adapting stimulus should be seen on the response to the test stimulus. The dose-response results were analysed using repeated measures ANOVA, and the adaptation results by a paired t-test (Statview) for differences between responses to different stimuli.

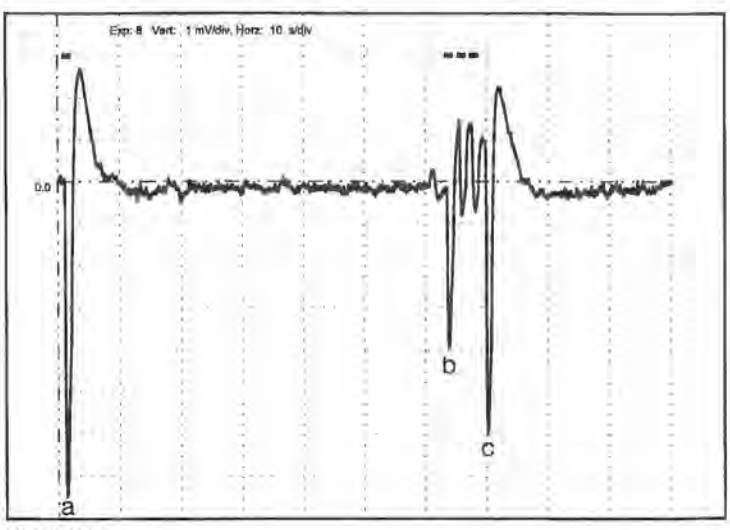

Fig.1. A cross-adaptation response. (a) response to stimulation with 100µg (*E,Z*)-2,6-nonadienal. After 1 min, the antenna is adapted by stimulating with 100µg *E*-2-hexenal three times consecutively (b), and within milliseconds restimulated with (*E,Z*)-2,6-nonadienal (c). The relative difference between nonadienal stimulations (a and c) is the adaptation effect of *E*-2-hexenal on nonadienal responsive receptors.

RESULTS

Mean EAG responses of *S. gregaria* to phenylacetonitrile (PAN), (*E,Z*)-2,6-nonadienal and *E*-2-hexenal increased in a dose dependent manner (Fig. 2). Responses elicited by different doses of (PAN) and (*E,Z*)-2,6-nonadienal did not different with a threshold value between 10 and 100 ng. Response curves to both compounds were, however, significantly different from the response curves to *E*-2-hexenal and nonanoic acid ($P<0.0001$, Fisher's PLSD test at 5% significance level). Responses to *E*-2-hexenal had a 100 times higher threshold value. Nonanoic acid elicited responses only at the highest concentrations, but then to a very low extent.

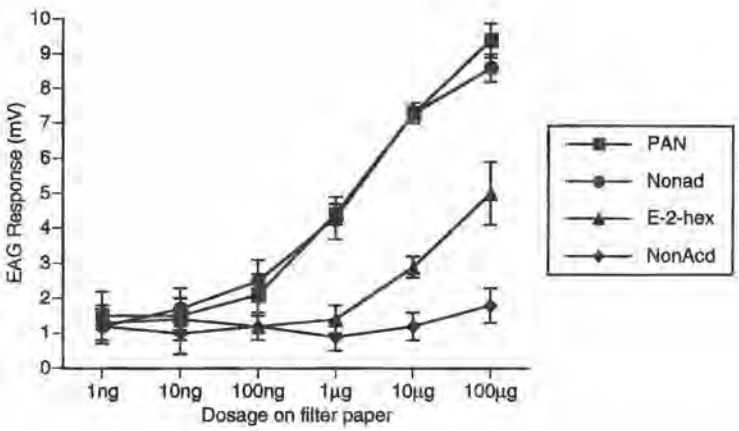

Fig. 2. Dose-response curves from stimulation of receptor neurones on the antennae of *Schistocerca gregaria* with four compounds. Each curve is constructed from ten replicates. The curves for phenylacetonitrile (PAN) and (*E,Z*)-2,6-nonadienal (Nonad) are similar in all doses and both are significantly different ($P<0.01$) from the curves for (*E*)-2-hexenal and nonanoic acid. The curves for (*E*)-2-hexenal and nonanoic acid are significantly different at the concentrations 10-100µg.

After three repetitive stimulations by 100 µg (self-adaptation stimulus), the response to (*E,Z*)-2,6-nonadienal was decreased to 58% of its preadaptation value, whereas the response to PAN decreased to 77% of its preadaptation value. Responses to *E*-2-hexenal and nonanoic acid were decreased to 67% and 69% of their preadaptation values respectively (Fig. 3).

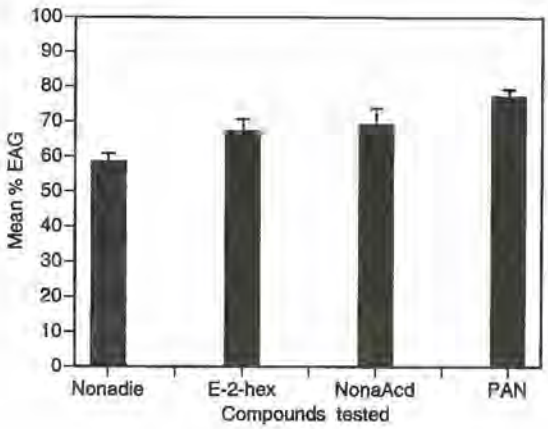

Fig. 3. Relative self-adaptation activities of the four compounds. The adaptation effect of each compound is expressed as a percentage of its initial response. A 100% EAG response means that no change has occurred after addaptation and 0% that the response was completely eliminated by adaptation. n=10 for each compound. Vertical bar = ± S.E. of mean.

When the different compounds were compared in cross-adaptation experiments, two response patterns emerged. Most compounds suppressed each other's responses only slightly (to between 77 and 95% of their preadaptation values), but when responses of *E*-2-hexenal and nonanoic acid were each cross-adapted by PAN, their respective responses were instead enhanced (Fig. 4).

The response to PAN was suppressed as much when adapted by (*E,Z*)-2,6-nonadienal as after self-adaptation (78%), whereas adapting with PAN suppressed the response to (*E,Z*)-2,6-nonadienal by 30% less than after self-adaptation (89%). Adaptation with *E*-2-hexenal and nonanoic acid reduced the responses to PAN stimulation to 86 and 84% respectively, while cross-adaptation with PAN increased the subsequent response to *E*-2-hexenal and nonanoic acid to 116 and 114% respectively. Cross-adaptation with *E*-2-hexenal before a test stimulus with nonanoic acid resulted in a 95% response, while the vice versa cross adaptation resulted in an 87% response. Cross adaptation between the compound pair nonanoic acid and (*E,Z*)-2,6-nonadienal resulted in a response reduction of between 5 and 20%, while in the pair *E*-2-hexenal and (*E,Z*)-2,6-nonadienal the reduction was between 15 and 20%.

DISCUSSION

In the present study, dose-dependent EAG responses were obtained to stimulations with pheromone compounds [PAN and (*E,Z*)-2,6-nonadienal], and a general plant odour (*E*-2-hexenal) whereas EAG responses to a component of the nymphal aggregation pheromone (nonanoic acid), were not dose-dependent. Self- and cross-adaptation

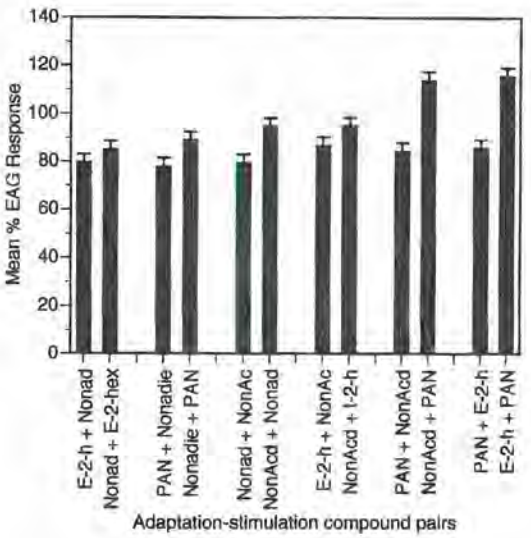

Fig. 4. Relative cross-adaptation activities of the four compounds. For each stimulus pair, the first is test and the second adapting compounds, respectively. The stimulation procedure was as in Fig. 1. A 100% response means that no change occurred after cross-adaptation, 0% that the response of the test stimulus was totally extinguished by the adapting stimulus, and >100% that the response to the test stimulus was enhanced by the adapting stimulus. n=10 for each compound. Vertical bar = ± S.E. of mean.

response values were relatively low to pheromone compounds.

As generally observed in adaptation studies, all adaptation induced changes are more evident with compounds that elicit high EAG amplitudes (Borroni and Atema, 1988; Borroni and O'Connell, 1992; Voigt and Atema, 1990). It follows that self-adaptations with PAN and (*E,Z*)-2,6-nonadienal should have elicited the same strong adaptation responses since their EAG amplitudes were higher and similar at all dosages tested. Instead, self-adaptation responses to PAN were significantly different from those of (*E,Z*)-2,6-nonadienal ($P<0.0001$, paired t-test), suggesting that these compounds are, at least partly, detected by different RNs.

In a single-sensillum study (Ochieng' and Hansson, submitted), it was demonstrated that aggregation pheromone compounds excited several olfactory RNs present in sensilla basiconica. These compounds did, however, inhibit responses of RNs tuned to plant odours and nymphal volatiles in s. coeloconica, whereas they did not affect RNs present in s. trichodea. It follows therefore, that EAG responses elicited by the major aggregation pheromone component (PAN) should result from excitation of RNs present in s. basiconica and possibly also from the inhibited neurones in the s. coeloconica, as it has been shown that also substances acting inhibitory on RN activity will produce an EAG (Van der Pers *et al.*, 1980).

Cross adaptation, in most cases, resulted in a lower reduction in response as compared to self adaptation. Only in the case of PAN being cross adapted with (*E,Z*)-2,6-nonadienal was a reduction as large as the self adaptation observed. Generally, PAN was strongly affected by cross adaptation, irrespective of the cross adapting compound.

In cross adaptations with nonanoic acid and *E*-2-hexenal the response to PAN was decreased to within 7 and 8 % of the self adaptation rate. However, from earlier investigations we know that RNs being excited by PAN are situated in another sensillum type than those detecting nonanoic acid and *E*-2-hexenal. The response decrease must thus, in these cases, be ascribed to another function than pure RN adaptation. A possibility is that the PAN-specific neurons are very sensitive to general antennal fatigue, i.e. depletion in energy sources or other essential elements for antennal function. Another possibility is interactions between RNs present in different sensilla through neural connections within the antennal nerve. From our data we can not conclude how the PAN-specific neurones are affected by adaptation with compounds detected by other types of RNs.

From the cross adaptation pairs including PAN, it is also clear how asymmetrical the interactions between two stimuli can be. Cross adaptation with (*E,Z*)-nonadienal reduces the PAN response to the self adaptation level, while the vice versa experiment results in a drastically lower reduction. In the two other PAN containing pairs, the relationship is the same, but even more pronounced as here the nonanoic acid and *E*-2-hexenal clearly adapt the response to PAN, while the response to these two compounds, after cross adaptation with PAN, is instead slightly enhanced.

Different neural assemblies must thus be operating, where one (the PAN-specific) is generally very susceptible to adaptation, whether on the neurone or the antenna level, while the other is very slightly affected by cross adaptation with PAN. PAN is a compound that is present in very high concentrations in a locust swarm (Deng *et al.* 1996), and the immunity of the systems detecting the other compounds from being adapted by PAN can possibly be an advantage.

The rest of the pairs tested show more or less identical reduction levels within the pairs, between 15 and 25% lower than the self adaptation rate. This implies that all compounds have, at least partly, separate detection systems that can remain operative despite cross adaptation with other compounds.

The low adaption rates of the tested compounds in general is not surprising as most are compounds that the locust is bound to experience in high concentrations in a swarm, thus the adaptation threshold of RNs tuned to these compounds should be higher than e.g. RNs tuned to detection of sex pheromones in moths, where neurones are tuned to detection of very low amounts of odorants. Neither is production and release of aggregation pheromones and plant odours limited by a strict time pattern as in the case of sex pheromones, thus olfactory RNs are bound to be bombarded with molecules for long periods, and hence adaptation with locust pheromones and host plant odours should not affect the system to an extreme degree.

It has been suggested for lobsters (Atema, *et al.*, 1989), that since they live in a chemically noisy environment, adaptation may be an important and necessary filter which

allows the animal to stop monitoring long lasting and uniform stimuli (background noise), and to extract only rapidly changing chemical stimuli as relevant chemosensory signals. Even though the substances tested in this study are in place over longer time and the locust is bound to associate with them more often, they are still of importance for locust behaviour, and hence the need for their continuous detection.

From this study we can conclude that all four compounds tested are most likely detected by a specific population of olfactory RNs. All compounds, except PAN, showed a substantially lower propensity to be adapted by compounds other than by itself.

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Aggregation pheromone detection by nymphal stages of the desert locust, *Schistocerca gregaria*

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Abstract: Neural activity was recorded from the olfactory receptor neurone assembly present in antennal sensilla basiconica in different nymphal stages of *Schistocerca gregaria*. The neurones were stimulated with individual components and blends of the adult and nymphal aggregation pheromones. Clearly dose-dependent responses were obtained to all the compounds tested. The sensitivity to the nymphal-produced pheromone blend did not differ between instars. The sensitivity did, however, differ for some single pheromone components and for the adult aggregation pheromone blend. Different components of the nymphal pheromone blend did also elicit responses at different threshold values, and different components were detected with different sensitivity. Adult-produced pheromones were generally detected at lower threshold values than nymphal-produced pheromones.

Key words: Hemimetabolous insect, Locust nymphs, Single sensillum, Receptor neurones, Electrophysiology, Aggregation pheromones

Introduction

In hemimetabolous insects, immature stages develop through several moults at which times cuticular parts of existing sensilla are replaced and new sensilla are added. This formation is usually coupled with an increase in the number and length of the antennal flagellar subsegments (Schafer and Sanchez, 1973). Sensillum numbers usually change at each moult, with the greatest increase occurring at the final moult (Chapman and Greenwood, 1986; Ochieng' *et al.* in press). However, it is not known whether the physiological characteristics of receptor neurons (RNs) change during development.

The sensory requirements of nymphal stages are more limited than those of adults. The nymphal olfactory receptors are usually only used for short-range orientation to food sources, and to aggregation or trail pheromones that are of limited range, as well as to detect temperature and humidity levels (Zacharuk and Shields, 1991). To sustain their growth and development, immature insects are voracious feeders, and in the desert locust, *Schistocerca gregaria* nymphs and migrating young adults are known to eat about their own weight of fresh vegetation each day (Steedman, 1988). This is in contrast to the requirements of adult insects whose antennal-sensilla have to monitor the environment for cues (both short- and long-range) to find conspecific mates and suitable oviposition sites (Zacharuk and Shields, 1991).

Earlier studies in different nymphal stages in hemimetabolous insects have been restricted to feeding responses, both behavioural and physiological, but similar investigations on olfaction are lacking. In recent years stage-specific, behaviourally active volatile compounds have been identified from live insects and faeces of *S. gregaria* (Obeng-Ofori *et al.*, 1993; Torto *et al.*, 1994, 1996; Njagi *et al.*, 1996). Sexually mature adults, specifically males, have been shown to produce adult aggregation-maturation pheromones that accelerate the maturation of young adults and aggregate older adults in a swarm (Torto *et al.*, 1994). Nymphal stages (second-to-fifth instars), on the other hand were found to produce a set of completely different semiochemicals that only aggregate nymphal stage locusts as well as retarding the maturation of young adults (Torto *et al.*, 1996). The first instar nymphs seem to have a different set of pheromones that is specific to this stage, and whose identification is yet unknown, but this stage is behaviourally indifferent to both adult-produced and second-to-fifth nymphal-produced pheromones (Njagi and Torto, personal communication).

The aggregation pheromones have been shown to be detected by antennal olfactory RNs. In studies where different body parts were incapacitated (Mordue, 1977; Gillett, 1983), it was demonstrated that gregarious locusts whose antennae were ablated in early stages developed morphological characteristics typical of solitarious phase individuals even though kept crowded. In physiological studies using adult locusts (Hansson *et al.*, 1996; Ochieng' and Hansson, submitted), it was shown that stage specific aggregation

pheromone compounds affected olfactory RNs housed in antennal sensillum types differently. Adult-produced pheromones were shown to excite RNs present in sensilla basiconica, each containing up to 30 sensory cells with massive dendritic branchings and multiporous sensilla wall. Receptor neurones in s. coeloconica that contains 2-4 sensory cells and spoke-channel systems of pores on the sensilla surface, were inhibited by these compounds. Nymphal-produced aggregation pheromone compounds, on the other hand, were found to excite RNs in both sensillum types.

In all studies that have been performed in locust nymphal stages, both behavioural and physiological, only a representative stage, mostly the fourth or fifth instar, have been used, and the results inferred to apply to all stages. In this study, we undertook to follow the response characteristics of antennal RNs to nymphal and adult aggregation pheromones in developmental stages from the second to the fifth instar.

Materials and Methods

Insects: Gregarious locusts that originated from Blades Biological (Edenbridge, UK) were bred under crowded conditions (50-100 per cage) in perspex cages (40 × 60 × 70 cm). First instars were isolated after hatching into 1-litre perspex jars with perforated lids in order to keep track of their age. The rearing room was well ventilated, temperature controlled (32 ± 2^0C day: 26 ± 2^0C night), and maintained on a light-dark cycle of 12:12 h and 45% relative humidity. Nymphs from second instar onwards were used 2-3 days after each moult.

Stimuli: The test compounds were obtained from Sigma Chemical Co. and were at least 98% pure. They included the following compounds that have been identified from the volatile collections of 5th instar *S. gregaria*: hexanal, octanal, nonanal, decanal, hexanoic acid, octanoic acid, nonanoic acid, decanoic acid, and a blend of these in the ratio 4:9:20:20:14:50:100:16 respectively (Torto *et al.*, 1996). In addition, we tested the effects of phenol and guaiacol, aromatic volatiles that have been identified in the nymphal frass (Torto *et al.*, 1994); mature male-produced aggregation pheromone compounds, phenylacetonitrile (PAN), benzaldehyde, guaiacol, phenol, and their blend in the ratio 100:15:4:4 respectively (Torto *et al.*, 1994; Njagi *et al.*, 1996), as well as a general green leaf odour, (*E*)-2-hexenal in our experiments. All the compounds were diluted in decadic steps in liquid paraffin from 100 µg/µl to 1 ng/µl.

Electrophysiology: The experimental set-up was as described by Hansson *et al.*, (1996). Insects were mounted in a plastic tube (6-10 mm i.d.) with the head exposed and the antennae fixed in place with dental wax. Recording of receptor neuron activity was

made using the penetration technique (Hubel 1957). Tungsten wire electrodes (Ø 0.1 mm) (Clarks, UK) were sharpened electrolytically (< 0.3 µm tip diameter) and inserted at the sensillum base using a Leitz micro manipulator. The recording electrode was connected to a high impedance AC/DC amplifier (Syntech, The Netherlands). The indifferent electrode (Ag/AgCl wire) was inserted into the locust abdomen. The signal was observed on a Phillips oscilloscope and recorded on a Vetter video cassette recorder, SLV-750HF (Vetter, New Jersey). The amplifier was also connected to a loudspeaker to provide an indication of contact quality. The recorded responses were analysed using Syntech Autospike software version 2.0.

A continuous flow of charcoal-filtered, humidified air was passed over the antennae through a glass tube (8 mm i.d.) at a speed of 0.5 m/s. The outlet of the tube was about 15 mm from the antenna. A Pasteur pipette containing a piece of filter paper (10 × 15 mm) loaded with 10 µl aliquotes (containing from 10 ng to 1,000 µg of each compound) served as stimulus source. Two millilitres of the Pasteur pipette atmosphere were injected into the continuous air stream through a small hole (2 mm diameter) in the wall of the tube, about 150 mm from the antenna, using a stimulus control device (Syntech). Stimuli were presented to the antenna in random order beginning with the lowest concentrations after a successful contact was made with a neurone. The interstimulus interval was 30 to 40 s. A pipette containing 10 µl liquid paraffin served as a control.

As reported before (Hansson *et al.*, 1996; Ochieng' *et al.*, in press), the s. basiconica contain up to 30 RNs, and single neurone responses could thus not be identified (Fig. 1). The response from the entire neurone assembly of single s. basiconica were therefore quantified. The total number of spikes were counted during a 1s stimulation period and the response intensity was determined as the difference between spikes 1s before and after stimulation minus the number elicited by control stimulation. The results were analysed using repeated measures ANOVA (Statview, for Macintosh), testing for significance differences between the stages.

Results

In the recordings from receptor neurones present in s. basiconia, a large number of neurones were activated by the different single stimuli (Fig. 1). The response to all compounds tested was clearly dose-dependent (Fig. 2a, b). In general, there was no distinct difference in sensitivity between different nymphal stages within a given class of compounds, as most dose-response curves overlapped. Some differences did, however, emerge after statistical treatment of the data. The responses to decanal and decanoic acid were significantly different between some stages and the response patterns to nymphal-produced aldehydes were different from the corresponding acids.

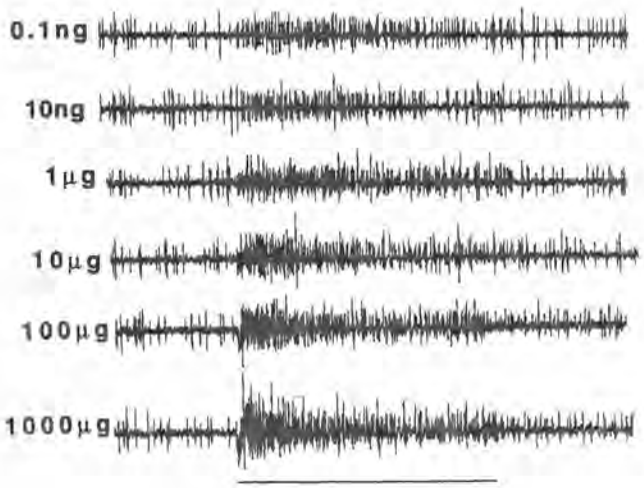

Fig. 1. Typical responses of receptor neurone assembly present in a sensillum basiconicum to stimulation with increasing doses of nymphal pheromone blend. The bar below the recordings indicates the duration of the olfactory stimulation (1 s).

Among the nymphal aldehydes, there was a clear response difference to stimulation with decanal, whereby the fifth instars displayed a significantly higher sensitivity than all other stages. Stimulation with nonanal yielded the opposite effect with the sensitivity of the fifth instars being lowest and significantly different from that of second and fourth instars. Response threshold values were also different between stimuli, with hexanal having the highest threshold at between 10-100 µg dosage whereas threshold values for the other stimuli were between 0.1-1 µg dosages.

Nymphal-produced acids elicited response patterns that were almost the reverse of the corresponding aldehydes. Decanoic acid elicited almost no response activity in any of the stages, even at the highest doses tested. The sensitivity of the second instar insects was, however, lower to this compound than in any of the other stages. Responses to hexanoic acid stimulations did not differ between the stages, but the response threshold was much lower (0.1 µg dosage) as compared to the corresponding aldehyde (10-100 µg dosage). A complete nymphal blend elicited responses that were not significantly different between any of the stages.

All the adult-produced pheromone compounds tested elicited mean responses with lower thresholds (0.01-0.1 µg dosage) than the responses to the nymphal-produced compounds. The sensitivity to the major adult pheromone compounds, PAN and guaiacol, did not differ from each other between any of the stages. When stimulated with

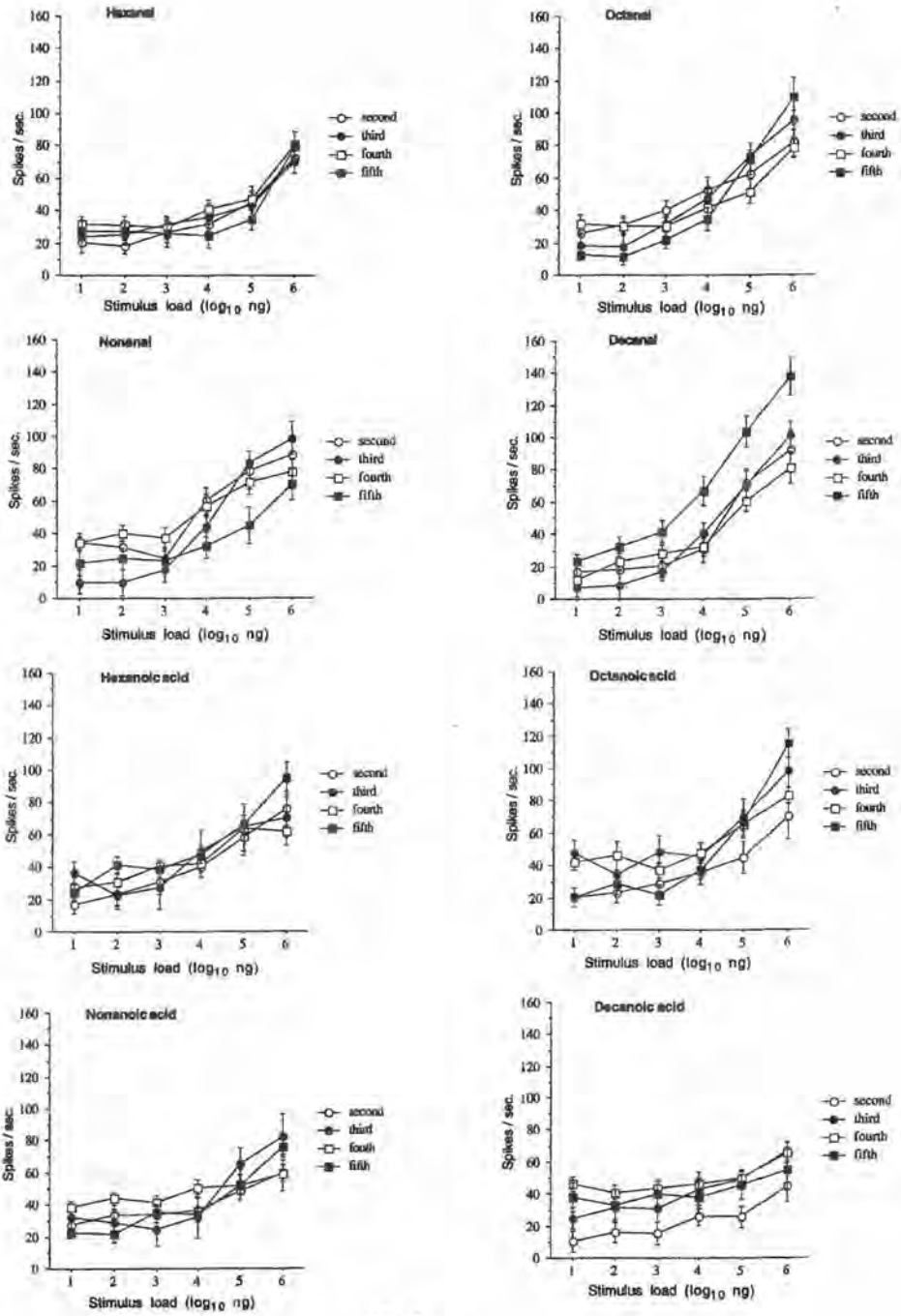
Hexanal
Octanal
Nonenal
Decanal
Hexanoic acid
Octanoic acid
Nonanoic acid
Decanoic acid
Spikes / sec.
Stimulus load (log_{10} ng)
second
third
fourth
fifth

Fig.2a

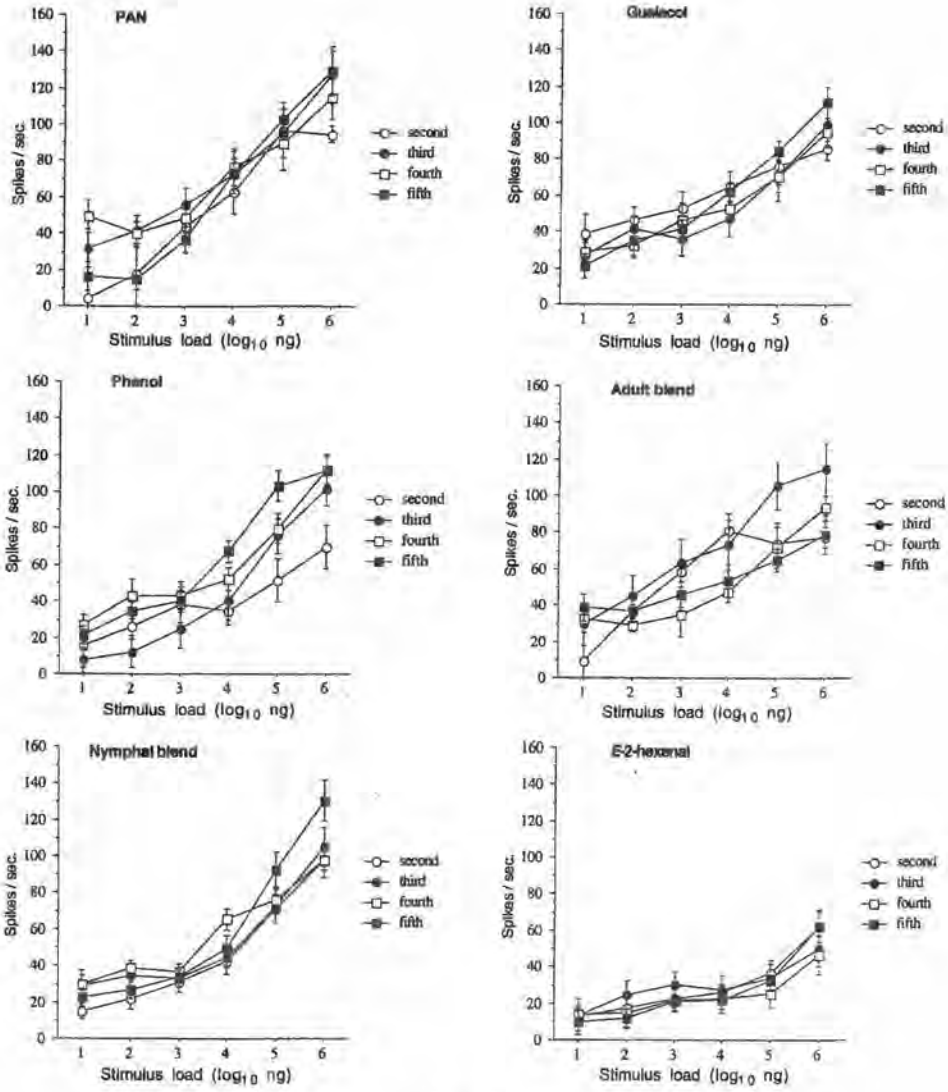

Fig. 2b

Fig. 2a, b. Dose-response curves from stimulation of receptor neurone assemblies present in sensilla basiconica of different nymphal stages. Stimuli are indicated above each graph. The x-axis indicates the stimulus load in the stimulus source. The y-axis indicates the net number of action potentials (spikes) ([spike # after stimulation - spike # before stimulation] - spike # elicited by control stimulation). n=10 for each compound. Vertical bar = ± S.E. of mean.

phenol, the second instar insects displayed a significantly lower sensitivity than the other stages, while for the complete adult pheromone blend, the third instar locusts had a higher sensitivity than the fourth and fifth (Table 1).

Stimulation with (*E*)-2-hexenal elicited responses only at the highest dosage. No difference in sensitivity was observed between the stages.

Discussion

These results show that although the general response pattern of olfactory RNs to stimulation with nymphal and adult aggregation pheromone components is similar in various stages, some variation in response to stimulations with individual pheromone components can be observed between the stages.

In nature, the locusts would normally experience a blend of all aggregation pheromone compounds. If there were a dramatic difference in response sensitivities between different stages, it would have emerged in response curves to the full nymphal blend. No significant difference was, however, observed between the different instars in response to the nymphal blend. From present behavioural data, such a difference would not be expected, as all instars, from 2nd to 5th, have been shown to be affected by this blend.

When the responses to single components are compared between the instars, it is evident that different instars respond strongly to different compounds. For the two nymphal pheromone components nonanal and decanal, the relative sensitivity of 5th instar RNs is totally opposite. When stimulated with nonanal, the 5th instar insects display the lowest sensitivity of all, while when stimulated with decanal, they display a significantly higher sensitivity than other instars. Similar differences were observed also for some of the other compounds tested. The behavioural significance of these differences is obscure. Very little is known regarding the behaviour of the different instars. Is it so that decanal has a more important function for the 5th instars, while the nonanal is relatively less important? This and similar questions have to be addressed in carefully designed behavioural assays.

Since the number of antennal olfactory sensilla increases at each moult (Chapman and Greenwood, 1986; Ochieng' *et al.* in press), the present results suggest that the overall sensory input to the insect brain is increased through the different stages, but the increase is not augmented by a simultaneous increase in RN sensitivity. Such a dual mechanism for sensitivity increase has earlier been shown in adult locusts of the solitary phase. This phase displays both a higher receptor neurone number and sensitivity than the gregarious phase (Ochieng' *et al.* in press; Ochieng and Hansson, submitted).

The output of the nymphal antenna could be tested further by performing

electroantennograms from insects of each stage to show whether the response intensity increases during development. The electroantennographic technique allows recording of responses from all RNs present on the antenna. Differences in olfactory sensitivity between different instars should also be investigated at the central nervous system level. In adult desert locusts, a sensitivity difference has been observed between antennal lobe neurons of individuals belonging to the gregarious and solitary phase (Anton and Hansson, unpubl). Also in fifth instar locusts a difference in antennal lobe neurone sensitivity between the phases has been established (Ignell, Anton and Hansson, unpubl.). In both cases, the solitary locusts are endowed with antennal lobe neurones of relatively higher sensitivity than the gregarious.

Adult produced pheromone compounds yielded response thresholds that were lower than the nymphal produced pheromones. Although the key adult-produced pheromone compound, PAN, is not part of the nymphal odours, it was detected at low concentrations by RNs in s. basiconica in all stages. PAN has been indicated as a behavioural antagonist to the nymphal aggregation pheromone, i.e. the nymphs would get dispersed by PAN (Njagi, personal communication). The neural processing of information regarding PAN must thus change profoundly from nymphs to adults. A signal that is repellent in the nymphal stages gets attractive in the adult locusts. Furthermore, the presence of guaiacol and phenol (that also occur in the nymphal frass) act synergistically on the effect of nymphal aggregation pheromones (Torto *et al.*, 1996). Heifetz *et al.*, (1996) demonstrated that guaiacol attracted 4th instars but did not affect the phase shift from solitarious to gregarious.

The fact that stimulations with (*E*)-2-hexenal resulted in responses with very low dose-dependency is in agreement with earlier findings in adult *S.gregaria*, where this compound is not detected by RNs in s. basiconica but by neurones associated with s. coeloconica (Ochieng' and Hansson, submitted). The present study will be expanded to include these RNs, tuned to food odour detection, to test whether their response sensitivities vary with developmental stage.

From the present results it can be concluded that no large differences in sensitivity to different aggregation pheromone components or blends are found between larval instars of gregarious desert locusts. To establish the effect of the pheromone odours on the aggregation system of *S. gregaria*, these data should be compared with a parallel study of nymphal stages belonging to the solitary phase.

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