Sex Pheromone Reception in the Scarab Beetle *Phyllophaga anxia* (Coleoptera: Scarabaeidae)

SAMUEL A. OCHIENG,[1,2] P. S. ROBBINS,[3] W. L. ROELOFS,[3] AND T. C. BAKER[2]

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ABSTRACT Antennal olfactory receptor neuron responses in the scarab beetle *Phyllophaga anxia* (LeConte) were investigated using tungsten microelectrodes. Morphological investigations revealed that antennal sensilla are distributed on the entire antennal club lamellae. The female-produced pheromones, L-valine and L-isoleucine methyl esters, were shown to affect two types of olfactory receptor neurons differently. One type of olfactory receptor neurons was excited with increasing pheromone concentrations at low doses (0.01–1 μg), but inhibited at higher doses. The second type was excited only at the high pheromone doses (100–1,000 μg stimulus loading). Both receptor neuron types were affected in the same way by the two pheromone components.

KEY WORDS *Phyllophaga anxia*, sex pheromone, single cell recording, L-valine methyl ester, L-isoleucine methyl ester

THE SCRABAEIDAE IS a large family of beetles containing nearly 1,500 species in North America. Scarabs are costly economic pests, both in the larval and adult stage, due to the damage they inflict below ground on roots and above ground on crops, plants, and trees. The cranberry white grub, *Phyllophaga anxia* (LeConte), also known as May or June beetles due to their flight period, is a serious pest damaging important crops including cranberry, strawberry, turfgrass, roses, corn, and tree nursery seedbeds in North America (Franklin 1950). The white larvae live in the soil feeding on root systems of cranberry. Larval feeding weakens the root system of the plant and predisposes the cranberry bog to weed invasion. Adult beetles cause damage to trees and other plant parts by eating flowers and foliage (Vittum et al. 1999).

As concerns for preserving the environment increase, emphasis on integrated pest management (IPM) as an alternative to insecticides in controlling insect populations is increasing. One IPM approach involves the use of sex pheromones emitted by mature females of a species to attract conspecific males, and thus lure the pest to a trap before it does considerable plant damage (Leal et al. 1992, Potter and Haynes 1993). The pheromone of *P. anxia* has been identified as a two-component blend composed of the methyl esters of the amino acids, L-valine and L-isoleucine (Zhang et al. 1997). In Massachusetts, adult females produce the L-valine and L-isoleucine methyl esters at a 3:1 ratio. In other regions of the United States, different ratios of these two compounds are used as pheromone components in *P. anxia* populations as well as over 40 other species of *Phyllophaga* (P.S.R. and W.L.R., unpublished data).

To investigate the mechanism of pheromone detection in *P. anxia* males, we examined morphological characteristics of the antennal club and physiological responses of single receptor neurons to the individual pheromone components.

Materials and Methods

Insects. Male beetles used in these experiments were light-trapped in Franklinville, NY, during the second week of June 2000. The males were kept individually in 30-ml disposable cups containing field soil at 10°C for several days. The beetles were shipped overnight to Ames, IA, in an insulated container to protect them from overheating.

Specimen Preparation for SEM. Antennae for scanning electron microscopy (SEM) were fixed in 70% ethanol for about one week before dehydration in a graded series of ethanol followed by critical-point drying. Antennal club lamellae were broken at the joint and both lateral and medial lamellae were mounted individually on aluminum stubs. Specimens were coated with gold/palladium (40:60) in a Desk II sputter coater (Denton Vacuum, Moorestown, NJ), and examined in a JEOL JSM-5800LV scanning electron microscope (Tokyo, Japan) operated at 10 kV. Both inner and outer surfaces of lateral and medial antennal club lamellae were examined.

Electrophysiological Recordings. For electrophysiological investigations, the beetle was restrained inside a specimen holder made from a cut barrel of a 10-ml hypodermic syringe. The head and exposed antenna were immobilized by dental wax and the club

[1] E-mail: sochieng@iastate.edu.
[2] Department of Entomology, Iowa State University, Ames, IA 50011.
[3] Department of Entomology, New York Agricultural Experimental Station, Geneva, NY 14456.

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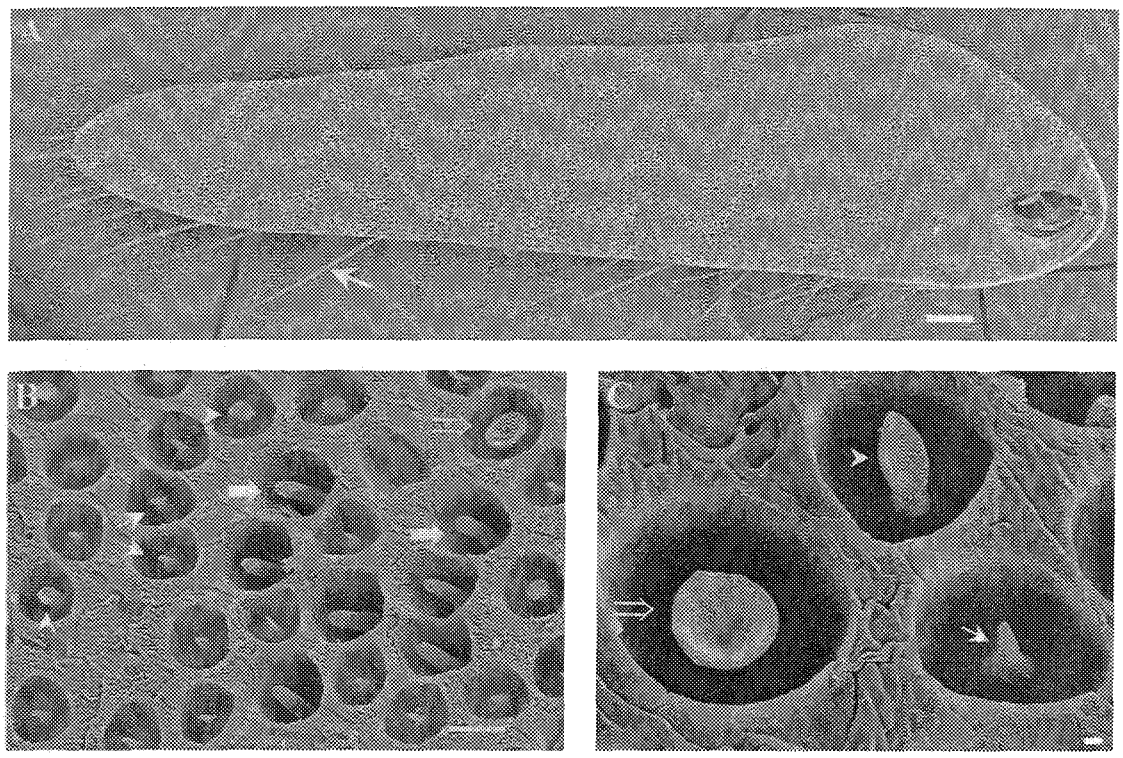

Fig. 1. Lateral antennal club lamella of *Phyllophaga anxia*. (A) The entire inner surface is covered by cuticular pits containing four sensilla types (visible only at higher magnifications). At the dorsal edge are long setae (arrow) that are possibly mechano-receptors. Bar = 100 μm. (B) Outer surface of the lateral lamella showing four sensilla types residing within cuticular depressions: sensilla auricillica (arrow heads), sensilla coeloconica type I (small arrows), sensilla coeloconica type II (large arrows), and spherical sensilla placodea (open arrow). Bar = 10 μm. (C) Higher magnification of the inner surface of the lateral antennal lamella showing sensilla auricillicum (arrowhead), sensilla coeloconicum type I (small arrow), and sensilla placodeum (open arrow). Bar = 1 μm.

lamellae set in an open position with an insect pin. The antenna was continuously flushed with a charcoal-filtered and moistened air stream flowing at 10 ml/s through an stainless steel tubing (8 mm i.d.) ending 2 cm before the preparation. A reference electrode made of silver wire was inserted in the insect abdomen through a hole on the specimen holder. The preparation was mounted on a Syntech portable recording unit, type INR-2 (Syntech, Hilversum, The Netherlands). We used Tungsten wire that was electrolytically sharpened to ≈1 μm tip as the recording electrode (Hubel 1957). The AC signal from the recording electrode was connected to the built-in amplifier of the portable recording unit and the AC output fed into a computer. We processed the data with a Syntech AutoSpike version 4.0 software.

The antennal club was probed with a recording electrode and in the event of a successful contact with a receptor neuron, dose-responses of L-valine and L-isoleucine methyl esters diluted in HPLC-grade hexane, beginning with the lowest concentrations were determined. Stimulus was applied as 10-μl aliquot on a piece of Whatman No.1 filter paper that was inserted into a Pasteur pipette. With a stimulus flow-controller device (Syntech), a 0.02-s air pulse at 40 ml/s-flow rates was injected through the odor cartridge and into the air stream flushing the antenna. We considered the response as the difference of spikes generated 100 ms before and after stimulus onset.

Results and Discussion

The antennal flagellum of *P. anxia* consists of three terminal plates: two outer and one middle lamellae, which can be folded together into a club shape. SEM images revealed that antennal sensilla are distributed fairly uniformly over both inner and outer surfaces of the club lamellae (Fig. 1). This is in sharp contrast to other scarab beetles like the chafers *Phyllopertha diversa* Waterhouse and *Anomala cuprea* Hope, and the Japanese beetle, *Popillia japonica* Newmann. In these beetles, the olfactory receptors have been shown to be restricted within narrow bands of the innermost surfaces of the antennal club lamellae (Leal and Mochizuki 1993, Hansson et al. 1999, Kim and Leal 2000). Five sensilla types were identified on the *P. anxia* antenna: long sensilla chaetica that occur along the peripheral edges and are most likely mechano-receptors; spherical sensilla placodea that varied in size between five and 10 μm in diameter, rabbit-ear-like

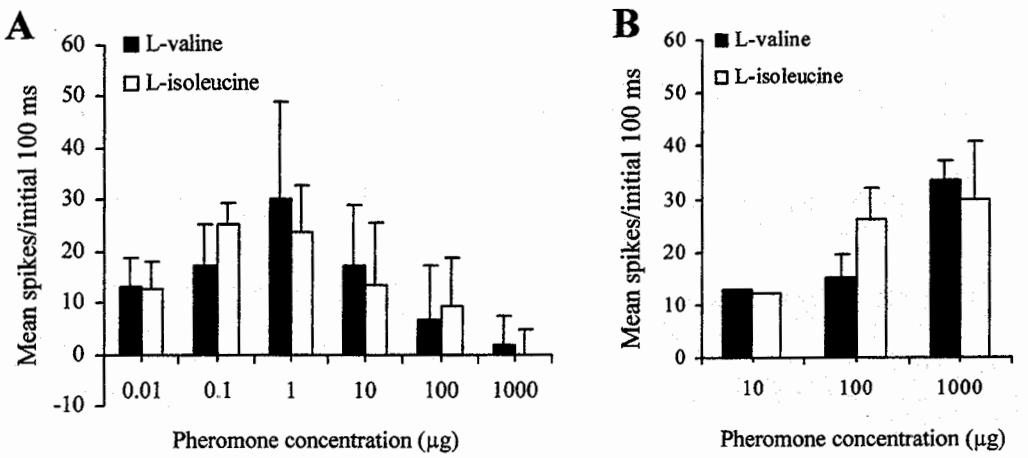

Fig. 2. Mean ± SE dose–response curves constructed from receptor neurons responsive to methyl esters of L-valine and L-isoleucine. (A) Responses of a neuron excited at low doses, but inhibited by higher pheromone concentrations ($n = 8$) (B) Responses of a receptor neuron type sensitive to pheromones only at higher doses (10–100 μg; $n = 6$).

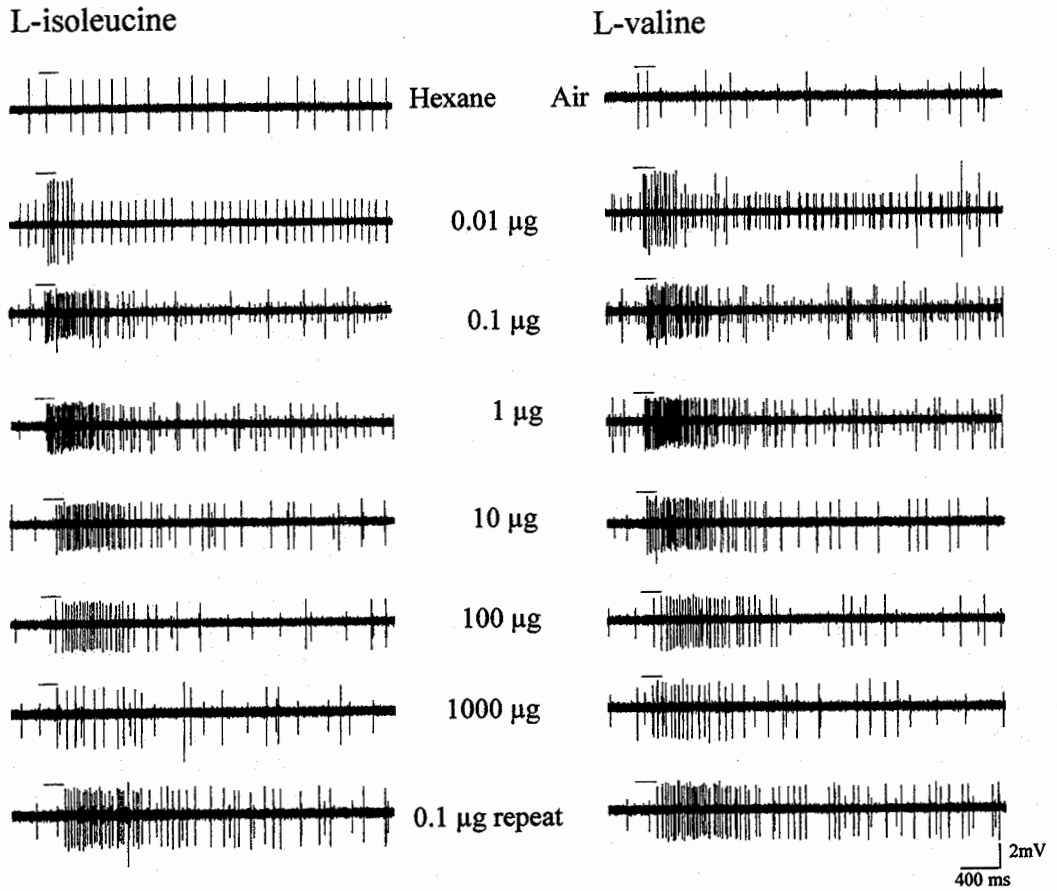

Fig. 3. Typical responses of a receptor neuron excited by lower doses (0.01–1 μg) of L-isoleucine and L-valine methyl esters, but which produced lower spike frequencies in response to higher concentrations. Notice the presence of a second neuron with a short spike amplitude that did not respond at the lowest tested dose (0.01 μg) of each stimulus. The large amplitude neuron is sensitive to lower concentrations as demonstrated by a repeat of 0.1 μg that resulted in higher spike frequency compared with the 1,000 μg dosage. Horizontal bars above each recording represent stimulus duration.

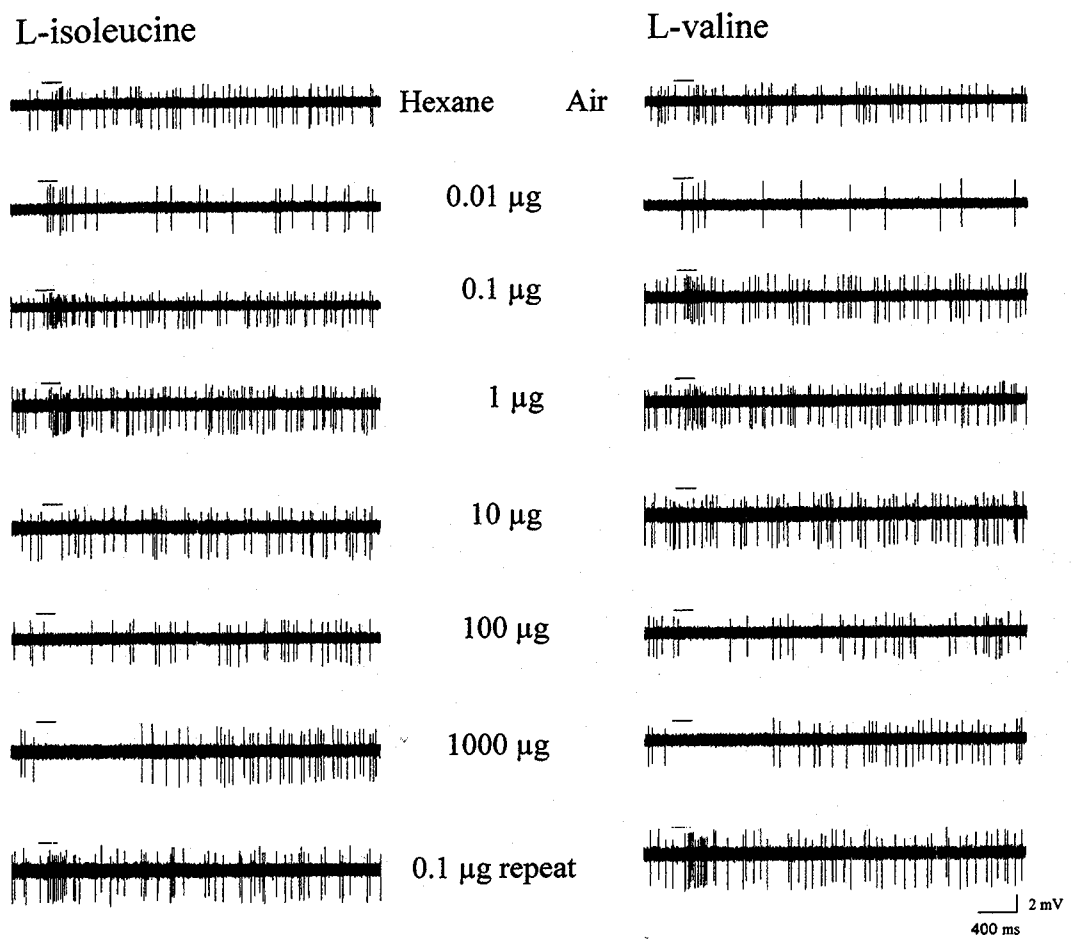

Fig. 4. Typical responses of a receptor neuron sensitive to lower doses (0.01–1 µg) but inhibited by higher doses of L-isoleucine and L-valine methyl esters. A repeat of 0.1 µg dosage confirms that the neuron is excited at lower concentrations. Horizontal bars above each recording represent stimulus duration.

sensilla auricillica, and sensilla coeloconica type I and type II. The last three types are most likely olfactory receptors. All are situated within cuticular depressions and are randomly distributed on both sides of all club lamellae, except for sensilla coeloconica type II that was observed only on the outer surface of the lateral lamella (Fig. 1B and C).

We obtained stable recordings from 18 sensilla from six male *P. anxia*. After contact had been established, a low frequency of spontaneous activity could be observed but no impulse was evoked by the control stimulation. Eight receptor neurons responded with increased stimulus intensity of both pheromone components at lower dosages, 0.01 µg reaching a maximum at 1-µg dosage. At higher dosages (≥10 µg), spike frequency of these receptor neurons were either decreased or completely inhibited (Figs. 2A, 3, and 4). Six receptor neurons responded by excitation in a dose-dependent manner only at higher stimulus dosages, (≥10 µg) (Figs. 2B and 5). Four neurons did not respond to the pheromone components despite establishing contacts with good spontaneous spikes. In most cases, contacts were made with just single receptor neurons, however, in some instances, spontaneous activities from a second neuron with different spike amplitude that did not respond to the stimuli were encountered (Fig. 3).

It was not possible to distinguish the various sensilla types under the dissecting microscope; hence the lamellar surface was randomly probed with the recording electrode to establish contacts with receptor neurons. We thus could not assign physiological roles to the individual sensillar types. Response patterns of receptor neurons were similar when presented with both pheromone components in all responding neurons contacted (Fig. 2).

The responses of the eight neurons that exhibited reduced responses at higher dosages and exquisitely

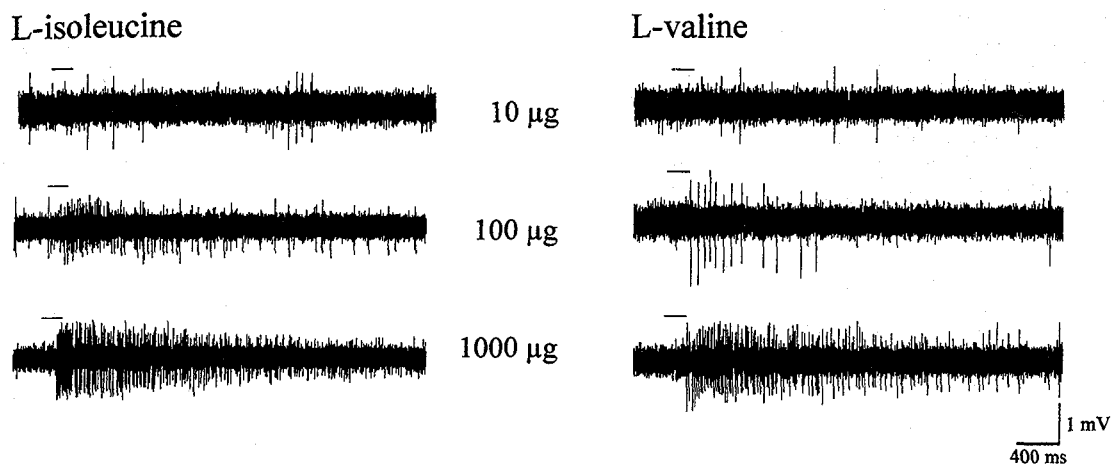

Fig. 5. Typical responses of a receptor neuron sensitive only at higher dosages (100–1,000 μg), of L-isoleucine and L-valine methyl esters. Horizontal bars above each recording represent stimulus duration.

high sensitivity to pheromones at low doses (0.01 μg) are unusual for sex pheromone receptors. Further behavioral or field studies are needed to verify whether these neuronal response profiles correlate with behavioral sensitivities. In earlier field-trapping experiments (Zhang et al. 1997), trap catches in one location showed a decrease (though not statistically significant) in total number of beetles at higher dosage compared with traps of low pheromone concentrations.

Multiple functions of a pheromone at different doses resulting in difference in behavior are common phenomena for aggregation pheromones in bark beetles. In the Douglas-fir beetles, *Dendroctonus pseudotsugae* Hopkins, at low doses these beetles use their aggregation pheromones to attract and recruit other members of their species. However, at higher pheromone emission rates (higher number of beetles colonizing the tree), they use the higher amounts of the same pheromone to disperse the colony in search for a new habitat (Rudinsky 1973). In *P. anxia*, pheromones have been demonstrated to originate only from mature females (Zhang et al. 1997), and pheromone-baited traps caught mostly male beetles, thus ruling out the possibility of them being aggregation pheromones.

Scarab beetles of the subfamily Melolonthinae, to which *P. anxia* belong, use amino acid derivatives and terpenoid compounds as sex pheromones. In the black chafer, *Holotrichia parallela* (Motschulsky), L-isoleucine methyl ester is the major sex pheromone, whereas L-valine methyl ester, though present in the gland extract, does not seem to play a significant role behaviorally (Leal et al. 1992). It is possible that the same pheromone compounds are used at different concentrations to avoid mating mistakes between individuals of the different species. In the future, in addition to examining the ultrastructure of pheromone-detecting sensilla, we will investigate the dose–response effects of pheromone mixtures as well as the influence of general plant odors on the reception of pheromones by *P. anxia*.

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