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Transient post-mating inhibition of behavioural and central nervous responses to sex pheromone in an insect

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Mating is costly for both male and female insects and should therefore only occur if it is likely to be successful. Within one scotophase, which is the dark period of the light cycle, male moths can only produce one single spermatophore, which is transferred to the female during mating. Remating within the same scotophase would thus be unsuccessful. We tested the hypothesis that newly mated males of the moth *Agrotis ipsilon* have developed an energy-saving strategy based on the transient inhibition of their sexual behaviour, thus avoiding unsuccessful remating. *Agrotis ipsilon* males do not copulate more than once during the same scotophase. Moreover, newly mated males do not respond behaviourally to the female sex pheromone although electroantennograms showed that their peripheral olfactory system is fully functional. However, intracellular recordings of antennal lobe neurons showed that the sensitivity for the synthetic sex pheromone blend decreased as compared with that of unmated males. Both the sexual behaviour and the sensitivity of the antennal lobe neurons were restored when tested during the next scotophase. Our results show a fast, transient neuronal plasticity that 'switches off' the olfactory system, which could prevent males from mating unsuccessfully.

Keywords: antennal lobe; central nervous processing; insects; post-mating; neuronal plasticity; olfaction

1. INTRODUCTION

Costs of reproduction have been demonstrated in a variety of species and considerable diversity exists both in the components of reproduction that are costly and the nature of the cost involved. Reproductive costs can be ecological in origin (e.g. Tuttle & Ryan 1981). They can also be physiological in origin and among them these include the costs of courtship (e.g. Simmons *et al.* 1992).

Mating is energy consuming for both males and females in most organisms and should therefore be avoided if it is unlikely to result in the successful production of offspring. Lifespan is reduced by courting in females of *Drosophila melanogaster* (Chapman 1992). Mating in moths causes a switch in the female's behaviour from being sexually receptive to the loss of receptivity and the onset of oviposition. The loss of receptivity in female moths, which is accompanied by depletion of sex pheromone, is temporary in species that mate more than once, such as the corn earworm *Helicoverpa zea* (Raina *et al.* 1986) or permanent in species that mate only once, such as the gypsy moth *Lymantria dispar* (Giebultowicz *et al.* 1991).

Mating in males has been shown to be energy consuming, as mated males often live for a shorter time than unmated males. Courtship and mating were shown to reduce the longevity of male *D. melanogaster* flies (Cordts & Partridge 1996). Virgin noctuid moth males (*Pseudaletia unipuncta*) live significantly longer than mated males (Fitzpatrick & McNeil 1989). On the other hand, multiply mated *P. unipuncta* females live longer than only once-mated females, thereby indicating an energy transfer from males to females (Svärd & McNeil 1994).

Sex accessory glands in male moths grow and produce proteinaceous material that is associated with sperm production and transfer (see Happ 1992). During mating, a spermatophore originating from the sex accessory glands of the male is transferred into the female where it has various modulatory functions on female behaviour and physiology (Leopold 1976). Males of *Agrotis ipsilon* show empty sex accessory glands just after mating (Duportets *et al.* 1998). They need one more day to refill their glands in order to be able to remate and, thus, to produce a new spermatophore (Duportets *et al.* 1998). However, nothing is known about the ability of males to respond behaviourally to the female-produced sex pheromone once they have mated.

Mating behaviour in moths is based on olfactory communication. Females produce species-specific sex pheromones in order to attract males. Males receive sex pheromone information through receptor neurons on their antennae and approach behaviour is elicited after central processing of the sex pheromone (for a review see Hansson 1995). Central nervous processing of the sex pheromone in *A. ipsilon* has previously been shown to be age and hormone dependent (Anton & Gadenne 1999; Gadenne & Anton 2000). The male olfactory system in this case is adapted in order to allow mate recognition and reproduction at the optimal time.

In the present study we tested the hypothesis that males have developed a strategy for avoiding unsuccessful remating attempts based on neuronal plasticity of their olfactory system. We studied the ability of newly mated males to remate and to respond behaviourally to sex

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pheromone within the same scotophase, which is the dark period of the light cycle. We also investigated the responses of the peripheral olfactory system and antennal lobe interneurons to the sex pheromone in mated and unmated males. Our experiments show that mating induced a fast transient inhibition of chemical communication-linked behaviour that is correlated with a decreased sensitivity of central neurons to sex pheromone in the male black cutworm moth *A. ipsilon*.

2. MATERIAL AND METHODS

(a) *Insects*

The colony of *A. ipsilon* originated from moths caught in southern France. Adults from field catches are introduced into the colony each spring. Larvae of the black cutworm were reared on an artificial diet (Poitout & Buès 1974) and maintained in individual plastic cups until pupation under a 16 L:8 D photoperiod at 21 ± 1 °C. Pupae containers were checked each day for newly emerged adults. Adults were held in plastic boxes and had access to 20% sucrose solution.

(b) *Remating experiments*

Analysis of the remating ability of newly mated males within the same scotophase was performed with 5 day old moths under a 16 L:8 D photoperiod at 21 ± 1 °C. Virgin males and virgin females were paired in cylindrical plastic containers before the onset of scotophase. Visual observations of matings were made every 30 min during the mating period at mid-scotophase (Swier *et al.* 1976). Once a mating was achieved (mating lasts between 1 and 2 h), the mated female was removed from the box and another virgin female was introduced into the box. We only took into account males that finished mating early enough that there was ample time for a remating attempt to occur if it was going to happen at all. Observation of any possible remating of the males continued until the onset of the photophase. In order to be sure that the male introduced a spermatophore during the first mating, all mated females were checked for the presence of the spermatophore. The experiment was performed twice at different dates with 60 pairs each.

(c) *Post-mating experiments*

Pairs were formed between 5 day old virgin males and females under the same conditions as described above. Once males had mated, they were quickly removed from the pairing box and submitted to wind tunnel experiments or electrophysiological tests within either 1 h (newly mated males) or the following day (24 h post-mated males).

(d) *Wind tunnel experiments*

The behavioural responses of mated and unmated males were tested in wind tunnel experiments. In order to check for effects on males of being exposed to females without mating, two different groups of unmated males were used: virgin males held separately from the females (isolated virgin males) and virgin males that had been exposed to females but that did not mate (female-exposed virgin males).

Newly mated, 24 h post-mated, female-exposed virgin and isolated virgin males were then tested for their ability to respond to the female sex pheromone in a wind tunnel according to a procedure described earlier (Picimbon *et al.* 1997). Briefly, pheromone glands of 4 day old virgin females were dissected at mid-scotophase. The glands were extracted in 50 µl hexane for 1 h. Experimental males were exposed to gland extracts (one female equivalent containing *ca.* 1 ng of the pheromone blend) that were dispensed on a filter paper and placed in the airflow upwind of the release site in the wind tunnel. Each experimental male was used only once and experiments were conducted blind with respect to mating status. The responsiveness of males to the pheromone gland extract was estimated using the following scale: no response (0), clasper eversion (1), interrupted flight (2), complete flight towards the source (3) and landing on the source (4). Statistical differences between groups were assessed by the *G*-test, ($p = 0.05$).

(e) *Electroantennograms*

Electroantennograms were performed in order to test the effect of mating on the peripheral olfactory system of the males. Female-exposed virgin males (together with females but not mated) were also tested during the same periods of time. Electroantennograms were performed on excised antennae mounted between two glass electrodes containing Beadle–Ephrussi Ringer (Hansson *et al.* 1995). The antennae were stimulated successively with clean air and the behaviourally active pheromone blend ((*Z*)-7-dodecenyl acetate, (*Z*)-9-tetradecenyl acetate and (*Z*)-11-hexadecenyl acetate at a ratio of 4:1:4) as used in field trapping experiments (Causse *et al.* 1988). Four doses of the pheromone blend were tested in order to create a dose–response curve (10, 100, 500 and 1000 ng total amount of pheromone components on filter paper). We calculated the mean electroantennogram amplitude (± s.d.) of all antennae in response to clean air stimulation for each group of experimental males. Statistical differences between the two dose–response curves were assessed using one-way ANOVA. Comparison of responses for each curve was performed by one-way ANOVA followed by the Mann–Whitney *U*-test ($p < 0.05$).

(f) *Intracellular recordings and stimulation*

Intracellular recordings were performed as described earlier (Anton & Gadenne 1999). Briefly, the brain of a male moth was exposed and the head cavity perfused with saline. Pheromone-sensitive antennal lobe interneurons were penetrated with a 2 M KCl-filled glass microelectrode in the male-specific macroglomerular complex. The antennae of the moths were stimulated with a 500 ms air pulse containing either the synthetic three-component pheromone blend or clean air as a blank. In order to separate plant-specific neurons from our study, a common green leaf volatile, (*E*)-2-hexenal, was also used as a stimulus. The pheromone blend (see § 2(e)) was applied at amounts of between 1 pg and 100 ng on a filter paper in a Pasteur pipette.

Responses (the number of spikes) were analysed manually as described elsewhere (Anton & Gadenne 1999). For statistical treatment, four groups of neurons were created: non-responding (no response), high-threshold (100 ng and 10 ng), medium-threshold (1 ng and 0.1 ng) and low-threshold neurons (0.01 ng and 1 pg). Neurons responding exclusively to the plant odour were not taken into account. A *G*-test for heterogeneity (Sokal & Rohlf 1995) was performed in order to compare the four sensitivity groups for the three groups of experimental animals (unmated, newly mated and 24 h post-mated males).

3. RESULTS

(a) *Remating ability*

A total of 32 and 40 males, respectively, mated once with a female within the entire scotophase in the two

remating experiments that were performed within the same scotophase (table 1). All mated males transferred a spermatophore during copulation. No mating was observed leading to a female without a spermatophore. Of these 32 and 40 mated males, those that had completed mating, at the latest 2 h before the end of the scotophase (22 and 29 newly mated males), were given a new virgin female. None of the males remated after the mated female had been replaced with a virgin female (table 1), showing that *A. ipsilon* males do not mate more than once during the same scotophase.

(b) *Behavioural sex pheromone responsiveness*

Newly mated, 24 h post-mated, female-exposed virgin and isolated virgin males were tested in the wind tunnel for their ability to respond to the female sex pheromone (table 2). A large proportion of female-exposed virgin, isolated virgin and 24 h post-mated males were attracted to the female sex pheromone. The behavioural responses of these three groups of experimental animals did not differ significantly (table 2). As female-exposed and isolated virgin males did not differ significantly in their sexual behaviour, only female-exposed virgin males were used as controls in the electrophysiological tests. Newly mated males never showed any behavioural response to the pheromone gland extract (table 2).

(c) *Antennal sex pheromone responsiveness*

Electroantennogram measurements were performed on 17 and 16 antennae of mated and unmated males, respectively, which were stimulated with different doses of the synthetic sex pheromone blend and with a clean air puff. A clear dose–response relationship was observed in the antennae of both mated and unmated males ($F = 13.24$ and $p < 0.001$ and $F = 9.23$ and $p < 0.001$, respectively) for the sex pheromone stimulation and the two curves were not statistically different (ANOVA, $p = 0.121$) (figure 1).

(d) *Central nervous sex pheromone responsiveness*

The responses of antennal lobe neurons to the synthetic pheromone blend of *A. ipsilon* were analysed in newly mated, 24 h post-mated and female-exposed virgin males (figure 2). Eighty-six antennal lobe interneurons in 16 control males, 78 antennal lobe interneurons in 15 24 h post-mated males and 76 antennal lobe interneurons in 15 newly mated males were tested. Most antennal lobe neurons responded to the sex pheromone blend with an increase in spike frequency after the onset of stimulation followed by an inhibitory period, as described earlier (Anton & Gadenne 1999; Gadenne & Anton 2000). Antennal lobe interneurons with different thresholds in response to the sex pheromone were found in males of all experimental groups (figure 2). However, the proportion of low-threshold antennal lobe neurons in newly mated males was significantly lower than in control males ($G = 73.64$, d.f. $= 3$ and $p = 0.001$). The proportion of low-threshold antennal lobe neurons in 24 h post-mated males did not differ significantly from the proportion in control males ($G = 9.92$, d.f. $= 3$ and n.s.) but was significantly different from newly mated males ($G = 84.39$, d.f. $= 3$ and $p = 0.001$).

4. DISCUSSION

In the present study we have shown that there is a transient inhibition of the behavioural and central nervous responses to sex pheromone after mating in the male moth *A. ipsilon*. This inhibition could prevent mating more than once during the same scotophase. However, the responses to pheromone are recovered by the beginning of the next scotophase in accordance with a 'recovery' of the ability to mate successfully (Duportets *et al.* 1998).

There is also a drastic change in sexual behaviour just after mating in female moths, which is characterized by a transient inhibition of sex pheromone production. This temporary inhibition was shown to be induced by a male factor acting on the female reproductive physiology during mating (Kingan *et al.* 1993). This factor was found to act on the release of pheromone biosynthesis-activating neurohormone, thus inhibiting sex pheromone biosynthesis in the pheromone gland (Raina *et al.* 1990).

The behavioural and central nervous responses to sex pheromones of *A. ipsilon* males are linked to the status of the sex accessory glands. When the males are sexually immature and do not respond to the pheromone (Gadenne *et al.* 1993), their sex accessory glands are empty and the amount of proteins present inside the glands is very low (Duportets *et al.* 1998). In addition, immediately after mating the sex accessory glands are empty (Duportets *et al.* 1998) and we show in this study that these newly mated males do not show any behavioural or physiological response to sex pheromone. While the relatively slow change from immature to mature pheromone-responding males with highly sensitive antennal lobe neurons was shown to be juvenile hormone mediated (Gadenne *et al.* 1993; Anton & Gadenne 1999; Gadenne & Anton 2000), the fast inhibition of the behavioural response to sex pheromone and the fast decrease in antennal lobe neuron sensitivity after mating is independent of the juvenile hormone titre, which does not decrease after mating (Duportets *et al.* 1998). Therefore, we conclude that there are different forms of plasticity in the olfactory pathway which modulate sex pheromone-guided behaviour. In both cases, plasticity is localized in the central nervous system. Electroantennogram recordings showed that the peripheral olfactory system is fully functional in males independently of the reproductive state. However, we cannot exclude the possibility that single receptor neurons change their response characteristics.

The fast inhibition of the behavioural response to sex pheromone shown in the present study could be mediated by hormones and/or biogenic amines or neuropeptides that are released in the central nervous system, which could modulate the sensitivity of pheromone-responding antennal lobe neurons. In the sense of Elekonich & Robinson (2000), the fast inhibition of the sex pheromone response described in the present paper would be a short-latency activational effect if it is hormone mediated, whereas the relatively slow effect of juvenile hormone described earlier could be called a long-latency activational effect.

Biogenic amines have been shown to be involved in regulating sex-specific behaviour and its central nervous

Table 1. Ability of newly mated, 5 day old *A. ipsilon* males to remate within the same night.

(Of the total males that mated throughout the scotophase (mated males), only those that had ended copulation at least 2 h before lights on were used for the remating experiments (mated males provided with new females). See § 2 for further details.)

	pairs	mated males	mated males provided with new females	remated males
first experiment	60	32	22	0
second experiment	60	40	29	0

Table 2. Pheromone responsiveness of *A. ipsilon* males.

(Five day old (unmated and newly mated) and 6 day old (post-mated) *A. ipsilon* males were submitted to gland extracts of 5 day old, virgin females. The behaviours of groups of males followed by the same letter are not statistically different (*G*-test, $p < 0.05$).)

males	number of males	no response (%)	clasper eversion (%)	interrupted flight (%)	complete flight (%)	landing on source (%)
unmated						
without females	34a	13 (38)	4 (12)	2 (6)	10 (29)	5 (15)
with females	36a	17 (47)	1 (3)	4 (11)	11 (31)	3 (8)
newly mated	32b	32 (100)	0	0	0	0
post-mated	36a	10 (28)	4 (11)	11 (31)	7 (19)	4 (11)

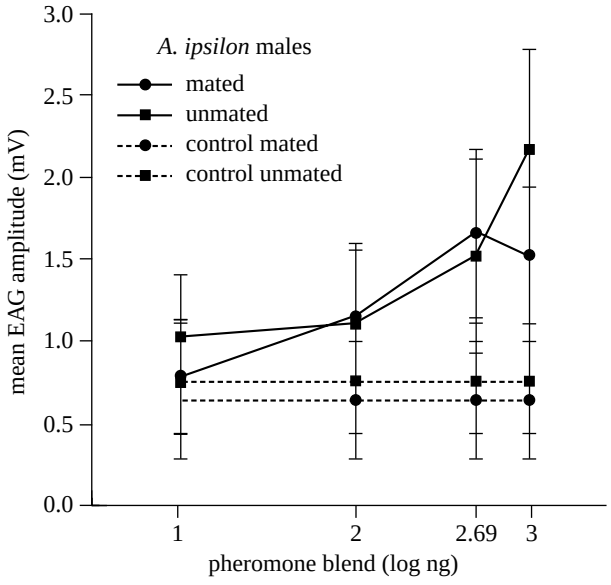

Figure 1. Electroantennogram dose–response curves for the pheromone blend of female-exposed virgin and newly mated *A. ipsilon* males. Hatched lines indicate pooled electroantennogram values for clean air stimulation in mated and unmated males. Means ± s.e.m.

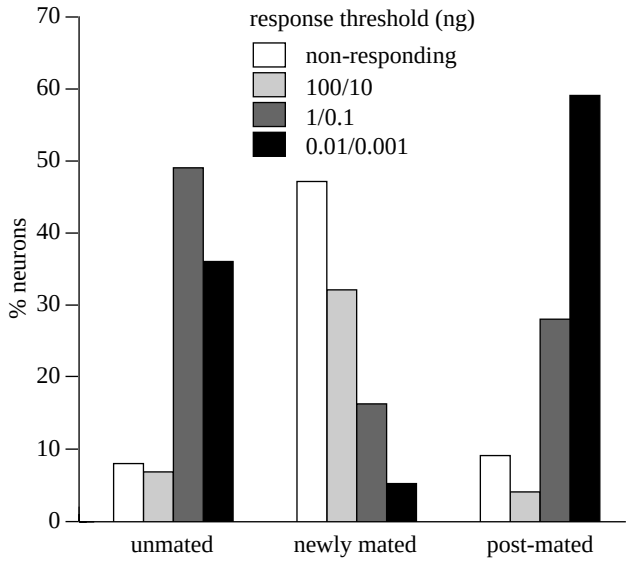

Figure 2. Effect of mating on the sensitivity of antennal lobe interneurons in *A. ipsilon* males for the sex pheromone blend. Percentage of antennal lobe neurons tested responding to the pheromone blend at different thresholds in female-exposed virgin (86 neurons in 16 males), newly mated (76 neurons in 15 males) and 24 h post-mated (78 neurons in 15 males) *A. ipsilon* males.

processing in both insects and vertebrates. Serotonin enhances antennal lobe interneuron responses to the sex pheromone in the sphinx moth *Manduca sexta* (Kloppenburg *et al.* 1999). Octopamine was shown to increase the behavioural pheromone responsiveness of the cabbage looper moth *Trichoplusia ni* (Linn & Roelofs 1992). In mammals, serotonin and dopamine have antagonistic functions: serotonin inhibits sexual behaviour, while dopamine promotes mating in male rats (for a review see Hull *et al.* 1999). The serotonin titre increases after ejaculation during the post-ejaculatory interval in the lateral hypothalamic area, thus inhibiting dopamine release in the nucleus accumbens (Lorrain *et al.* 1999).

The process of emptying the sex accessory glands during copulation in *A. ipsilon* could thus possibly inhibit or enhance the release of neuromodulators acting on central olfactory neurons. It would therefore be very interesting to measure brain serotonin or other biogenic

amine levels that are linked to mating status. Surgical treatments leading to sex accessory gland-deprived males could also allow us to test the ability of the antennal lobe interneurons of these males to respond to the pheromone.

By reducing the sensitivity of sex pheromone-responding neurons, the fast modulatory system of *A. ipsilon* males prevents wasting of energy by unsuccessful matings. Recovery of the central nervous processing of sex pheromone and sexual behaviour allows successful matings during the following days that will increase the male moth's reproductive success, i.e. the number and heterogeneity of their offspring.

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REFERENCES

Anton, S. & Gadenne, C. 1999 Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *Proc. Natl Acad. Sci. USA* **96**, 5764–5767.

Causse, R., Buès, R., Barthès, J. & Toubon, J. F. 1988 Mise en évidence expérimentale de nouveaux constituants des phéromones sexuelles de *Scotia ipsilon* et *Mamestra suasa*. In *Médiateurs chimiques: comportement et systématique des Lépidoptères. Application en agronomie* (ed. C. Descoins & B. Frérot), pp. 75–82. Paris: Institut National de la Recherche Agronomique.

Chapman, T. 1992 A cost of mating with males that do not transfer sperm in female *Drosophila melanogaster*. *J. Insect Physiol.* **38**, 223–227.

Cordts, R. & Partridge, L. 1996 Courtship reduces longevity of male *Drosophila melanogaster*. *Anim. Behav.* **52**, 269–278.

Duportets, L., Dufour, M. C., Couillaud, F. & Gadenne, C. 1998 Biosynthetic activity of corpora allata, growth of sex accessory glands and mating of the male moth *Agrotis ipsilon*. *J. Exp. Biol.* **201**, 2425–2432.

Elekonich, M. M. & Robinson, G. E. 2000 Organizational and activational effects of hormones on insect behavior. *J. Insect Physiol.* **46**, 1509–1515.

Fitzpatrick, S. M. & McNeil, J. N. 1989 Lifetime mating potential and reproductive success in males of the true armyworm, *Pseudaletia unipuncta*. *Funct. Ecol.* **3**, 37–44.

Gadenne, C. & Anton, S. 2000 Central processing of sex pheromone stimuli is differentially regulated by juvenile hormone in a male moth. *J. Insect Physiol.* **46**, 1195–1206.

Gadenne, C., Renou, M. & Sreng, L. 1993 Hormonal control of sex pheromone responsiveness in the male black cutworm, *Agrotis ipsilon*. *Experientia* **49**, 721–724.

Giebultowicz, J. M., Raina, A. K., Uebel, E. C. & Ridgway, R. L. 1991 Two-step regulation of sex pheromone decline in mated gypsy moth females. *Arch. Insect Biochem. Physiol.* **16**, 95–105.

Hansson, B. S. 1995 Olfaction in Lepidoptera. *Experientia* **51**, 1003–1027.

Hansson, B. S., Blackwell, A., Hallberg, E. & Lofqvist, J. 1995 Physiological and morphological characteristics of the sex pheromone detecting system in male corn stemborers *Chilo partellus*. *J. Insect Physiol.* **41**, 171–178.

Happ, G. M. 1992 Maturation of the male reproductive system and its endocrine regulation. *A. Rev. Entomol.* **37**, 303–320.

Hull, E. M., Lorrain, D. S., Du, J., Matuszewich, L., Lumley, L. A., Putnam, S. K. & Moses, J. 1999 Hormone–neurotransmitter interactions in the control of sexual behavior. *Behav. Brain Res.* **105**, 105–116.

Kingan, T. G., Thomas-Laemont, P. A. & Raina, A. K. 1993 Male accessory gland factors elicit change from 'virgin' to 'mated' behavior in the female corn earworm moth *Helicoverpa zea*. *J. Exp. Biol.* **183**, 61–76.

Kloppenburg, P., Ferns, D. & Mercer, A. R. 1999 Serotonin enhances central olfactory neuron responses to female sex pheromone in the male sphinx moth *Manduca sexta*. *J. Neurosci.* **19**, 8172–8181.

Leopold, R. A. 1976 The role of male accessory glands in insect reproduction. *A. Rev. Entomol.* **21**, 199–221.

Linn, C. E. & Roelofs, W. 1992 Role of photoperiod cues in regulating the modulatory action of octopamine on pheromone-reponse thresholds in the cabbage looper moth. *Arch. Insect Biochem. Physiol.* **20**, 285–302.

Lorrain, D. S., Riolo, J. V., Matuszewich, L. & Hull, E. M. 1999 Lateral hypothalamic serotonin inhibits nucleus accumbens dopamine: implications for sexual satiety. *J. Neurosci.* **19**, 7648–7652.

Picimbon, J. F., Gadenne, C., Bécard, J. M., Clément, J. L. & Sreng, L. 1997 Sex pheromone of the French black cutworm moth, *Agrotis ipsilon* (Lepidoptera: Noctuidae): identification and regulation of a multicomponent blend. *J. Chem. Ecol.* **23**, 211–230.

Poitout, S. & Buès, R. 1974 Elevage de plusieurs espèces de lépidoptères sur milieu artificiel simplifié. *A. Zool Ecol. Anim.* **2**, 79–91.

Raina, A. K., Klun, J. A. & Stadelbacher, E. A. 1986 Diel periodicity and effect of age and mating on female sex pheromone titer in *Heliothis zea* (Lepidoptera: Noctuidae). *A. Entomol. Soc. Am.* **79**, 128–131.

Raina, A. K., Bird, T. G. & Giebultowicz, J. M. 1990 Receptivity terminating factor in *Heliothis zea* and its interaction with PBAN. In *Insect neurochemistry and neurophysiology* (ed. A. B. Borkovec & E. P. Masler), pp. 299–302. Clifton, NJ: Humana Press.

Simmons, L. W., Teale, R. J., Maier, M., Standish, R. J., Bailey, W. J. & Withers, P. C. 1992 Some costs of reproduction for male bushcrickets, *Requena verticalis* (Orthoptera: Tettigoniidae): allocating resources to mate attraction and nuptial feeding. *Behav. Ecol. Sociobiol.* **31**, 57–62.

Sokal, R. R. & Rohlf, F. J. 1995 *Biometry.* New York: W. H. Freeman.

Svärd, L. & McNeil, J. N. 1994 Female benefit, male risk: polyandry in the true armyworm *Pseudaletia unipunta*. *Behav. Ecol. Sociobiol.* **35**, 319–326.

Swier, S. R., Rings, R. W. & Musick, G. J. 1976 Reproductive behavior of the black cutworm, *Agrotis ipsilon*. *A. Entomol. Soc. Am.* **69**, 546–550.

Tuttle, M. D. & Ryan, M. J. 1981 Bat predation and the evolution of frog vocalizations in the Neotropics. *Science* **214**, 677–678.