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THE PHYSIOLOGICAL BASIS FOR THE TERMINATION OF PHEROMONE-RELEASING BEHAVIOUR IN THE FEMALE BROWN-BANDED COCKROACH, *SUPELLA LONGIPALPA* (F.) (DICTYOPTERA: BLATTELLIDAE)

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Abstract—The factors contributing to the termination of calling (pheromone release) in adult female *Supella longipalpa* were examined. Calling ceases after mating and does not resume over the duration of the insect's life. Calling was suppressed and basal oöcyte growth was stimulated by the transient presence of a spermatophore, by the implantation of an artificial spermatophore, and by mating with vasectomized males. Calling resumed after oöthecal deposition in females mated with vasectomized males. Ventral nerve cord transections, either immediately following copulation or after oöthecal deposition, restored calling in mated females. The experimental evidence suggests that the termination of calling is mediated neurally by a two-stage process, initiated by placement of the spermatophore in the bursa copulatrix and maintained by the presence of sperm in the spermatheca.

Key Word Index: *Supella longipalpa;* calling behaviour; mating behaviour; spermatophore; spermatheca; nerve transection; pheromone release

INTRODUCTION

After copulation, females of many insect species exhibit a shift from virgin to mated behaviour. These behavioural changes include increased egg production and oviposition, changes in food preferences, loss of sexual receptivity, and termination of pheromone production and pheromone-releasing behaviours ("calling") (Sasaki and Riddiford, 1984; Webster and Cardé, 1984; reviews: Barth and Lester, 1973; Chen, 1984; Truman and Riddiford, 1974).

Mating factors that mediate the behavioural switch may be mechanical or chemical in nature, or a combination of the two. Presence of the spermatophore in the bursa, sperm or seminal fluid in the spermatheca, and/or eggs in the oviducts or brood sac have been reported as mechanical cues in the termination of virgin behaviour in the Orthoptera and Dictyoptera (Hartmann and Loher, 1974; Grillou, 1973; Loher and Huber, 1966; Stay and Gelperin, 1966; Roth, 1964; Roth and Stay, 1961) and in the Lepidoptera (Stringer *et al.*, 1985). Chemical factors associated with the sperm, with male accessory gland secretions, or with the bursa copulatrix may mediate the change to mated behaviour in the Diptera (review: Leopold, 1976; Chen, 1984), the Coleoptera (Boucher and Huignard, 1987), the Orthoptera (Friedel and Gillott, 1977; Stanley-Samuelson and Loher, 1986), and in the Lepidoptera (Riddiford and Aschenhurst, 1973; Yamaoka and Hirao, 1977; Stringer *et al.*, 1985). A combination of both chemical and neural factors has been proposed in mediating the switch to mated behaviour in two lepidopteran species (Karpenko and North, 1973; Stringer *et al.*, 1985) and possibly in the drosophilids (Manning, 1967; Leopold, 1976).

Among the cockroaches, female calling behaviour has been described in only a few species (see Schal and Bell, 1985; Schal and Smith, 1990). In *Supella longipalpa*, the behaviour is characterized by elevated wings, extended metathoracic legs, and a recurved abdomen (Hales and Breed, 1983). Calling is associated with the release of a volatile sex pheromone, it is initiated at an adult age of 6 days, and it ceases after mating (Smith and Schal, 1990).

In the present study, we present evidence that the termination of calling is mediated serially by a two-stage process initiated by the placement of the spermatophore in the bursa copulatrix, and maintained by the presence of sperm in the spermatheca. Moreover, experimental results from manipulations involving artificial spermatophores, vasectomies, and ventral nerve cord transections suggest that the effect is mediated through neural channels.

MATERIALS AND METHODS

Insects

Groups of late-instar nymphs were isolated from a colony and maintained at 27 ± 1°C under a 12 h light:12 h dark photoperiodic regime. Adults were collected within 24 h after the imaginal moult (day 0) and maintained in groups under the same light and temperature conditions. Dry dog food and water were provided *ad libitum*.

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Assays of calling

Calling was observed directly after experimental manipulation by placing females individually in Petri dishes and conducting observations during the scotophase in a temperature- and light-controlled room (27 ± 1°C; 12 h light:12 h dark). During the scotophase, observation was facilitated by illumination with low intensity, photographic darkroom red lights.

Dissections

All insects were immobilized on ice for 3 min and secured on a paraffin wax dish with plasticene. Specific surgeries and manipulations are discussed in the appropriate section of the results. Oöcyte dimensions were taken using an ocular micrometer.

RESULTS

Spermatophore removal

Although calling begins at a mean adult age of 6 days, the youngest age at which nearly all females will mate is 8 days (unpublished). Mated females ovulate and oviposit on days 12–13. Consequently, a window of only 4–5 days is available for observations after copulation before oöthecal extrusion interrupts calling. However, if a virgin female is permitted to deposit the first infertile oötheca, a period of about 12 days before the production of the next oötheca allows sufficient time for experimental manipulation and observation.

Three sets of calling, virgin females that had oviposited oöthecae 3 days earlier were obtained. Two sets were permitted to copulate during the next scotophase. The spermatophores were removed from 20 mated females within 4 min after the termination of copulation; 21 mated females and 21 virgin females were manipulated in the same manner without spermatophore removal. The insects were observed for 2 days, then dissected and their basal oöcytes were measured. To confirm that removal of the spermatophore 4 min after the termination of copulation precludes sperm transfer to the spermatheca, the spermatophores were removed from 10 mated females as described previously and the females were dissected 2 days later. No sperm were present in any of the spermathecae.

Of females from which the spermatophore was removed, 29% called during the following 2 days, compared with 100 and 5% of the virgin- and mated-control females, respectively (Table 1). Additionally, statistically significant oöcyte growth occurred 2 days after manipulation in mated-control females as well as in those females that experienced only the transient presence of the spermatophores.

Table 1. Effect of the transient presence of the spermatophore on the incidence of calling and on oöcyte development

Treatment	*n*	Females calling *n*	Females calling (%)	Basal oöcyte length* (mm) (X ± SE)
Mated + spermatophore removed†	20	1	(5)	2.30 ± 1.17 a
Mated control	21	6	(29)	1.89 ± 0.71 b
Virgin control	21	21	(100)	1.23 ± 0.54 c

*Means having no letter in common were significantly different at the 5% level (Tukey's studentized range test).
†Spermatophores were removed 4 min after the termination of copulation.

Implantation of artificial spermatophores

To test the hypothesis that the mechanical placement of the spermatophore mediates the termination of calling, 18 virgin females that had deposited oöthecae 24 h earlier, received implants of spermatophore-sized sand grains into the bursa copulatrix. Eleven sham-virgin females were manipulated in the same manner without receiving the artificial spermatophores. The insects were observed throughout the scotophase during the next 48 h, the maximal length of time that the spermatophore normally remains in the bursa copulatrix (unpublished), then dissected for basal oöcyte measurement.

As predicted, only 17% of the females with implanted artifical spermatophores called, compared with 100% of the sham-operated females (Table 2). Moreover, as for mated females (Table 1), artificial spermatohore implantation significantly stimulated basal oöcyte growth (Table 2).

Ventral nerve cord transections

To determine whether information from insertion of the spermatophore ascends via the ventral nerve cord, 8-day-old virgin females were permitted to copulate. A portion of the ventral nerve cord between the first and second abdominal sternites was removed 4 min after termination of copulation in 9 females. Sham-operated females ($n = 10$) served as controls. The females were observed for 7 days. Eight of 9 ventral nerve cord-transected females resumed calling 1–3 days (2.0 ± 0.73 days) after surgery, whereas none of the sham-operated control females called (Table 3).

Because the spermatophore remains in place for less than 48 h after copulation, it is unlikely that the spermatophore alone sustains the mated, noncalling behaviour. Presence of the sperm in the spermatheca may provide the continued stimulation to prevent the resumption of calling. Consequently, ventral nerve cord transection was performed 4 days after copulation, a period of time which allowed for the movement of sperm to the spermatheca, extrusion of the spermatophore, and deposition of a viable ootheca. Calling was restored in 9 out of 10 ventral nerve cord-transected females 2–5 days after surgery (3.8 ± 1.05 days), but not in the 10 sham-operated controls, suggesting that feedback information from the spermatheca ascends via the ventral nerve cord.

Table 2. Effect of artificial spermatophore implants on the incidence of calling and on oöcyte development

Treatment	*n*	Females calling *n*	Females calling (%)	Basal oöcyte length* (mm) (X ± SE)
Implanted females	18	3	(16.7)	1.58 ± 0.58
Sham females	11	11	(100)	1.22 ± 0.49

*Means of basal oöcyte lengths are significantly different at the 5% level (*t*-test).

Table 3. Effect of ventral nerve cord transection on the incidence of calling

Treatment	n	Females calling n	(%)
Post-copulation*	9	8	(89)
Sham-control	10	0	(0)
Post-oötheca†	10	9	(90)
Sham-control	10	0	(0)

*The ventral nerve cord was transected within 4 min after mating.
†The ventral nerve cord was transected within 24 h after oöthecal deposition.

Mating with vasectomized males

Castration of *S. longipalpa* males is a difficult procedure because the testes are diffuse within the abdomen. However, transection of the vas deferens can be achieved by cutting through the lateral margins of the 5th or 6th abdominal segments of 0-day adult males. After copulation with test females, examination of the spermathecae for the presence of sperm confirmed the success of the surgery. Vasectomized males produce a spermless spermatophore of dimensions (1.0 × 0.5 mm) identical to those of normal or sham-operated males (unpublished). Eight-day virgin females were permitted to copulate with vasectomized or sham-operated males. Each female was observed for 21 days.

Both control and experimental females ceased calling for a period of 3–5 days after which the first oöthecae were extruded and deposited (Table 4), demonstrating again that spermatophore insertion mediated the switch to mated, noncalling behaviour. There was no significant difference (t-test; $P > 0.05$) between the 2 groups in the number of days to oötheca production (Table 4), indicating that spermatophore insertion also stimulated oöcyte growth. Enforced virginity resulted in deposition of the first inviable oöthecae on day 12.87 ± 1.40 ($n = 40$). After deposition of the first oötheca, females that copulated with vasectomized males resumed calling, whereas control females did not (Table 4). Furthermore, during the 21-day observation period, control females produced oöthecae at a rate identical to that of normal, mated females (3.46 ± 0.79 oöthecae; $n = 15$); experimental females showed a rate of oöthecal production characteristic of normal, virgin females (1.60 ± 0.53 oöthecae; $n = 20$). Apparently, presence of sperm in the spermatheca was necessary to maintain the mated behaviour. Oothecae of both sets of females were observed for viability; only those from the control females bore nymphs.

DISCUSSION

Termination of calling in *S. longipalpa* is mediated by a serial process involving mechanical stimulation exerted by the spermatophore in the bursa copulatrix, and the presence of sperm in the spermatheca. The conclusion that the spermatophore acts mechanically through neural pathways is supported by the observation that calling is terminated by the brief presence of a spermatophore without sperm transfer to the spermatheca (Table 1), by implantation of artificial spermatophores (Table 2), and by ventral nerve cord transection immediately following copulation (Table 3). Copulations with vasectomized males which produce normal-sized spermatophores, resulted in the transient cessation of calling (Table 4), providing further evidence that the spermatophore serves as the initial mechanical stimulus in the switch to mated behaviour. Because vasectomized males produce normal-sized spermatophores, accessory gland substances appear to play no role in the termination of calling. Moreover, implantations of spermatophores and sperm into the abdomen have no effect on calling in virgin females (data not shown).

A number of parallels exist between the factors that regulate the termination of sexual receptivity in *Nauphoeta cinerea* (Roth, 1964) and those that regulate the termination of calling in *S. longipalpa*. In *N. cinerea*, sexual receptivity, defined as female palpatation of the male tergum, ceases after mating and, as in *S. longipalpa*, the effect may be imitated in virgin females through the implantation of artificial spermatophores or by mating with castrated males. Similarly, a return to virgin behaviour (calling and sexual receptivity in *S. longipalpa* and *N. cinerea*, respectively) is observed after ventral nerve cord transection immediately following copulation. Both species exhibit a switchover to mated behaviour (termination of calling and of sexual receptivity, respectively) and accelerated oöcyte development when the spermatophore is permitted to remain in place for only minutes following copulation. Moreover, when *S. longipalpa* females are mated with vasectomized males, oöthecal production occurs after an interval of time identical to that of normally mated females, and calling ceases over that pre-ovipositional period. Hence, it appears that in *S. longipalpa* and *N. cinerea*, the initial termination of calling and sexual receptivity, respectively, is mediated by the placement of the spermatophore into the bursa, which exerts its influence through ascending neural channels.

The mechanical stimulation by the spermatophore in effecting the change from virgin to mated behaviour has been reported for other insects. In the moth *Manduca sexta*, mechanical distension of the bursa copulatrix induces the switch to mated ovipositional behaviour (Stringer *et al.*, 1985) and calling ceases for 1–2 days following copulation with castrated males (Sasaki and Riddiford, 1984). In the cockroach *Diploptera punctata*, oöcyte development characteristic of mated females is triggered by the mechanical presence of the spermatophore in the

Table 4. Effect of mating to vasectomized males on the incidence of calling and oöthecal production

Treatment	Call before 1st oöthecae n	Call after 1st oöthecae n	Days after mating to 1st oöthecae X ± SE	Days between oöthecae X ± SE	Number of oöthecae in 21 days X ± SE
Mated to vasectomized males	0/10	10/10	3.60 ± 0.97 a	11.89 ± 1.24 a	1.90 ± 0.75 a
Mated to sham-operated males	0/10	0/10	3.56 ± 0.73 a	6.11 ± 0.78 b	3.44 ± 0.73 b

Means within a column having no letter in common are significantly different at the 5% level (t-test).

bursa copulatrix (Roth and Stay, 1961). In females of the grasshopper *Gomphocerus rufus*, maintenance of sexual unreceptivity for 6–10 days between successive copulations is mediated neurally by stimuli arising from the presence of the spermatophore (Loher and Huber, 1966). In the mosquito *Aedes aegypti*, the initial loss of sexual receptivity is mediated by the mechanical distension of the bursa copulatrix from filling with sperm and seminal fluid (Gwadz, 1972). Thus, the termination of calling or sexual receptivity may be initially mediated by the presence of the spermatophore or seminal fluid in the bursa copulatrix in insect species representing several orders.

Because the spermatophore has only a transient effect, maintenance of mated behaviour requires an additional factor. In ovoviviparous species, distension of the brood sac by the internally incubated egg case can serve as the stimulus in maintaining sexual unreceptivity in mated females (e.g. *N. cinerea*, Roth, 1964). In the oviparous *S. longipalpa*, a sperm-bearing spermatheca is necessary for maintaining mated, noncalling behaviour. Females that mated to vasectomized males resumed calling after oöthecal deposition and produced subsequent oöthecae at intervals characteristic of virgin females (Table 4). Moreover, females with sperm-bearing spermathecae resumed calling after ventral nerve cord transection (Table 3) indicating that mated behaviour is maintained by the presence of sperm in the spermatheca which is communicated via ascending neurones of the ventral nerve cord.

Unmated females of the bisexual strain of *Pycnoscelus surinamensis* (=*P. indicus*) abort the extruded egg case after ovulation, whereas mated females retract and internally incubate the egg case in the brood sac. By conducting spermathecal excisions, spermathecal duct and ventral nerve cord transections, spermathecal denervations, and matings to castrated males, Stay and Gelperin (1966) demonstrated that mated ovipositional behaviour in *P. surinamensis* requires the presence of sperm in the spermatheca. As in *S. longipalpa*, the sperm-laden spermatheca exerts its influence through the nervous system. Similarly, when *Trichoplusia ni* females are mated to castrated or irradiated males, in which only apyrene sperm and subnormal quantities of accessory gland fluids are produced, virgin ovipositional behaviour is maintained (Karpenko and North, 1973). Apparently, the presence of viable sperm and male accessory gland secretions is necessary to mediate the switch from virgin to mated behaviour, and to sustain the latter.

An intact ventral nerve cord is required for the switchover to mated behaviour in *S. longipalpa*, but input from higher nervous centres to the terminal abdominal ganglia is not needed for the expression of calling. This is radically different from the situation in most lepidopterans, where ventral nerve cord-transected females do not call normally (Sasaki and Riddiford, 1984; Itakagi and Conner, 1986, 1987; Tang *et al.*, 1987). It is unknown, however, whether this difference is due to a more anterior location of pheromone glands in the cockroach. In *S. longipalpa* pheromone production appears to involve abdominal tergites (unpublished), whereas moth glands are usually associated with the ovipositor.

Signals ascending through the ventral nerve cord arrive at the target site (e.g. anterior regions of the central nervous system) and trigger the events that terminate calling behaviour in a yet unknown manner. One model may involve the neural inhibition of a motor pattern residing in the brain or anterior ganglia. An alternate scenario may involve tropic neurosecretory factors which are released during the scotophase in mature virgin females to elicit calling, but the release of which is inhibited by neural signals arising from spermatophore insertion and sperm-distention of the spermatheca. Based on removal and reimplantation of the corpora allata, and administration of juvenile hormone to virgin female *Byrostria fumigata*, Bell and Barth (1970) suggested that high titres of juvenile hormone associated with mating may mediate the switch to mated behaviour. However, in *S. longipalpa*, corpora allata from virgin females attain similar rates of juvenile hormone release *in vitro* as do corpora allata from mated females (Smith *et al.*, 1989). Moreover, intact virgin females continue to call when corpora allata reach their peak release rates *in vitro*. Studies entailing the administration of nervous tissue and corpora cardiaca extracts and the ablation of regions of the brain and other ganglia should further elucidate the mechanisms controlling the termination of calling in the brown-banded cockroach.

The results presented in this study indicate that, in addition to terminating calling behaviour, mating stimulates oöcyte development. Smith *et al.* (1989) also showed that activation of the corpora allata to peak synthesis rates *in vitro* (and subsequent ovulation) is faster in mated than in virgin *S. longipalpa*. However, Gadot *et al.* (1989) showed that corpus allatum activity *in vitro*, the rate of oöcyte development, and oviposition were faster in grouped than in individually isolated *Blattella germanica* females. This grouping effect, and an unintended selection through mating of females that exhibit faster oöcyte maturation than females that refuse to mate, largely accounted for differences that were previously attributed to mating. However, recent data from our laboratory indicate that grouping has no effect on oöcyte maturation in *S. longipalpa*, and that females grouped with normal males (i.e. grouped mated females) exhibit significantly faster oöcyte development than females grouped with phallomerectomized males (i.e. grouped virgin females).

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REFERENCES

Barth R. H. and Lester L. J. (1973) Neuro-hormonal control of sexual behaviour in insects. *Ann. Rev. Entomol.* **18,** 445–472.

Bell W. J. and Barth R. H. (1970) Quantitative effects of juvenile hormone on reproduction in the cockroach *Byrsotria fumigata*. *J. Insect Physiol.* **16,** 2303–2313.

Boucher L. and Huignard J. (1987) Transfer of male secretions from the spermatophore to the female insect in *Caryedon serratus* (Ol.): analysis of the possible trophic role of these secretions. *J. Insect Physiol.* **33,** 949–957.

Chen P. S. (1984) The functional morphology and biochemistry of insect male accessory glands and their secretions. *Ann. Rev. Entomol.* **29,** 233–255.

Friedel T. and Gillott C. (1977) Contribution of male produced proteins to vitellogenesis in *Melanoplus sanguinipes*. *J. Insect Physiol.* **3,** 145–151.

Gadot M., Burns E. and Schal C. (1989) Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica:* effects of grouping and mating. *Archs Insect Biochem. Physiol.* **11,** 189–200.

Grillou H. (1973) A study of sexual receptivity in *Blabera craniifer* Burm. (Blattaria). *J. Insect Physiol.* **19,** 173–193.

Gwadz R. W. (1972) Neuro-hormonal regulation of sexual receptivity in female *Aedes aegypti*. *J. Insect Physiol.* **18,** 259–266.

Hales R. A. and Breed M. D. (1983) Female calling and reproductive behavior in the brown-banded cockroach, *Supella longipalpa* (F.) (Orthoptera: Blattellidae). *Ann. Entomol. Soc. Am.* **76,** 239–241.

Hartmann R. and Loher W. (1974) Control of sexual behaviour pattern 'secondary defence' in the female grasshopper, *Chorthippus curtipennis*. *J. Insect Physiol.* **20,** 1713–1728.

Itakagi H. and Conner W. E. (1986) Physiological control of pheromone release behaviour in *Manduca sexta* (L.) *J. Insect Physiol.* **32,** 657–664.

Itakagi H. and Conner W. E. (1987) Neural control of rhythmic pheromone gland exposure in *Utetheisa ornatrix* (Lepidoptera: Arctiidae). *J. Insect Physiol.* **33,** 177–181.

Karpenko C. P. and North D. T. (1973) Ovipositional response elicited by normal, irradiated, F_1 male progeny, or castrated male *Trichoplusia ni* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* **66,** 1278–1280.

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

Loher W. and Huber F. (1966) Nervous and endocrine control of sexual behaviour in a grasshopper (*Gomphocerus rufus* L.) *Symp. Soc. exp. Biol.* **20,** 381–400.

Manning A. (1967) The control of sexual receptivity in female *Drosophila*. *Anim. Behav.* **15,** 239–250.

Riddiford L. M. and Ashenhurst J. B. (1973) The switchover from virgin to mated behaviour in female cecropia moths: the role of the bursa copulatrix. *Biol. Bull.* **144,** 162–171.

Roth L. M. (1964) Control of reproduction in female cockroaches with special reference to *Nauphoeta cinerea*—I. First pre-oviposition period. *J. Insect Physiol.* **10,** 915–945.

Roth L. M. and Stay B. (1961) Oocyte development in *Diploptera punctata* (Eschscholtz) (Blattaria). *J. Insect Physiol.* **7,** 186–202.

Sasaki M. and Riddiford L. M. (1984) Regulation of reproductive behaviour and egg maturation in the tobacco hawk moth, *Manduca sexta*. *Physiol. Entomol.* **9,** 315–327.

Schal C. and Bell W. J. (1985) Calling behavior in female cockroaches (Dictyoptera: Blattaria). *J. Kan. Entomol. Soc.* **58,** 261–268.

Schal C. and Smith A. F. (1990) Neuroendocrine regulation of pheromone production in cockroaches. In *Cockroaches as Models for Neurobiology: Applications in Biomedical Research* (Edited by Huber I., Masler E. P. and Rao B. R.), Vol. 2, pp. 179–200. CRC Press, Roca Raton, Fla.

Smith A. F. and Schal C. (1990) Corpus allatum control of sex pheromone production and calling in the female brown-banded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). *J. Insect Physiol.* **36,** 251–257.

Smith A. F., Yagi K., Tobe S. S. and Scal C. (1989) *In vitro* juvenile hormone biosynthesis in adult virgin and mated female brown-banded cockroaches. *Supella longipalpa*. *J. Insect Physiol.* **35,** 781–785.

Stanley-Samuelson D. W. and Loher W. (1986) Prostaglandins in insect reproduction. *Ann. Entomol. Soc. Am.* **79,** 841–853.

Stay B. and Gelperin A. (1966) Physiological basis of ovipositional behaviour in the false ovoviviparous cockroach, *Pycnoscelus surinamensis* (L.). *J. Insect Physiol.* **12,** 1217–1226.

Stringer I. A. N., Giebultowicz J. M. and Riddiford L. M. (1985) Role of the bursa copulatrix in egg maturation and reproductive behaviour of the tobacco hawk moth, *Manduca sexta*. *Int. J. Invert. Reprod. Develop.* **8,** 83–91.

Tang J. D., Charlton R. E., Cardé R. T. and Yin C.-M. (1987) Effect of allatectomy and ventral nerve cord transection on calling, pheromone emission and pheromone production in *Lymantria dispar*. *J. Insect Physiol.* **33,** 469–476.

Truman J. W. and Riddiford L. M. (1974) Hormonal mechanisms underlying insect behavior. *Adv. Insect Physiol.* **10,** 297–352.

Webster R. P. and Cardé R. T. (1984) The effects of mating, exogenous juvenile hormone and a juvenile hormone analogue on pheromone titre, calling, and oviposition in the omnivorous leafroller moth (*Platynota stultana*). *J. Insect Physiol.* **30,** 113–118.

Yamaoka K. and Hirao T. (1977) Stimulation of virginal oviposition by male factor and its effect on spontaneous nervous activity in *Bombyx mori*. *J. Insect Physiol.* **23,** 57–63.