Sex Pheromone of the Mealworm Beetle (*Tenebrio molitor*)

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ABSTRACT Sex pheromone activity from female mealworm beetles (*Tenebrio molitor*) may be extracted and quantified by fractional population response of virgin adult males, which attempt copulation with any pheromone-treated object. Extracts of mature males can be shown to elicit identical behavioral activity, although at lower individual concentration. Since response to the pheromone is sex-specific, its apparent production by both sexes of *T. molitor* is especially noteworthy. It has been shown that the presence of pheromone in males cannot be due to contamination by females.

Both pheromone production and the male response are undetectable in newly-emerged adults; both rise to their maximum extent within one week of eclosion.

In a number of insects the odor of a sex pheromone, with few or no co-stimuli, is sufficient to initiate the species-typical mating behavior pattern. Biological assays based on such behavior patterns may be utilized for behavioral studies and for the isolation and characterization of the pheromone. Sex pheromones have been isolated from a number of insects, primarily Lepidoptera, and an extensive review of the subject has recently appeared (Jacobson, '65).

An early study by Valentine ('31) suggested the presence of a chemical sex attractant in *Tenebrio molitor* L. We wish to report the development of a quantitative biological assay for this pheromone, and to discuss some points of interest in its natural history.

MATERIALS AND METHODS

The *T. molitor* colony was maintained on wheat bran in a chest of screen-topped drawers, from which pupae were collected and carefully separated according to sex (Konok, '55). The emerging adults were kept in two cabinets, one reserved exclusively for males, the other for females. Both cabinets were kept at room temperature, in darkness, and under continuous positive internal air pressure, so that no outside odors could enter. One drawer of the male cabinet was reserved for the group of 300–600 males routinely used for the bioassay. This group was sorted at frequent intervals to eliminate all but vigorous individuals in good physical condition.

The assays were carried out on a surface of clean brown wrapping paper in a darkroom under red light, since the beetles are strongly photonegative to most other wavelengths. For each test point, a random sample of 20 beetles was taken from the tray of test males, and each beetle was placed under an individual 100 mm petri dish cover. About five minutes were allowed for the beetles to become accustomed to their surroundings; during this period they characteristically engaged in intense exploratory behavior. Short pieces of glass rod, 3 mm in diameter and fire-polished at both ends, were washed in chromic acid and kept in ethanol until needed. They were then wiped clean, dipped in the test solution to a uniform depth, and placed on a small rack. Exactly 30 seconds after each rod was dipped, it was placed under a petri dish cover containing a test beetle. The dipping was carried out at a regular rate, keeping all time intervals constant.

The time elapsed since introduction of the rod was noted as soon as a beetle responded positively by attempting to copulate with the rod. A copulation attempt was minimally defined as a bending of the tip of the abdomen downward and anteriorly around the end of the rod, as shown in figure 1b. After 25 minutes, any beetle not having responded was re-

corded as a negative; this introduces very little error, since only a small proportion of positive responses was ever observed after 25 minutes of exposure. No beetle was used more than once during a 24-hour period.

RESULTS

Male response. The sex pheromone of mature whole females is easily extractable in ethanol. A glass rod dipped in this extract will elicit a typical mating response (cf. Materials and Methods): the male beetle approaches the rod, examines it with his antennae, mounts the rod and bends its abdomen until it contacts the end of the rod, while the antennae and forelegs beat a rapid tattoo along the sides. This behavior is indistinguishable from the initial stages of copulation with a female (fig. 1a–b). Normal copulation with mature females usually lasts from 2 to 6 minutes; attempts to copulate with a treated glass rod have been observed to continue for as long as two hours. Presentation of a glass rod dipped only in ethanol never elicited a male response.

The strength of a female extract is scored for the proportion of copulation responses (toward treated glass rods) elicited within one or more groups of 20 virgin males, tested individually. When a series of dilutions are scored and the proportions of responding males are plotted (on a probit scale) against the log of the dilution factor, the points yield a straight line, indicating a normal distribution of response thresholds (fig. 2). The concentration of pheromone giving a 50% response in the males has been assigned an arbitrary value of one unit per milliliter.

This bioassay for the *T. molitor* pheromone is similar in principle to those reported for the pheromones of the lepidopterans *Bombyx mori* (Butenandt, Beckmann and Hecker, '61) and *Trichoplusia ni* (Noctuidae) (Shorey, Gaston and Fukuto, '64); all three are assays of the "quantal" type, based on the fraction of a test population showing a defined response. However, there are differences both in effective concentration range and in sensitivity to concentration differences. The concentration of bombykol has been determined reproducibly within a power of ten, whereas the *T. molitor* assay reliably detects differences well under a factor of two, and the *T. ni* assay appears to be only slightly less sensitive. The effective range of the *B. mori* assay was not reported, but is presumably much greater than the 100-fold range of concentration over which the *T. ni* assay is effective. In contrast the *Tenebrio* assay reported here has a range of 20- to 30-fold variation in pheromone concentration, resulting in a steeper slope of

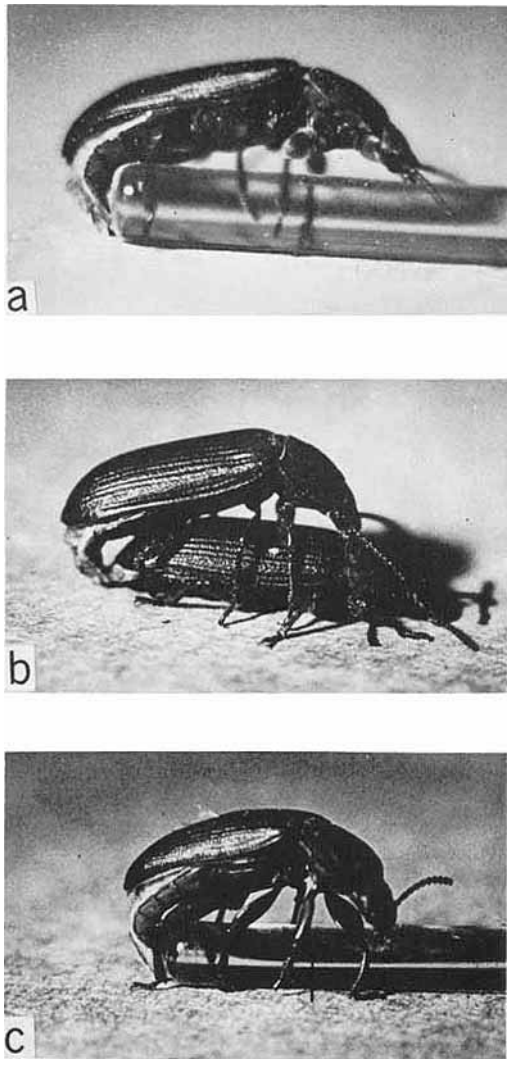

Fig. 1 Response of male *Tenebrio molitor.* (a) to a glass rod treated with female pheromone extract. (b) to the live female of *Tenebrio molitor.* (c) to a glass rod treated with male pheromone extract.

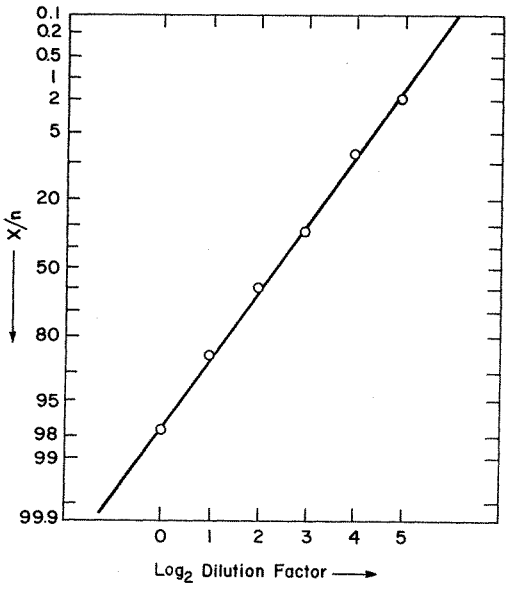

Fig. 2 Biological assay for the female sex pheromone of *Tenebrio molitor*. Probit of the fraction of males responding is plotted against the relative concentration.

the assay curve, and a correspondingly increased sensitivity to concentration differences.

Stability and chemical characteristics. The pheromone is only slightly soluble in water; it may be completely extracted from neutral or weakly acidic aqueous solution by most organic solvents. Contact with solutions of sodium hydroxide results in a rapid loss of activity, which is not regained on subsequent acidification. Methylene chloride extracts of the pheromone are stable at room temperature (22°C), whereas ethanol extracts slowly lose activity under these conditions, dropping to about 30% of their original value in two weeks. The pheromone is stable to reflux temperatures (65°C) in tetrahydrofuran for at least 16 hours. On a glass rod, it has a low volatility; a 24-hour exposure to open air results in a barely detectable drop in activity. This would be consistent with the observation (Cotton and St. George, '29) that *T. molitor* females mate repeatedly throughout adult life with no obvious behavioral cycles.

Physiological development: Secretion of pheromone. The level of extractable pheromone rises from nearly zero (almost undetectable) in newly eclosed females to the mature level of somewhat more than one unit/female after only one week (fig. 3). Dissection of the females showed that the pheromone reached the mature level at about the same time that the first ova are released from the ovarioles into the oviducts.

Attempts to locate the organ(s) or cells secreting the pheromone have indicated the region of the metathoracic sternum and first two abdominal terga. Using the method of extracting and assaying pieces of female beetles, it has not been possible to determine the area more precisely. Only young females which have just begun to secrete pheromone are suitable for localization experiments, because the pheromone does not remain in the vicinity of the source, but gradually spreads out over most of the surface of the beetle.

It is not uncommon to observe males attempting copulation with other males, even in all-male colonies, and the same phenomenon has been noted among other tenebrionid beetles: *Tribolium castaneum* and *T. confusum* (Sokoloff, personal com-

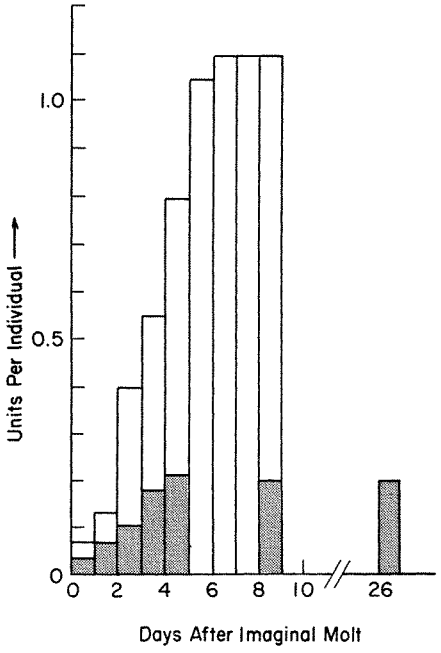

Fig. 3 Levels of ethanol-extractable sex pheromone in immature *T. molitor* adults. Females: open bars; males: solid bars.

munication); *Zophobas rugipes* (Tschinkel, unpublished). These observations prompted us to prepare and test extracts of male *T. molitor*. When whole males (from strictly all-male colonies) are extracted in ethanol, and the extract tested on other males, the latter attempt copulation with the glass rod in a manner indisinguishable from that toward rods treated with female extract (fig. 1c). Plotted as a function of time following imaginal molt, and in the same units as those used for the female extracts, the production of pheromone *per* individual male is seen to attain a maximum after about five days (fig. 3). The shape of the curve roughly parallels the rise in pheromone concentration *per* individual female over the same period of time, but the average level of pheromone *per* male at the mature level is only about 18% of that *per* female

DISCUSSION

Tenebrio molitor is, to our knowledge, the first case in which a sex pheromone is unquestionably secreted by both sexes but appears to act only on the male. Rogoff et al. ('64) have reported that a sex pheromone could be extracted from both male and female houseflies (*Musca domestica*). However, only one of their preparations was derived from virgin adult males, and its activity was extremely low. The authors suggest that the considerably higher activities found in non-virgin male extracts might therefore be due to contamination. The method of rearing *T. molitor* in the present instance allows dismissal of the contamination hypothesis.

Pheromones may be sex-specific in secretion, effect, or both, and it is possible to construct a table of all possible combinations of specificity and effects (table 1).

Secretion of sex pheromone by the sexes of a given species is therefore not necessarily mutually exclusive, but may be only a quantitative predominance of one sex over the other as in the case of *T. molitor* (class III). This implies the existence of a spectrum of ratios of secretory predominance, ranging from the all-and-none situation (classes I and II) to an approximately equal secretion by both sexes (possibly most of class IX).

Sex pheromones most frequently act as attractants but the same compounds may have additional functions, such as sexual recognition or stimulation, or even effects other than the release of a behavior pattern (cf. Loher, '60). A pheromone might act as an attractant at low concentrations, but become primarily an excitant at high concentrations, releasing typical mating behavior patterns in the affected sex (Wilson and Bossert, '63). Attractants which have little function as sex-excitants have also been reported (Bornemissza, '64). In

TABLE 1

Classification of pheromones by sex specificity

Class	Secreted by	Sex affected	Illustrative examples	Reference
I	♀	♂	Numerous	
II	♂	♀	*Anthonomus grandis*	(Keller, '64)
			Harpobittacus australis	(Bornemissza, '64)
			Harpobittacus nigriceps	
III	both	♂	*Tenebrio molitor*	(Valentine, '31)
IV	both	♀	None reported	
V	♀	both	*Dendroctonus pseudotsugae*	(Rudinsky, '63)
VI	♂	both	*Ips confusus*	(Wood, '62)
			Lycus loripes	(Eisner and Kafotos, '62)
VII	♂	♂	None reported	
VIII	♀	♀	Most hymenopteran social pheromones	
IX	both	both	Numerous	

such cases the release of mating behavior patterns must be due to other stimuli, possibly to other pheromones.

In this regard we have found the sex pheromone of *Tenebrio molitor* to be effective as a male attractant only over a few centimeters, and to act primarily as a sex excitant of the male. It is apparently not the body form of the females alone which releases the copulatory response in the male. Thus, males do not copulate with female corpses from which the pheromone has been extracted, but will do so if the leached female corpse has first been daubed with a pheromone extract, or if pheromone is present within 2–3 cm of the corpse. Since any treatment so far devised which removes the pheromone is also lethal to the beetle, it was not possible to study the role of female posture or motion in the relative absence of the pheromone except in the case of young females, before these had produced an adult level of extractable pheromone. Males did not copulate either with young females or young males (0–1 days old) although they frequently attempted copulation with other mature males. Furthermore, the vague resemblance of the glass rod to the female body form is not necessary for excitation, since the same pheromone extracts on the surface of a cover slip will elicit the unmistakable "tattoo" behavior characteristic of male sexual excitement.

These observations, then, suggest that the pheromone is a weak attactant, and that its major role is sexual excitation of the male. It appears to be the principal, if not the only stimulus for mating behavior, and as such might also be considered a female recognition substance, despite its apparent production in smaller amounts by the male.

Attempts to purify this pheromone are currently being undertaken in our laboratory and also by G. M. Happ (personal communication) at the Catholic University of America.

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