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EFFECTS OF MALE AND FEMALE SCENT ON REPRODUCTIVE MATURATION IN YOUNG FEMALE *TENEBRIO MOLITOR*

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Abstract—In the first 7–10 days after emergence from the pupal cuticle, oöcyte length and emission of sex attractant increase in female mealworm beetles. Shortly after ecdysis, females were isolated and exposed to one of three air-streams containing: (1) unscented air, (2) scent of mature males, and (3) scent of mature females. Within 3 days, females exposed to male scent exhibited greater growth of their terminal oöcytes and a higher level of sex pheromone emission than either of the other two groups. At 3 days, females exposed to female scent emitted significantly more sex attractant than those females in unscented air, but only at 7 days were significant effects of female scent reflected in oöcyte growth of isolated females. Apparently both male and female scents contain primer pheromones.

INTRODUCTION

WHEN in the vicinity of the opposite sex, it is of some advantage for an organism to be reproductively mature. In some species of insects, population density may influence the rate of maturation of individual members and thus may promote reproductive synchrony. The stimuli involved in these interactions are best understood for the desert locust, *Schistocerca gregaria*. Pheromones, produced by mature male locusts, accelerate sexual maturation in young adults (LOHER, 1961; HIGHNAM and LUSIS, 1962). In female mealworm beetles, *Tenebrio molitor*, both crowding and mating increase the rate of reproductive maturation. Oöcyte growth is more rapid in crowded females and in mated females, and the two influences are additive (MORDUE, 1965). Sex attractant is emitted at a higher titre by crowded virgins than by isolated ones (HAPP and WHEELER, 1969). It was the purpose of the present study to determine whether pheromones are responsible for these group effects in female *Tenebrio*.

PROCEDURE AND RESULTS

Tenebrio molitor were obtained from a stock culture and sexed in the pupal stage. Upon emergence from the pupal cuticle, each young female was placed in a 2 oz. glass jar, supplied with food and moisture (potato), and randomly assigned

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to one of three experimental groups. Series M females were exposed to the scent of 100 mature males; series F, to that of 100 mature females; and series C, the controls, to ambient laboratory air. For each group, air was pulled by water vacuum through a desiccator containing oatmeal and slices of potato (and the appropriate beetles for the M and F series) and then, via a system of rubber and glass tubing, through jars containing the isolated females. The isolated females were in parallel to one another; they were exposed only to the airstream from the desiccator and could not smell each other. In any set of experiments, all series were run simultaneously and thus all beetles were exposed to the same scent contaminants in the laboratory air.

Each group contained at least 7 females. After appropriate periods of exposure to the scent from the desiccators, the young females were removed from the airstream and the three groups compared in three respects: sex attractant emission, metabolic rate, and ovarian development.

Emission of sex attractant

Each young female was tested for attractiveness by bioassay on male beetles. For this bioassay procedure, 10 males were confined in a small Lucite chamber. While unscented air passed through the chamber, the males aggregated near the outflow end. When the airstream contained female scent, the males moved upwind and attempted to mount one another. The percentage of males responding varied with the potency of the scent (details in HAPP and WHEELER, 1969).

Prior exposure to the scent of mature beetles enhanced sex attractant release in the young females. Both male and female scent produced this effect, and the influence of male scent was most dramatic. At 3 and at 7 days after ecdysis all three series were significantly different from one another (Table 1).

TABLE 1—PERCENTAGE OF MALES RESPONDING TO YOUNG FEMALES PREVIOUSLY EXPOSED TO MALE SCENT, FEMALE SCENT, OR AMBIENT AIR

Age of females (after emergence) (days)	M series	F series	C series
3		$\chi^2 = 44{\cdot}20,\ P < 0{\cdot}005$	
	79·7%	56·2%	43·1%
		$\chi^2 = 20{\cdot}14,\ P < 0{\cdot}005$	$\chi^2 = 5{\cdot}51,\ P < 0{\cdot}025$
7		$\chi^2 = 22{\cdot}38,\ P < 0{\cdot}005$	
	85·7%	70·4%	56·3%
		$\chi^2 = 5{\cdot}02,\ P < 0{\cdot}025$	$\chi^2 = 5{\cdot}82,\ P < 0{\cdot}025$

Metabolic rate

The metabolic rate of each young female was measured by Warburg respirometry (25°C, 2 hr). As shown in Table 2, there was no simple relationship between exposure to the scents and oxygen consumption at 3, 5, or 7 days. The only pattern which emerged from the data was a negative correlation ($R = -0{\cdot}873$) between the number of full-term oöcytes and metabolic rate, i.e. when most ovarioles have completed the formation of the first oöcyte, the metabolic rate of these isolated virgins declines.

TABLE 2—RATE OF OXYGEN CONSUMPTION OF YOUNG FEMALES PREVIOUSLY EXPOSED TO MALE SCENT, FEMALE SCENT, OR AMBIENT AIR

Age of females (after emergence) (days)	μl O_2/mg per hr		
	M series	F series	C series
3	0·345 ± 0·0165	0·340 ± 0·009	0·313 ± 0·010
5	0·365 ± 0·0167	0·331 ± 0·034	0·427 ± 0·034
7	0·276 ± 0·055	0·291 ± 0·046	0·318 ± 0·058

Oöcyte growth

The extent of ovarian development was measured by combining the lengths of terminal oöcytes and ripe eggs in the left ovary (MORDUE, 1965). The scent of mature beetles accelerated ovarian growth (Fig. 1). At 3 days after emergence the terminal oöcytes of females of the M series were significantly larger ($P < 0{\cdot}01$) than those of the other two series, whereas the F and C series were indistinguishable. By 4 days, a few ripe eggs had already appeared in the sac of the lateral oviduct of females in the M series. At 5 days, oöcyte length in the M series was greater than in the controls ($P < 0{\cdot}01$) while beetles exposed to female scent were intermediate and were not significantly different from either. By 7 days, most females in both the M and F series had produced ripe eggs, but oöcyte length in the controls was only slightly greater than at 5 days.

DISCUSSION

On the basis of their mode of action, WILSON and BOSSERT (1963) have suggested that pheromones fall into two broad categories: the releasers and the primers. Releaser pheromones act primarily at the behavioural level by producing an immediate and reversible change in the behaviour of the recipient. In contrast, primer pheromones induce a more prolonged shift in the physiology of the recipient.

Tenebrio produces pheromones with at least three releaser effects on mating and oviposition (Fig. 2). The scent of adult females attracts and sexually excites the males (VALENTINE, 1931; TSCHINKEL *et al.*, 1967; HAPP and WHEELER, 1969). Male scent is attractive to females and releases oviposition behaviour (HAPP, 1969). Both of these pheromones act only on the opposite sex. The third releaser action

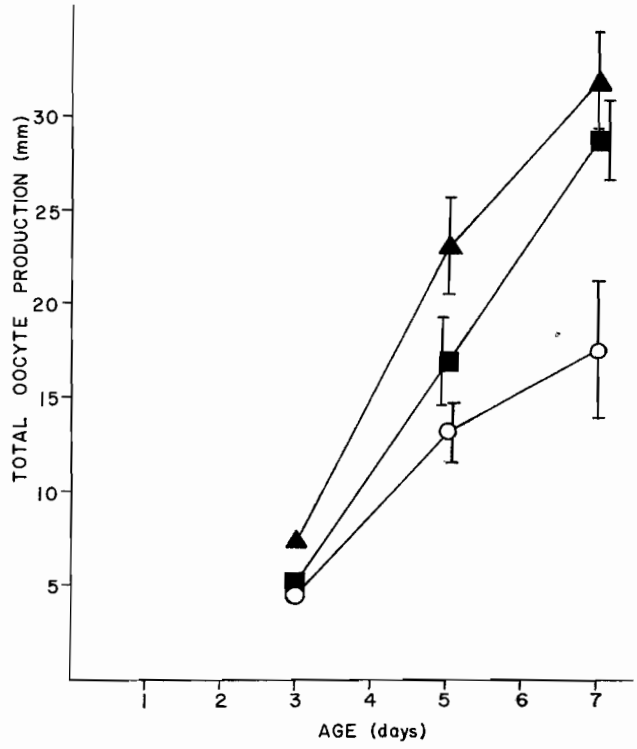

FIG. 1. Rate of oöcyte production in isolated females exposed to male scent, ▲; female scent, ■; ambient air, ○.

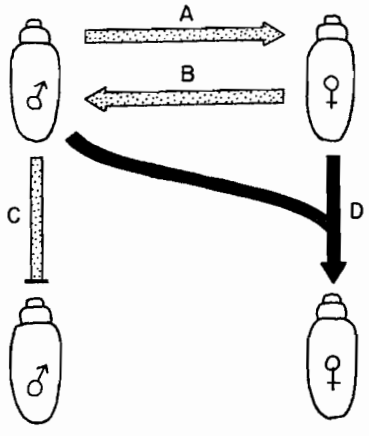

FIG. 2. Tentative representation of pheromones acting between male and female *Tenebrio molitor*. (A) Releaser produced by the male which attracts the female. (B) Releaser produced by the female which attracts and excites the male. (C) Releaser produced by the male which inhibits the response of other males to B. (D) Primers produced by both sexes which accelerate reproductive maturation in young females.

is antiaphrodisiac; male scent may inhibit the response of other males to the attractant produced by females. This inhibitory pheromone is emitted only in the presence of female scent, and some is transferred to the female during mating (HAPP, 1969). In addition to these releasers, the present study has shown that the scents of mature males and females have primer activity toward young virgin females. The site of pheromone synthesis in these beetles is not clear at present, but perhaps the sternal pit glands, which have been shown to be sexually dimorphic, are responsible for pheromone production (WIGGLESWORTH, 1948).

We do not know how many different molecules with pheromone activity are regulating reproduction in *Tenebrio*. The three releasers are distinct in their action and may be three separate molecules. On the other hand, two molecules could account for the releaser effects if one assumes that there is a pronounced increase in the emission of male pheromone when the males are exposed to female scent. Several possibilities are raised by the demonstration of primer activity in male and female scents. The fact that male scent was the more potent may suggest that the two sexes produce distinct primers of unequal potency. A simpler alternative is that one primer is emitted at differing rates in males and females. It is also conceivable that one or both of the attractants described earlier act as primers. Final resolution of these questions must await chemical identification of the pheromones involved.

Both oöcyte growth and emission of sex attractant are enhanced by the primer. Previous evidence indicates that these two phenomena are controlled by separate physiological regulatory mechanisms. MORDUE (1965) reported that the rate of oöcyte growth increased with crowding and mating and that eventually more ripe eggs were produced by crowded-mated females than by any other experimental group. In contrast, while isolated virgins produced the lowest titres of sex attractant and crowded virgins released much higher titres, the two parallel mated groups (crowded and isolated) were very similar to each other and fell in between these extremes (HAPP and WHEELER, 1969). In the case of oöcyte length, the measurement is a cumulative outcome of all the rates of growth over several days up to the point at which the beetle is dissected. The test for attractiveness towards males gives essentially an instantaneous rate, for it measures emission only during a test period of 5 min. Although these two aspects of reproductive physiology in the female appear to vary independently, the primer acts similarly on both—to increase the rate of the process. It might be added that the percentage of males responding to C series females is similar to that for isolated females and the response to F series females is similar to crowded virgin females described previously (HAPP and WHEELER, 1969). However, the M series females excited as many as 86 per cent of the males at 7 days. This level of pheromone emission has never been previously found in live females, and these data emphasize the very marked accelerating effect of male scent.

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