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DEFENSIVE AND PHEROMONAL SECRETION OF THE TERGAL GLAND OF *Aleochara curtula*[1] II. Release and Inhibition of Male Copulatory Behavior

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Abstract—At high concentrations, the defensive tergal gland secretion (TGS)[3] of the staphylinid beetle, *Aleochara curtula*, inhibits the male copulatory response (grasping with parameres). Inhibitory chemicals, for which a function as alarm substances is assumed, are *n*-undecane, 1-undecene, *n*-dodecanal, toluquinone, and 2-methoxy-3-methyl-1,4-benzoquinone. When emitted in small amounts, however, the TGS releases the male grasping response. The main components with aphrodisiac effect are (*Z*)-4-tridecene, *n*-dodecanal, and (*Z*)-5-tetradecenal. These supplementary mating stimulants, which are not sex specific, work synergistically with the aphrodisiac female sex pheromone from the epicuticular lipids and are discussed as alerting pheromones of short-term effect. Antennal movements of resting males as an indication of the recognition of a female and the approach to the mate are released at somewhat longer distances, when the TGS is additionally present.

Key Words—*Aleochara curtula* (Goeze), Coleoptera, Staphylinidae, tergal gland secretion, defense, mating stimulants, female sex pheromone, hydrocarbons, *n*-aldehydes, substituted 1,4-benzoquinones.

INTRODUCTION

The composition of the defensive secretions of beetles often is very complex and species specific, and the chemical patterns have been used as an additional criterion for exemplary taxonomic investigations (e.g., Dettner, 1980; Eisner et al., 1977; Moore and Wallbank, 1968; Schildknecht et al., 1968). However,

[1]Coleoptera: Staphylinidae (Aleocharinae).
[3]Abbreviations: TGS: tergal gland secretion; FE: female equivalent; ME: male equivalent.

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in the functional interpretation of the chemical complexity, the chief obstacle often is the absence of knowledge of the selective advantage of each of the various compounds (Tschinkel, 1975). Some authors have interpreted the constant occurrence of minor secretion compounds as a subsidiary result of biochemical pathways (Dettner, 1980). Besides the defensive properties and their physical chemical requirements, additional functions in intraspecific communications have been demonstrated for the defensive secretion compounds of some insects (e.g., Ayre and Blum, 1971; Keville and Kannowski, 1975; Löfqvist, 1976; Melber, 1977; Regnier and Wilson, 1968). The results of our present studies of the tergal gland secretion (TGS) of *Aleochara curtula* (Goeze) also indicate the additional use of byproducts of the defensive secretion in communication.

For Aleocharinae, a subfamily of staphylinid beetles, the defensive function of the TGS, as observed in the repulsing of ants, was demonstrated for different species (Hölldobler, 1970; Jordan, 1913; Pasteels, 1968). The composition of the secretion from the gland reservoir has been chemically investigated in *Lomechusa strumosa* (Blum et al., 1971), *Drusilla canaliculata* (Brand et al., 1973), and *A. curtula* (Peschke and Metzler, 1982). 1,4-Benzoquinones and series of aliphatic hydrocarbons and aldehydes have been established as compounds common in these beetles. Quantitative comparisons of males and female secretions of *A. curtula* revealed no sex specificity (Peschke and Metzler, 1982).

Kemner (1923) provided the first evidence that TGS possibly plays a role in the intraspecific communication of *A. curtula*, an abundant species living on carcasses. He reported that the beetles emitted a pungent odor, probably from the tergal gland, when activated at high temperatures in the field. The mating behavior then was released more obviously in males. In our cultures of mixed sexes, copulatory activity was also evidently increased when the beetles were slightly disturbed. Then even homosexual behavior was observed in groups of separated males; this was normally not seen with untroubled males of the same cultures. On the other hand, Peschke (1978a) demonstrated in *A. curtula* the occurrence of an aphrodisiac female sex pheromone, which is spread over the entire surface and trapped by epicuticular waxes. In preliminary experiments, long-chain hydrocarbons, which do not occur in the TGS, have been identified as releasers of the male grasping response; however, TGS compounds have been trapped from the air by Porapak Q (Peschke, in preparation).

In contrast with the observations after slight disturbance, which indicate a releasing or activating effect of the TGS in male copulatory behavior, males did not show sexual responses if the female was badly injured and emitted large amounts of the defensive secretion. The males fled from such females and cleaned the antennae. Other beetles, formerly resting nearby, started to rush around.

In the present paper, experiments on the role of the TGS of *A. curtula* in male copulatory behavior are reported. The preliminary observations have indicated contrary functions depending on concentration. Is the TGS able to release the male copulatory behavior (the grasping with parameres) at low concentrations, and does it inhibit the sexual response at high doses? What are the supplementary functions of the TGS compounds, in contrast to those of the epicuticular pheromone with its short-range and long-lasting effect? To answer these questions, the sex specificity, range, and rate of evaporation of active TGS compounds and their suspected synergism with the epicuticular pheromone in particular were investigated. In addition, experiments on the stimulatory effect on male behavioral patterns prior to the grasping response are reported.

METHODS AND MATERIALS

Rearing. A. curtula was reared in the laboratory according to Fuldner (1968) and Peschke (1978a). Immediately after emergence, sexes were separated, and the beetles were kept in groups of ten. After three weeks they were extracted or used in the bioassay.

Extracts. The tergal gland secretion (TGS) was obtained by inserting a triangle of filter paper between the sixth and seventh abdominal tergites of 20 day-old beetles of separated sexes freshly killed by freezing (45 min at −17° C). The contents of the gland reservoirs were absorbed by the filter papers which were extracted with *n*-hexane (gland contents from 100 beetles in 1 ml). These solutions were used for bioassay without further preparation.

Individual components from the TGS were identified and quantitatively determined by Peschke and Metzler (1982). For the bioassay, *n*-alkanes, 1-alkenes, dodecanal, and toluquinone were purchased from Fluka, Roth, and Sigma in >99.9% purity. (*Z*)-5-Tetradecenal and (*Z*)-4-tridecene were obtained by preparative GLC of the natural secretion, and 2-methoxy-3-methyl-1,4-benzoquinone was synthesized according to Peschke and Metzler (1982). The latter substance was dissolved in acetone; for all other compounds, *n*-hexane was used as the solvent.

The amounts of the total secretion or its single components were specified in terms of female or male equivalents (FE or ME). One FE means the total content of the reservoir of one female obtained by the filter paper method or the total content of individual components. These values represent averages of the gland contents of 100 beetles per preparation. As a control, quantitative GLC gave reproducible results from different extractions.

Extracts of the epicuticular sex pheromone were obtained by washing 100 freshly killed females (20 days old) in 50 ml chloroform–methanol (2:1 v/v) for 15 min at 22° C and evaporation to 1 ml. The concentrations of surface washings were also specified in terms of female equivalents.

Models. The following models for bearing the different extracts were prepared and glued to the tips of glass needles according to Peschke (1978a): (a) acetone extracted beetles (24 hr Soxhlett; male and female extracted beetles alone did not release male copulatory behavior but caused the same response rates after contamination with active agents); (b) 20-day-old males or (c) 20-day-old females kept isolated from each other, freshly killed by freezing (45 min at −17° C).

Samples of 0.01 ml solutions of the TGS, its single compounds, or surface washings were dropped onto the different models. Within 5 min after evaporation of the solvent (the shiny model became dull), the models were tested, then stored at room temperatures and tested for 5 min again at 1 hr, 24 hr, and 48 hr after contamination.

Grasping Reaction. Single males from groups kept in tens were repeatedly tested three weeks after emergence and sexual isolation. A model was held ten times in 1/2-sec intervals before a male running along the margin of a glass dish, the bottom of which was covered with moistened filter paper. The grasping with parameres towards the model was used as the criterion for the release of the male copulatory response (for further details of the test procedure see Peschke, 1978a). The number of males responding sexually at any of the ten encounters was recorded, pooled for identical models, and the response rate was specified in percent males showing the grasping reaction. Significance of differences was established by the chi-square test (fourfold table with original values) or by the exact test of Fisher, if any field of the fourfold table was filled with a value $\leqq 3$ (Sachs, 1969). The exact confidence limits (95%) drawn in the figures were taken from the tables of Hald (1965).

RESULTS

Behavioral Pattern of Grasping Response. Acetone-extracted beetles do not release the male mating behavior ($N_{\delta\delta} = 162$, 20 models). These models were contaminated with 0.01-ml hexane solutions of the TGS separately obtained from male or female beetles and were tested for the release of male sexual behavior 0–5 min after evaporation of the solvent. At certain percentages, the models contaminated with TGS released the same behavioral pattern of the grasping response as shown towards freshly killed females or models contaminated with female surface washings: a few millimeters before contact by the antennae, the males bent their abdomens over the backs of their heads and protruded the genitalia with the tonglike parameres (Figure 5c; Peschke, 1978a,b). In addition, perfect contact orientation along the female abdomen (Peschke, 1979) and attempts to couple the genitalia were also observed, but were not evaluated quantitatively.

Sex Specificity of TGS. The grasping response rate depends on the

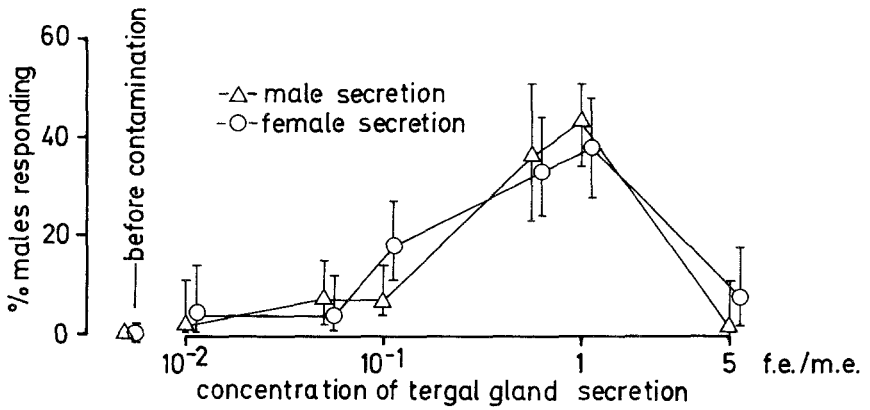

FIG. 1. Copulatory responses of *A. curtula* (% males grasping with parameres) to acetone-extracted beetles 0–5 min after contamination with male or female tergal gland secretion at various concentrations (for each point 50–140 males tested with 2–5 models; vertical lines: 95% confidence limits).

concentration of the TGS (Figure 1); however, significant behavioral differences between male and female secretion could not be established. This was congruent with the chemical comparison which revealed no sex specificity, either qualitatively or quantitatively (Peschke and Metzler, 1982). Therefore, in the subsequent experiments, it was not necessary to test the secretions of both sexes, and the investigations were restricted to the female secretion as releaser of male copulatory behavior.

Release of Grasping Response Depending on Concentration of TGS. The maximum releasing effect of the TGS was observed with 38% of the males responding at a concentration of 1 FE ($N_{\delta\delta}$ = 98, 4 models, Figure 1). This response rate, however, was low in comparison to that obtained with freshly killed females (98%, $N_{\delta\delta}$ = 982, 33 models) or 1 FE surface washings of females spread on acetone-extracted beetles (85%, $N_{\delta\delta}$ = 974, 37 models). At the unnaturally high concentration of 5 FE of TGS, most of the males fled the model and cleaned the antennae; only a few males showed the grasping response (8%, $N_{\delta\delta}$ = 49, 2 models). Dilution to 0.1 FE (18%, $N_{\delta\delta}$ = 101, 4 models) or less caused significant reductions in the response rates in comparison to 1 FE ($P < 0.001$).

Contamination of Freshly Killed Males. In the former experiments, the releasing effect of pure TGS was demonstrated by contamination of acetone-extracted beetles. However, in the natural situation, the secretion is contaminated onto a wax-covered surface. Therefore, freshly killed males (>20 days old, kept isolated) which did not release the male grasping reaction prior to

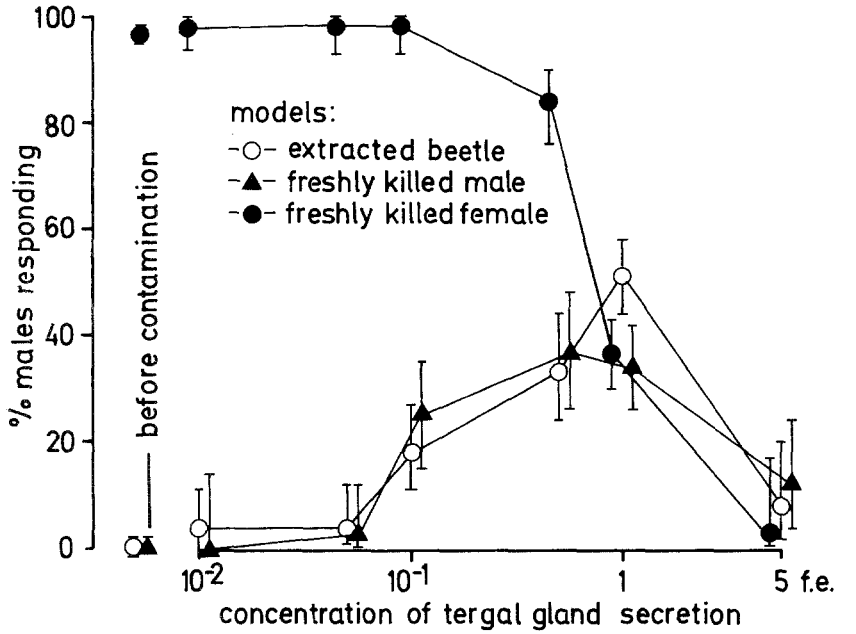

FIG. 2. Copulatory responses of *A. curtula* (% males grasping with parameres) to different models (acetone-extracted beetles, freshly killed males or females) 0–5 min after contamination with the tergal gland secretion at various concentrations (for each point 50–240 males tested with 2–10 models; vertical lines: 95% confidence limits).

contamination (0%, $N_{\delta\delta} = 319$, 17 models) were treated with different concentrations of the TGS and tested 0–5 min after evaporation of the solvent. The response rates were quite similar to those from experiments using extracted beetles as models (Figure 2).

Inhibitory Effect by Contamination of Freshly Killed Females. Freshly killed females released the full male response prior to contamination (98%, $N_{\delta\delta} = 982$, 33 models). In the first 5 min after contamination with the TGS, concentrations up to 0.1 FE caused no significant effects (Figure 2). However, at 0.5 FE a slight but significant decrease to 84% male response was observed ($N_{\delta\delta} = 133$, 3 models; $P < 0.001$). Only 36% or 3% of the males responded sexually to contaminated females at concentrations of 1 and 5 FE, respectively ($N_{\delta\delta} = 234$, 9 models; or $N_{\delta\delta} = 31$, 1 model; $P < 0.001$ in comparison to untreated female).

Synergism of TGS with Female Epicuticular Sex Pheromone. In the former experiment, it was not possible to demonstrate synergism of the TGS with the epicuticular pheromone by contamination of freshly killed females because these already yielded a response rate near 100% prior to contamination. Also a surface washing from females, containing 1 FE of the epicuticular

sex pheromone, had produced a male grasping response rate of 85% when applied to an acetone-extracted beetle. After its dilution to 0.1 FE, however, only 55% of the males responded with the grasping reaction in the first 5 min after evaporation of the solvent ($N_{\delta\delta}$ = 178, 7 models). The TGS was then added at different concentrations to the 0.1 FE surface washing. In comparison to the 55% level, significant increases of the male response rate (Figure 3, $P < 0.001$) were obtained by adding the TGS in concentrations from 1 FE to 10^{-3} FE; at the latter, the TGS alone was not capable of releasing the male reaction. The optimal response rate of 99% was produced by the admixture of 0.05 FE or 0.1 FE of the TGS. ($N_{\delta\delta}$ = 62, 2 models; or $N_{\delta\delta}$ = 66, 2 models). At 10^{-4} FE no further significant synergistic effect could be established (56%; $N_{\delta\delta}$ = 54, 2 models). On the other hand, addition of 5 FE

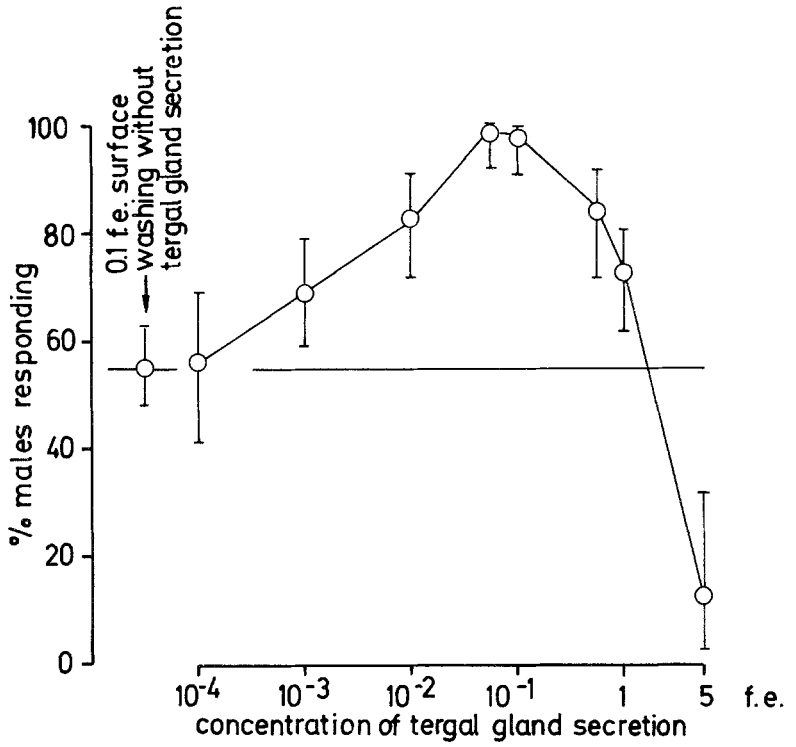

FIG. 3. Copulatory responses of *A. curtula* (% males grasping with parameres) to acetone-extracted beetles 0–5 min after contamination with mixtures of 0.1 FE female surface washing and various concentrations of the tergal gland secretion (for each point 50–100 males tested with 2–3 models; vertical lines: 95% confidence limits; horizontal line: level of response to 0.1 FE female surface washing without admixture of the tergal gland secretion).

caused a striking decrease of the response rate to 13% ($N_{\delta\delta}$ = 24, $P < 0.001$).

Releasing and Inhibitory Effects in Relation to Time. In the former experiments, models contaminated with TGS were used for only 5 min immediately following evaporation of the solvent, because later on the response rate declines according to preliminary observations. However, natural females released the male grasping response at 94% ($N_{\delta\delta}$ = 203, 7 models) even 1 day after being killed by freezing. One FE of a female surface washing spread on an extracted beetle one day earlier still released the male copulatory behavior at a rate of 45% ($N_{\delta\delta}$ = 195, 7 models). Therefore the evaporation of active TGS compounds was evaluated by contamination of different models with 1 FE of the female secretion and tests after 0–5 min at 1 hr, 24 hr, and 48 hr (Figure 4).

In the first 5 min after contamination with 1 FE of the TGS, the extracted beetles produced somewhat higher response rates (56%, $N_{\delta\delta}$ = 144, 6 models) in this new series of experiments than equally contaminated freshly killed males (34%, $N_{\delta\delta}$ = 136, 6 models, $P < 0.001$). However, 1 hr after treatment, the extracted beetles failed to release grasping (0%, $N_{\delta\delta}$ = 95, 3 models), whereas contaminated, unextracted males still yielded response rates of 14%

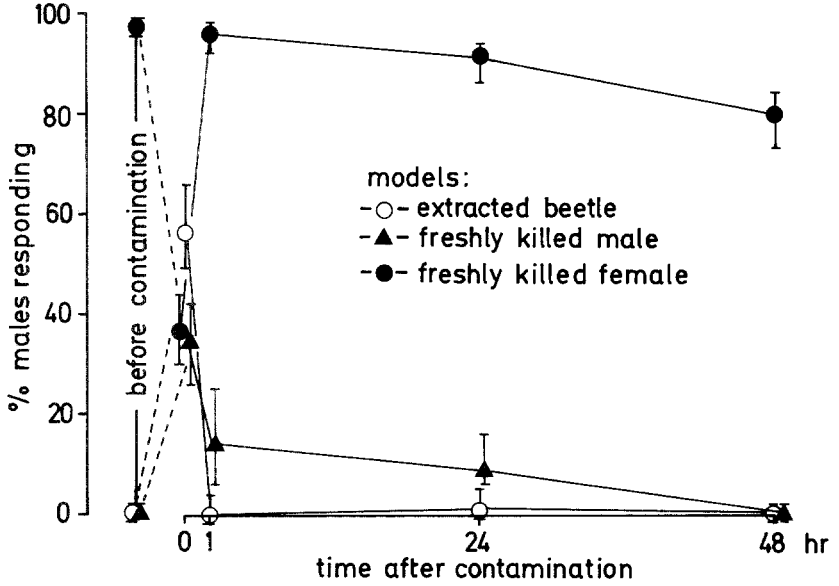

FIG. 4. Copulatory responses of *A. curtula* (% males grasping with parameres) to different models (acetone-extracted beetles, freshly killed males or females) in relation to time after contamination with 1 FE of the tergal gland secretion (for each point 50–240 males tested with 2–9 models; vertical lines: 95% confidence limits).

($N_{\delta\delta}$ = 57, 2 models, $P < 0.001$), and one day later of 9% ($N_{\delta\delta}$ = 175, 6 models, $P < 0.001$). Perhaps this delay of evaporation is caused by the trapping of TGS components in the natural wax cover of the male model. However, this is of little effect when compared to the permanence of the female epicuticular sex pheromone.

Immediately after contamination of freshly killed females with 1 FE of TGS, the grasping response rate was only 36%; ($N_{\delta\delta}$ = 234, 9 models); however, 1 hr after treatment the initial response rate of 96% was reestablished ($N_{\delta\delta}$ = 239, 6 models, $P < 0.001$, Figure 4). Then, 24 or 48 hr later, no significant differences with the very slow decrease of attractiveness of an untreated freshly killed female could be ascertained.

Releasing and Inhibitory Effects of Single Components. In order to determine which single components of the TGS release the male grasping response, the main compounds were tested individually at concentrations of 1 or 0.1 FE, simulating the plentiful and moderate emission of the secretion, respectively. The latter concentration was chosen because it did not reduce the female attractiveness, but gave sufficient response when the complete TGS was tested on acetone-extracted beetles. Acetone-extracted beetles were contaminated and tested for 5 min only, following evaporation of the solvent (Table 1A). Toluquinone and 2-methoxy-3-methyl-1,4-benzoquinone did not release the male copulatory response at either concentration. Also the main hydrocarbons, *n*-undecane and 1-undecene, yielded no response. From the C_{12} hydrocarbons, only *n*-dodecane was available in sufficient amounts; a few males responded sexually to it at a concentration of 1 FE. (*Z*)-4-Tridecene was the most effective hydrocarbon and yielded response rates of 12%. *n*-Pentadecane did not release male grasping at either concentration; however, a few males did respond to *n*-hexadecane at 1 FE. Both aldehydic compounds, *n*-dodecanal and (*Z*)-5-tetradecenal, were quite effective.

In a second test series, freshly killed females were also contaminated with synthetic or purified natural compounds of the TGS (0.1 and 1 FE) and, after evaporation of the solvent, tested 0–5 min for release of the male grasping response (Table 1B). *n*-Hexane and acetone, which were used as solvents, did not reduce the female attractiveness. At a concentration of 1 FE, significantly fewer males responded sexually to females after contamination with toluquinone, 2-methoxy-3-methyl-1,4-benzoquinone, *n*-undecane, 1-undecene, (*Z*)-4-tridecene, and *n*-dodecanal, respectively. At 0.1 FE, the inhibitory effect of all these chemicals was significantly weaker, with more than 80% of the males responding sexually to the models. Only one exception was found: at 0.1 FE of toluquinone, the response rate was still drastically reduced to 39%. Moreover, striking differences of response rates (100% or 5%) were observed with females treated with *n*-dodecanal at concentrations of 0.1 and 1 FE, respectively. These effects demonstrate that the test of individual chemicals could not

TABLE 1. COPULATORY RESPONSES OF *A. curtula* (% MALES GRASPING WITH PARAMERES: %GR) TO ACETONE-EXTRACTED BEETLES (A) OR FRESHLY KILLED FEMALES (B), BOTH CONTAMINATED WITH INDIVIDUAL COMPONENTS OF TERGAL GLAND SECRETION AT CONCENTRATIONS OF 1 OR 0.1 FE

Substance	0.1 FE				1 FE				1 FE (μg/gland)[d]
	%GR	$N_{\delta\delta}$	N_{models}	P^a	%GR	$N_{\delta\delta}$	N_{models}	P^a	
A. *Contamination of acetone-extracted beetles*									
Untreated model	*0*	162	20		*0*	162	20		
Solvents									
n-Hexane	*0*	162	20		*0*	162	20		
Acetone	*0*	42	2		*0*	42	2		
Hydrocarbons									
n-Undecane	*0*	49	2	N.S.[c]	*0*	68	3	N.S.[c]	96.9
1-Undecene	*0*	49	2	N.S.[c]	*0*	67	3	N.S.[c]	10.9
n-Dodecane	*0*	69	3	N.S.[c]	*3*	71	3	N.S.[c]	2.7
(*Z*)-4-Tridecene	*11*	62	3	+++[c]	*12*	59	3	+++[c]	2.9
n-Pentadecane	*0*	45	2	N.S.[c]	*0*	72	3	N.S.[c]	2×10^{-2}
n-Hexadecane	*0*	65	3	N.S.[c]	*6*	67	3	++[c]	2×10^{-2}
Aldehydes									
n-Dodecanal	*38*	48	2	+++[c]	*15*	114	5	+++[c]	21.4
(*Z*)-5-Tetradecenal	*9*	45	2	++[c]	*35*	46	2	+++[c]	4.7
Quinones									
Toluquinone	*0*	75	3	N.S.[c]	*0*	69	2	N.S.[c]	24.0
2-Methoxy-3-methyl-1,4-benzoquinone	*0*	77	3	N.S.[c]	*0*	52	2	N.S.[c]	130.0

B. *Contamination of freshly killed females*									
Untreated model	*97*	982	33		*97*	982	33		
Solvents									
n-Hexane	*93*	406	14	+++[b]	*93*	406	14	+++[b]	
Acetone	*97*	78	3	N.S.[b,c]	*97*	78	3	N.S.[b,c]	
Hydrocarbons									
n-Undecane	*84*	50	2	+	*56*	84	3	+++	96.9
1-Undecene	*90*	62	2	N.S.	*32*	72	3	+++	10.9
n-Dodecane	*85*	59	2	+	*66*	58	2	+++	2.7
(*Z*)-4-Tridecene	*97*	30	1	N.S.[c]	*75*	55	2	+++	2.9
n-Pentadecane	*98*	58	2	N.S.[c]	*97*	60	2	N.S.[c]	2×10^{-2}
n-Hexadecane	*97*	63	2	N.S.[c]	*99*	73	2	N.S.[c]	2×10^{-2}
Aldehydes									
n-Dodecanal	*100*	117	3	+++	*5*	98	4	+++	21.4
(*Z*)-5-Tetradecenal	*96*	113	3	N.S.	*94*	71	2	N.S.	4.7
Quinones									
Toluquinone	*51*	104	4	+++	*62*	124	5	+++	24.0
2-Methoxy-3-methyl-1,4-benzoquinone	*90*	94	3	N.S.[c]	*64*	154	6	+++[c]	130.0

[a] Statistical significances (+++: $P < 0.001$, ++: $P < 0.01$, +: $P < 0.05$, N.S.: not significant) were calculated by chi-square test relative to models contaminated with the solvent, except in *b*)

[b] Models contaminated with the solvent were compared to the untreated model.

[c] Exact test of Fisher used, otherwise normal fourfold table.

[d] Quantitative chemical determination for 20-day-old females after Peschke and Metzler (1982).

exactly simulate the effects of the complex TGS with the concert of compounds of variable functions.

Distance of Release of Antennal Movements and Grasping. Until this point in the study, the only criterion for the degree of sexual excitement was the capability of a female or of a contaminated model to release the male grasping response. The aphrodisiac compounds of the TGS are more volatile than those of the epicuticular sex pheromone. Therefore, it was assumed that the additional mating stimulants of the TGS might act at longer ranges. In the following experiments, we measured the distances from which the grasping response was trigged when the TGS was present or not. When a female ran towards a resting male in our cultures we incidentally observed typical antennal movements of the male just before the grasping response was released. This behavior was apparently a first indication of sexual excitement, and we also measured the distance from which the rapid antennal movements were released.

Special Methods. Single males were allowed to rest at the margin of an 8-cm-diam Petri dish, the bottom of which was covered with moistened filter paper. Directly 180° opposite, a freshly killed female glued to a needle was fixed outside and hung into the dish near the bottom and margin and was preexposed for 1 min in order to allow the development of an odor gradient. Models contaminated with 1 FE of the TGS were used 0–5 min after evaporation of the solvent only. The dish was rotated and thereby moved the marginal male with head first at a speed of 2 cm/sec towards the longitudinal axis of the fixed model. The behavior of the resting male was recorded as it approached by a National video system, and the distance between the freshly killed female and the front of the male's head, when it showed the first indication of antennal movements or grasping with parameres, was measured by analyzing single exposures. Significance of differences between mean distance values was established by t test (Sachs, 1969). In another series of experiments the male beetles were allowed to run freely along the margin of a fixed dish.

RESULTS

At first, freely running males were observed while approaching a freshly killed female. The first indication of the grasping response (opening the genital segment) was observed at a distance of 2.1 ± 0.5 mm ($N = 26$) from the male head to the body of an untreated female. If the female was contaminated with a solution of 0.1 FE of the TGS, the grasping response was released at a distance of 2.2 ± 0.7 mm ($N = 24$). This difference was not significant, and we also could not observe any other change of behavior of the quickly approaching male. With a resting male, however, which was passively advanced to an uncontaminated female, a new male behavioral step prior to

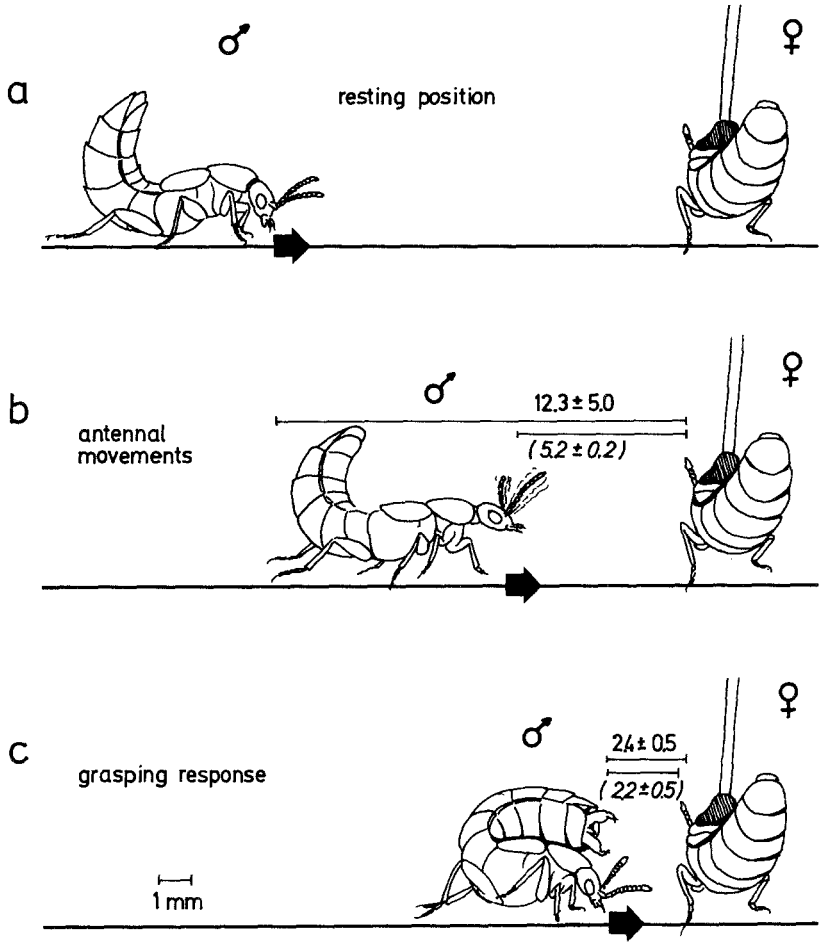

FIG. 5. Behavior of resting *A. curtula* males, passively advanced to a fixed female freshly killed by freezing: (a) resting male with its head bent down and motionless antennae; (b) the male raises its head and vibrates the antennae; (c) grasping with parameres. The distances (mm) to the female, at which the respective male behavior has first been observed, is noted for females 0–5 min after contamination with 0.1 FE of the tergal gland secretion (upper lines) and for untreated females (lower lines, in brackets).

the grasping reaction could be observed. Usually, a resting male bent its head down to the ground and held the antennae horizontal and motionless (Figure 5a). When a female approached to a distance of 5.2 ± 0.2 mm ($N = 29$), the male raised its head and vibrated its antennae (Figure 5b). This behavior was released at a significantly longer distance (12.3 ± 5.0 mm; $N = 18$; $P < 0.001$), if the female had borne 0.1 FE of the TGS. In a separate experiment,

where the approach of the model was stopped at the moment of the release of antennal movements, the males started to walk towards the female within a few seconds. Normally, the male was drawn towards the female at constant speed and the grasping reaction was observed at a distance of 2.4 ± 0.5 mm ($N = 24$) from a control female (Figure 5c) and of 2.2 ± 0.5 mm ($N = 14$) from a female contaminated with 0.1 FE of the TGS. This difference was not significant.

DISCUSSION

Hölldobler (1970), Jordan (1913), and Pasteels (1968) have shown the primary function of the tergal gland secretion (TGS) of different Aleocharinae to be that of defense. This could apply as well for the closely related species *Aleochara curtula*, which has a TGS of quite similar chemical composition (Peschke and Metzler, 1982). One can assume that recognition of the defensive secretion, plentifully emitted by a badly injured beetle could enhance the chances of a conspecific individual escaping a predator. In these situations, copulatory behavior would be inappropriate. Indeed, at high concentrations of TGS, the male grasping response to females is inhibited in our experiments, even though this is a rather brief effect.

The aphrodisiac sex pheromone from the epicuticular hydrocarbons of *A. curtula* females alone is capable of releasing the male grasping response, since components of the TGS could not be detected in these preparations (Peschke, 1978a). However, TGS compounds were trapped from air currents over a group of beetles by Porapak absorption (Peschke, in preparation). At present, the actual concentration of the secretion cannot be measured in the immediate surroundings of the beetles in different short-term behavioral situations. However, male copulatory behavior of *A. curtula* is enhanced when the odor of the TGS is present, thus confirming former observations of Kemner (1923) in the field. The stimulatory effect of the TGS alone is rather weak in comparison to the epicuticular pheromone, but it works synergistically to that sex pheromone even at very low concentrations.

Comparative chemical investigations revealed no sex specificity of the composition of the TGS of *A. curtula* (Peschke and Metzler, 1982), and male or female secretions alone release equivalent copulatory responses. However, the homosexual reactions to these substances are rare when the essential information from the epicuticular sex pheromone is lacking. In a combat situation, where the defensive secretion is emitted, it was occasionally observed that the males switch from fighting to grasping with the parameres.

The additional function of the TGS of *A. curtula* when emitted at low concentration would fit the definition of a supplementary mating stimulant or aphrodisiac. The information of the female sex pheromone from the

epicuticular hydrocarbons is very permanent and therefore difficult to alter. In contrast, the volatile secretion from the tergal gland can be used in sending short-term information, because the releasing as well as the inhibitory effects decline within minutes. In addition, it is believed that males perceive the volatile releasing compounds of TGS from distances somewhat greater than those observed for the epicuticular pheromone alone. The distance from which the male grasping response is triggered by a female was not influenced by the TGS; however, the male antennal movements as a first sign of excitement and the approach to a female then is released at a 2.4 times greater distance, when the odor of the TGS is additionally present. In the first instance, this extension of range might seem to be unimportant; however the beetles live in small irregular cavities under carcasses, particularly those of mammals, and the probability of encounters of males and females of *A. curtula* in those labyrinths would be increased after emission of the TGS. Long-distance attractants emitted by the beetles themselves have not yet been demonstrated; it is, however, assumed that odors from the carcasses bring both sexes of the abundant species together in the small habitat.

For the repellent TGS compounds, which inhibit the male copulatory response at high concentrations, the definition of a "pheromone" seems not to be appropriate, since the responding beetles only secondarily use information apparently not selected for them, and the advantage for the signaler is not established (Burghardt, 1970). However, according to Atema (1977) the neutral term "alarm substance" can be used without any implication of communicative or social function. The capability of the receiver to perceive TGS compounds, however, presupposes the use of the secretion as a pheromone as a mating stimulant when emitted at low concentrations. Then the female is able to send a distinct and obviously adaptive signal to the mate. Perhaps the essential function of the TGS in the male mating behavior of *A. curtula* is generally a stimulatory or activating one, alerting the male to receive the essential information from the female epicuticular pheromone. In further investigations the alerting function of the TGS compounds in intraspecific communication, other than that of mating, will be analyzed in connection with chemical measurements of the actual emission of the secretion.

The gradual emission of the TGS is accomplished by the morphology of the gland reservoir, an invagination of the intersegmental membrane between the sixth and seventh abdominal tergites (Araujo, 1978; Peschke and Metzler, 1982). The morphology of the abdominal musculature was described by Peschke (1978b): supported by a fold of the intersegmental membrane, the opening of the reservoir is closed when the sixth and seventh tergites are pressed together by the strong tergosternal muscle of tergite VI (M8). This closure is so tight that compounds of the TGS could not be detected by GLC in

surface washings of beetles carefully killed by freezing. The gradual opening of the reservoir could be accomplished by tilting the seventh tergite in its transverse axis; this motion is produced by the action of modified tergosternal muscles upon the anterior rim of tergite VII (M8). An extensive ejection of the TGS could be supported by a small muscle, which inserts at the reservoir itself (derived from the tergal muscle M2), and by increasing the pressure of the abdominal hemolymph.

In many arthropods 1,4-benzoquinones have evolved independently to essential defensive agents with deterrent or toxic effects towards a proposed enemy (Eisner and Meinwald, 1966; Schildknecht et al., 1968; Weatherston and Percy, 1970). Conforming with this obvious function, toluquinone and 2-methoxy-3-methyl-1,4-benzoquinone of *A. curtula* do not release the male copulatory behavior; on the other hand, when a female is badly injured, the plentiful emission of quinones diminishes the releasing effect of the epicuticular sex pheromone. However, the inhibitory effect seems not to be restricted to the quinones, because *n*-undecane, 1-undecene, (*Z*)-4-tridecene, and *n*-dodecanal also reduce the female attractiveness at high concentrations. On the other hand, at low concentrations simulating the moderate emission of the TGS, some minor hydrocarbons, such as (*Z*)-4-tridecene, *n*-dodecane, and even hexadecane, as well as *n*-dodecanal and (*Z*)-5-tetradecenal, can individually release the male grasping response.

While not assessed in each case experimentally, the function of additional lipid compounds of defensive secretions, especially hydrocarbons, as solvents or spreading the penetration agents is discussed by many authors (Blum et al., 1968; Calam and Youdeowei, 1968; Regnier and Wilson, 1968; Remold, 1962; Tschinkel, 1975; von Endt and Wheeler, 1972; Waterhouse and Gilby, 1964; Wilson and Regnier, 1971). Supplementary to their proposed physico chemical function, the significance in chemical intraspecific communication of the hydrocarbons from defensive secretions has been proved with some insects (e.g., Ayre and Blum, 1971; Löfqvist, 1976; Melber, 1977; Regnier and Wilson, 1968). Similar to *A. curtula*, an additional aphrodisiac function of hydrocarbons has been demonstrated for the defensive secretion of the pygidial gland of *Tribolium confusum* (Tenebrionidae) by Keville and Kannowski (1975).

Because of their lipid character and their pungent odor, the aldehydes from the TGS of *A. curtula* might also act as solvents and deterrents. Long-chain aliphatic aldehydes occurring in arthropod defensive secretions have been found previously in other Aleocharinae (Brand et al., 1973) and Myriapoda (Wheeler et al., 1964). Similar aldehydes have been identified as male or female sex pheromones of moths, but they are produced in quite different glands and are not connected with a defensive function (Dahm et al., 1971; Leyrer and Monroe, 1973; Röller et al., 1968; Roelofs et al., 1974; Underhill et al., 1977; Weatherston et al., 1971).

In summary, minor compounds of the TGS of *A. curtula*, which were formerly considered as byproducts of the biochemical pathway of defensive agents only (Peschke and Metzler, 1981), appear to have additional functions in intraspecific communication. These findings provide new insight into the selective advantage of the complexity and species specificity of the defensive secretion of these beetles.

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