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0014-4754/88/030216-05$1.50 + 0.20/0

o-Aminoacetophenone, a pheromone that repels honeybees (*Apis mellifera* L.)

R. E. Page, M. S. Blum and H. M. Fales

Department of Entomology, The Ohio State University, Columbus (Ohio 43210, USA), Department of Entomology, University of Georgia, Athens (Georgia 30602, USA), and Laboratory of Chemistry, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda (Maryland 20892, USA)
Received 3 November 1987; accepted 16 December 1987

Summary. *o*-Aminoacetophenone is a pheromone produced by virgin honeybee queens and released with feces. In small social groups, the pheromone repels and is used to terminate agonistic interactions between queens and workers.
Key words. Pheromone; social behavior; honeybee.

The feces of virgin queen honeybees, *Apis mellifera* L., was recently reported to be a source of a pheromone that repels workers and releases autogrooming behavior[1]. When confined in small social groups, virgin queens *occasionally* release 10–30 µl of rectal fluid (feces) when workers behave agonistically toward them. They *frequently* release rectal fluid when they fight with other virgin queens. The release of feces usually terminates the agonistic behavior[1,2].
The chemical constituents of virgin queen rectal fluid have recently been identified[3] and include: 1. octyl decanoate, 2. decyl octanoate, 3. decyl decanoate, 4. tetradecyl decanoate, 5. decanoic acid, 6. dodecanoic acid, 7. octanoic acid, 8. benzoic acid, 9. 1-dodecanol, 10. octyl octanoate, 11. *o*-aminoacetophenone, and other higher molecular weight organic acids and hydrocarbons.
A 'strong floral odor' is associated with virgin queen feces and with the repellency observed[1]. Of these identified compounds *o*-aminoacetophenone (*o*-AAP) smells like grapes and is the most likely compound responsible for the floral odor. Two other compounds, octanoic acid (OA) and 1-dodecanol (1-DD) are sufficiently volatile, and unlikely to be normal metabolic constituents of feces, to warrant further investigation. Therefore, these compounds were selected as potential repellent pheromones and were assayed.
Materials and methods. Compounds were obtained from Sigma Chemical Company. Individual product numbers are C 2875, A 5158, and L 5375 for OA, *o*-AAP, and 1-DD, respectively. Each was diluted to a concentration of 1:1000 in mineral oil, Sigma product number M 3516.
Behavioral assays were identical to those previously reported[1]. Groups of 10 workers were collected from colonies into 235-ml cups. Each cup had a piece of filter paper attached to the bottom with a 1.8-cm diameter circle marked in the center. Cups were taken into the laboratory where all assays of groups of 10 bees were completed within 1 h.
For each replicate, a test and control observation arena (cup) were paired. Behavior of all bees in each arena was observed for 60 s before and after 10 µl of the specific compound diluted in mineral oil was placed in the center of the circle. The other arena of each pair received similar observational time before and after introduction of 10 µl of mineral oil. Two behavioral activities were recorded: 1) the number of times bees walked through the circle, and 2) the number of bees autogrooming after each 60-s interval. These correspond to activities that are altered significantly by virgin queen feces[1]. The responses of the workers to the tests and controls before and after addition of the test compound or the mineral oil control were summed for twenty replicate sets of trials for each compound and analyzed using a chi-square test of independence[4].
Results. Only *o*-AAP repels bees (table 1). The degree of repellency is equal to that previously shown for virgin queen feces[5]. No treatment compound increased autogrooming behavior significantly (table 2). However, mineral oil alone increased autogrooming significantly when the number of autogrooming workers before and after exposure to mineral oil (36 and 64, respectively) in all assays were considered ($\chi^2 = 7.84$, $p < 0.01$, df = 1). These results suggest that the higher molecular weight hydrocarbons found in virgin queen feces may be responsible for the increase in autogrooming reported previously.
As compared to the controls, octanoic acid reduced autogrooming behavior significantly. Whether this result is spurious or has real biological significance remains to be determined.

Table 1. Results and analyses of behavioral assays of different compounds for the number of worker trips through the circle on the bottom of the observation arena. Twenty replicate trials were conducted for each 60-s period before and after the introduction of control (mineral oil only) and treatment compounds.

		Number of trips	
		Control	Treatment
o-Aminoacetophenone			
	Before	309	312
	After	266	150
$\chi^2 = 20.29$, $p < 0.01$, df = 1			
Octanoic acid			
	Before	348	420
	After	324	355
$\chi^2 = 0.84$, $p > 0.05$, df = 1			
1-Dodecanol			
	Before	348	340
	After	306	317
$\chi^2 = 0.28$, $p > 0.05$, df = 1			

Table 2. Data and analyses for the autogrooming behavioral response

		Number autogrooming Control	Treatment
o-Aminoacetophenone			
	Before	5	7
	After	15	42
$\chi^2 = 1.13$, $p > 0.05$, df = 1			
Octanoic acid			
	Before	6	14
	After	28	22
$\chi^2 = 3.87$, $p \approx 0.05$, df = 1			
1-Dodecanol			
	Before	25	39
	After	21	39
$\chi^2 = 0.22$, $p > 0.05$, df = 1			

Discussion. o-Aminoacetophenone is the first repellent pheromone reported for honeybees. The effectiveness of this compound in small social groups is now well documented; its function in the more complex social environment of a colony still needs to be investigated. In small social groups, queens release large quantities of fecal material under conditions of agonistic social interactions with workers and other queens; however, small quantities are continuously released and sometimes appear to be attractive to workers (personal observations). The higher molecular weight hydrocarbons could serve as carriers for *o*-AAP as well as releasers of autogrooming.

o-Aminoacetophenone is also produced by the sawfly, *Cephalcia lariciphila*[6], and the primitive fungus-growing ant, *Mycocepurus goeldii*[7]. As with worker honeybees, worker ants are attracted to this compound in low concentrations and repelled at higher concentrations. These are the only three arthropods known to produce this compound[8].

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0014-4754/88/030270-02$1.50 + 0.20/0

Ultrasonic vocalizations by adult prairie voles, *Microtus ochrogaster*

J. J. Lepri[a,*], M. Theodorides[a] and Ch. J. Wysocki[a,b]

[a] *Monell Chemical Senses Center, 3500 Market Street, Philadelphia (Pennsylvania 19104, USA), and* [b] *Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, Philadelphia (Pennsylvania 19104, USA)*
Received 24 July 1987; accepted 22 October 1987

Summary. Male and female *Microtus ochrogaster* were presented with anesthetized and awake conspecifics while ultrasonic vocalizations (USVs) were monitored. Males produced significantly more USVs than females during 5-min testing sessions. Males tended to produce more USVs to unfamiliar females than to familiar female siblings. Sexual experience led to increased USV scores by males. These results suggest that USVs by male prairie voles communicate to females the male's gender and his availability for reproductive behavior.
Key words. Prairie voles; *Microtus ochrogaster*; ultrasonic vocalizations; reproductive behavior; sexual experience.

Ultrasonic vocalizations (USVs) occur above the normal range of human hearing and are produced by a variety of mammalian species. Many insectivore species use these vocalizations for prey-finding and navigational purposes (e.g., bats), whereas many rodent species appear to use them as communication signals. Both immature and adult rodents emit USVs; pups emit them as distress signals to attract parental care[1] and adults emit them during social interactions[2], suggesting that they are components of a communication system. Demonstrations of the true functional significance of USVs by adults have been few, but some general parameters concerning the behavioral contexts in which they are emitted have emerged. For example, in pine voles, *Microtus pinetorum*, males emit USVs much more frequently than females, and the emission of USVs is regulated by gonadal androgens[3], as in many other species of muroid rodents[4]. In golden hamsters, *Mesocricetus auratus*, Floody et al.[5] demonstrated that following brief exposure to males, tape-recorded playbacks of USVs by males prolong lordosis in estrous females. Furthermore, sexual experience increases rates of USV and facilitates reproductive behavior in male house mice[6]. These findings support the hypothesis that USVs are important components of male reproductive behavior.

Prairie voles, *Microtus ochrogaster*, have attracted research attention in an effort to determine the factors which influence the striking population fluctuations shown by this and many other species of voles and lemmings. Particular attention has been focused on reproductive biology. Like most species of voles that have been studied, prairie voles are induced ovulators. The reproductive system of female prairie voles is generally quiescent in the absence of stimulation from males. Following introduction to unfamiliar conspecifics of the opposite sex, male and females prairie voles immediately engage in extensive investigation of each other[7], resulting in the exchange of sensory information, especially chemosignals, that stimulates reproduction. Mating usually initiates 24–48 h after pair formation[8] and copulation induces ovulation[9].

The reproductive unit of prairie voles appears to be the monogamous pair. Field studies have demonstrated that a particular pair of breeding prairie voles can be consistently found together in the same nest[10]. Following the successful production of a litter, laboratory-housed males and females show aspects of mate-fidelity, including aggression to unfamiliar animals and increased latency to engage in sexual behavior with them[11]. Furthermore, inbreeding avoidance between familiar siblings is profound in laboratory set-