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PROPAGANDA SUBSTANCES IN THE CUCKOO ANT *Leptothorax kutteri* AND THE SLAVE-MAKER *Harpagoxenus sublaevis*

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Abstract—This paper reports the first discovery of "propaganda substances" in a workerless inquiline ant, the European myrmicine *Leptothorax kutteri* Buschinger. These substances are used by the parasite queen as a chemical weapon for defense against hostile workers of the host species *L. acervorum.* The substances also have an unusual behavioral effect: they cause host workers to attack each other, and they therefore appear to override nestmate recognition in host colonies. Laboratory experiments show that the source of these substances is the Dufour's gland of the *L. kutteri* queen. Our experiments also confirm the hypothesis that the closely related slave-making ant *Harpagoxenus sublaevis* uses its Dufour's gland secretions as a chemical weapon during slave raids and colony foundation. The behavioral effect of these slave-maker secretions is identical to that of *L. kutteri* queens.

Key Words—Dufour's gland, Formicidae, Hymenoptera, inquiline ant, slave-making ant, *Harpagoxenus sublaevis, Leptothorax kutteri, Leptothorax acervorum*, pheromone, nestmate recognition.

INTRODUCTION

Inquiline ants have the unusual ability to infiltrate colonies of ants of other species and to become accepted by their members. However, compared to our knowledge of the colony infiltration methods of myrmecophiles (e.g., see Hölldobler 1970, 1971; and review of Kistner, 1979), there is relatively little known about how inquiline queens accomplish these feats (see reviews of Wilson, 1971; Dumpert, 1981). For instance, it is not known how the inquiline ant *Leptothorax kutteri* (Buschinger, 1965) manages to infiltrate colonies of its host *Lep-*

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tothorax acervorum. In laboratory introductions the parasite queens are usually attacked by *L. acervorum* workers from the target colony (Buschinger, 1965, and personal communication; Franks, unpublished observations). In such introduction experiments, *L. kutteri* queens display none of the behaviors previously recorded in inquiline foundresses (see Discussion). Instead, this paper demonstrates that they deploy a "propaganda substance" as a defense against the attacks of the target colony workers. *L. kutteri* is the first inquiline in which such substances have been found. Nevertheless, the manner in which the parasite queens follow up this strategem and come to be tolerated by their hosts remains an unsolved problem.

Propaganda substances were first described in slave-making *Formica* from North America (Regnier and Wilson, 1971). On slave raids these ants broadcast mimics of alarm pheromones among resisting host workers, causing them to panic and flee. The substances used by *L. kutteri* have a different behavioral effect. They cause host workers to attack each other, and they therefore appear to override nestmate recognition in host colonies.

In addition, this paper shows that the slave-making ant *Harpagoxenus sublaevis*, another parasite of *L. acervorum* and also a close relative of *L. kutteri*, possesses substances which have a similar effect on *L. acervorum* workers during conflicts between the slave-makers and host colonies, that is during slave raids by *H. sublaevis* workers and in colony foundation by the queens. The most obvious adaptation of *H. sublaevis* females for fighting is their possession of large and secateur-like mandibles, with which they dismember their opponents. The existence of chemical weapons in this species was first suggested by Buschinger (1974a) when he recorded that *L. acervorum* workers may attack each other following contact with slave-raiding or colony-founding *H. sublaevis* females. Similar phenomena have been observed by Alloway (1979) during slave raids by *H. americanus* on its North American *Leptothorax* hosts. We also observed an effect of this type in laboratory introductions of *L. kutteri* queens to *L. acervorum* colonies. In *H. sublaevis* it was suggested (Buschinger, 1974a) that the effect is due to a pheromone from the Dufour's gland which the slave-maker wipes onto its opponent with its sting. The Dufour's gland secretion of *H. sublaevis* has also been proposed to act as a repellent which the slave-makers smear over captured brood in order to deter would-be *L. acervorum* rescuers (Winter, 1979; Buschinger et al., 1980).

Remarkably, in both *H. sublaevis* and *L. kutteri*, the Dufour's gland is hypertrophied (Buschinger, 1974b). Therefore our first experiment was to test under standardized conditions whether or not contact with *L. kutteri* caused *L. acervorum* workers to be attacked by their nestmates. Following a positive result, our second experiment tested the hypothesis that it is the Dufour's gland secretions of *L. kutteri* and *H. sublaevis* that contain the parasites' propaganda substances.

METHODS AND MATERIALS

All the ants used in the experiments came from colonies collected in sourthern Sweden in July 1983. Both *L. acervorum* and *H. sublaevis* are widespread in central and northern Europe, but *L. kutteri* is known only from sites in West Germany, the Alpine region, and Sweden (Buschinger, 1965; Douwes and Buschinger, 1983). The colonies were found in pine forests in hollow dead twigs on the ground. They were maintained in the laboratory, and the experiments were carried out between October 1983 and June 1984.

Experiment 1. Single *L. acervorum* workers were removed from unparasitized colonies and confined in arenas (100 cm^2) with either (1) single newly inseminated *L. kutteri* queens, (2) single *L. acervorum* queens from another nest, or (3) single *L. acervorum* workers from another nest. The behavior of the pairs of ants was then observed. After 60 min the workers were returned to their own nests, and the behavior of their nestmates towards them was recorded.

Experiment 2. We tested the response of *L. acervorum* workers to nestmates treated with extracts of the Dufour's gland of *L. kutteri* queens, *H. sublaevis* females, and, as controls, queens of *L. acervorum*. The *H. sublaevis* females were not classified as workers or queens because in this species most queens are identical in external morphology to workers. The colonies tested in the trials were unparasitized colonies of *L. acervorum*. For every trial, a set of four replicate portions was made from each colony by selecting at random four groups of three workers and housing each group in its own small arena (4 cm^2). We then added to three of these replicate portions a nestmate treated with either (1) an extract of Dufour's gland contents, (2) an extract of poison vesicles, or (3) solvent alone.

The glandular extracts were prepared by first dissecting ants in distilled water. The glands were then removed and crushed whole in liquid paraffin (mineral oil). This solvent was used because, unlike hexane, for example, it was not toxic to the ants. Since the Dufour's gland volumes of *L. kutteri* and *H. sublaevis* are, respectively, about 40 and 60 times that of the *L. acervorum* queen, the *L. kutteri* Dufour's gland extracts contained 6 glands/10 μl of solvent (giving a concentration of 1.5×10^{-2} μl gland content/μl of solvent), and the *H. sublaevis* Dufour's gland extracts contained 4 glands/10 μl of solvent (concentration 1.6×10^{-2} μl gland contents/μl solvent). In the *L. acervorum* queen trials (since here the Dufour's gland is very small), we used single glands crushed in single paraffin droplets (concentration 1.3×10^{-3} μl gland contents/μl solvent). Poison extracts contained the same number of glands as corresponding Dufour's gland extracts. Poison extract was used in these trials as an additional control, to test whether other endogenous substances, in general, could induce hostility.

All the ants which were introduced to the groups of workers were first marked with paint dots on their heads. The glandular extracts (or liquid paraffin alone) were then applied to the thorax with a pinhead. The average volume applied was 0.50 ± 0.15 μl (determined by weight). Therefore, in the case of the ants treated with Dufour's gland extract, each ant received the equivalent of either 0.29 of an average *L. kutteri* gland, or 0.21 of a *H. sublaevis* gland, or a whole queen *L. acervorum* gland (since here the whole paraffin droplet in which the gland had been crushed was applied). Sixty minutes after the addition of each treated ant to the arena of nestmates, we recorded whether or not it was being bitten. This we defined as an attack. The time interval guaranteed that any attack was not a temporary response to a new object in the arena. Finally, a worker from a different unparasitized *L. acervorum* colony was added to the fourth replicate portion of the trial colony. This worker was also marked with a paint dot, and its reception in the arena was recorded in the same way as that of the treated ants. Only the results from trials in which this alien was attacked were considered valid. This ruled out trials in which the unfamiliar surroundings of the test arena might have lead to a lack of hostility to any introduced worker.

RESULTS

Experiment 1. As regards response of workers to nestmates confined with *L. kutteri* queens or alien conspecifics: the *L. acervorum* workers always fought with the ant with which they were confined, whether it was a queen *L. kutteri*, an alien *L. acervorum* queen, or an alien *L. acervorum* worker. In these fights the *L. kutteri* queens were seen to daub the *L. acervorum* workers with a clear viscous fluid which they released from the tip of the abdomen. This behavior was not observed in *L. acervorum* workers or queens. When the *L. acervorum* workers which had been with the *L. kutteri* queens were returned to their home colonies, all of them were attacked by their nestmates (Table 1). Workers which had been with either worker or queen alien conspecifics were attacked only very rarely when returned to their own nests (Table 1). This suggests that a *L. kutteri* queen can contaminate a hostile host worker with a secretion which causes the worker to be attacked by its nestmates.

Experiment 2. As regards response of workers to nestmates treated with gland extracts: *L. acervorum* workers treated with extracts of the Dufour's gland contents of *L. kutteri* and *H. sublaevis* were significantly more likely to be attacked by their nestmates than were workers treated with either extracts of the contents of the poison vesicles or with liquid paraffin alone (Table 2, columns A and B). Furthermore, extracts of the Dufour's gland substances of *L. acervorum* queens did not induce attack by nestmates at a significantly greater frequency than did the control solvent (Table 2, column C). These results suggest that the hostility-inducing substances of *L. kutteri* and *H. sublaevis* originate in

TABLE 1. RESPONSE OF WORKERS TO NESTMATE CONFINED WITH *L. kutteri* QUEEN OR ALIEN CONSPECIFIC

	Leptothorax acervorum workers[a]	
	Attacked	Not attacked
Exposed to:		
L. kutteri Queen	9	0
L. acervorum Queen	1	8
L. acervorum Worker	0	9

[a] $\chi^2 = 23.2$ $P < 0.001$.

the Dufour's gland and that the active agents in these secretions are either absent or present in only very small amounts in the Dufour's glands of *L. acervorum* queens.

DISCUSSION

The substances used by *L. kutteri* queens when they encounter foreign host colonies and their behavioral effects are, as far as we know, unique among inquilines. Other species of inquiline, in which single queens enter large, mature host colonies, use a variety of techniques, but none of them involve substances which cause host workers to fight one another. For example, the inquiline queen *Anergates atratulus* appears to play dead when met by a worker of the host species *Tetramorium caespitum*. She then clings with her jaws onto the worker's antenna and is dragged into the nest (Wheeler, 1910; Gösswald, 1954). *Epimyrma stumperi* either plays dead in a similar manner, or captures a *Leptothorax tuberum* host worker, rubs it with her forelegs, and grooms herself, apparently acquiring the host's colony odor in this way (Kutter, 1969). Another parasite of *Tetramorium caespitum*, *Teleutomyrmex schneideri*, employs a third type of entry method. She seems to appease the host workers with surface glandular secretions (Stumper, 1950; Gösswald, 1953).

How *L. kutteri* queens penetrate host colonies in the field has never been observed. The possibility cannot be excluded that the parasite queen enters colonies at the founding stage, as suggested in the inquilines *Strumigenys xenos* (Brown, 1955) and *Pogonomyrmex colei* (Rissing, 1983). It is also conceivable that *L. kutteri* queens transfer to daughter colonies during budding of the host colony, as may occur in *Plagiolepis xene* (Passera, 1964). However, the occurrence of single *L. kutteri* queens in isolated *L. acervorum* colonies (Buschinger, personal communication) suggests that at least some parasite colonies are founded by lone *L. kutteri* queens entering mature host colonies. From our

TABLE 2. RESPONSE OF WORKERS TO NESTMATES TREATED WITH VARIOUS EXTRACTS

	(A) *L. kutteri* gland extracts			(B) *H. sublaevis* gland extracts			(C) *L. acervorum* gland extracts		
	Attacked	Not attacked	Total trials	Attacked	Not attacked	Total trials	Attacked	Not attacked	Total trials
Dufour's extract	19	11	30	15	15	30	4	11	15
Poison extract	3	27	30	6	24	30	2	13	15
Liquid paraffin control	4	26	30	4	26	30	3	12	15
	$\chi^2 = 26.2, P < 0.001$			$\chi^2 = 11.4, P < 0.01$			$\chi^2 = 0.8, P > 0.05$		

results, we would expect these invading queens always to be attacked by the target colony workers. In all of the 15 laboratory introductions of single *L. kutteri* queens to *L. acervorum* colonies we have performed to date, such attacks have proved fatal (Franks, unpublished observations). However, it is likely that the probability of successful colony foundation in nature is very low. When attacked by the target colony workers, the parasite could use its propaganda substances to confuse its aggressors, allowing it to escape immediate danger and to proceed to gain the acceptance of its hosts by means which are at present unknown. It could seek to enter a cavity in the host nest where it could remain undetected, in order to pick up the colony odor from the nest material. Or it may deliberately court attack in order to acquire the colony odor by cuticular contact, as does the myrmecophilous beetle *Myrmecaphodius excavaticollis* (Vander Meer and Wojcik, 1982), deploying the propaganda substance only if the attack becomes too severe. There is evidence that *L. acervorum* workers who have not met the parasite can pick up the propaganda substance from nestmates which have. This would speed up the spread of the substances through the defending force, potentially vital for a single parasite pitting itself against an entire colony.

The use of Dufour's gland secretions as chemical weapons in the penetration of host colonies may also occur in the inquiline *Doronomyrmex pacis*. Buschinger (1974b) discovered the Dufour's gland to be hypertrophied in this species as well. Intriguingly, it is also a close relative of *L. kutteri*, and parasitizes the same host species, *L. acervorum*. However, another related parasite of *L. acervorum*, *L. goesswaldi*, appears to have a Dufour's gland only slightly larger relative to its body than that of *L. acervorum* (Buschinger, 1974b).

In the slave-maker *H. sublaevis*, our results confirm the hypothesis (Buschinger, 1974a) that this ant possesses a chemical weapon produced in quantity by the Dufour's gland. In this species there is no difficulty in seeing how substances which provoke internecine battles in host workers serve the needs of the parasite, since both colony-founding *H. sublaevis* queens and workers on slave raids aim to drive all adult host ants from the brood of their target colonies. However, an explanation remains to be found for the strong similarity both in the behavioral effects of the propaganda substances of *L. kutteri* and *H. sublaevis* and also in their glandular origin. This similarity may be due to a convergent evolution, especially since the parasites share the same host. Alternatively, the close phylogenetic relatedness of these two parasites, and of both to their host (Buschinger, 1981), argues for common ancestry as the explanation. Chemical analysis of Dufour's gland secretions could be used to discriminate between these alternatives. Since these secretions appear to override nestmate recognition, the analysis of these substances may also provide special insights into the chemical basis of colony-specific odor.

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