J. Insect Physiol. Vol. 30, No. 4, pp. 265–270, 1984
Printed in Great Britain.

0022-1910/84 $3.00 + 0.00

COLONY-SPECIFIC TERRITORIAL MARKING WITH THE METAPLEURAL GLAND SECRETION IN THE ANT *SOLENOPSIS GEMINATA* (Fabr)

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(*Received* 30 *June* 1983; *revised* 6 *September* 1983)

Abstract—*S. geminata* workers mark the areas they explore with a secretion from the metapleural gland. The territorial mark lasts for more than 6 h. Territories without the chemical mark induce the workers to initiate recruitment to that territory. Ants on their own territory or on territories impregnated with metapleural gland extract from workers of their colony, initiate more intraspecific combats than ants on territories of a different colony or on territories without the chemical mark.

Key Word Index: Territorial marking, pheromone, metapleural gland, ant, *Solenopsis geminata*

INTRODUCTION

A territory as defined by Hölldobler and Wilson (1977) is "an area occupied more or less exclusively by an animal by means of overt defense or advertisement". A "true territorial pheromone is a substance deposited on a portion of the home range that induces aversive or agonistic behaviour in intruders belonging to the same species". These definitions do not offer simple criteria which allow one to decide experimentally if an organism uses a territorial pheromone or not. Therefore we suggest 4 criteria in order to define a territorial pheromone: (1) A territorial pheromone has to be a chemical secreted by the organism onto the substrate on a portion of the home range. (2) The chemical(s) has to have intraspecific differences detectable by the organism. The organism has to recognize its own mark and has to differentiate it from marks of conspecifics. (3) The presence of the territorial signal has to give some advantage to the organism producing the signal. (4) A marked area should be respected by conspecifics. The end result should be a reduction in aggressive interactions between organisms (Wilson, 1976), because "individual animals or groups are spaced out more than would be expected from a random occupation of suitable habitats" (Davies, 1978). This is a sufficient, but not necessary, condition for territory. Criteria 3 for example could be fulfilled without criteria 4.

In the case of social insects, the colony will play the role of the individual organism.

Few reports on territorial behaviour or territorial pheromones in ants are known (Hölldobler, 1979). Jaffe *et al.* (1979) reported colony-specific territorial marking in *Atta cephalotes*. The territorial marking pheromone of this ant is produced in the "valves gland" (Bazire-Benazet and Zylberberg, 1979; Jaffe *et al.* 1979). For *Oecophylla longinoda*, territorial marking with rectal sac secretions has been reported (Hölldobler and Wilson, 1977). Cammaerts *et al.* (1977, 1978, 1981) proposed a function as territorial marking for the Dufour's gland secretion in *Myrmica* ants. For *Pogonomyrmex badius* (Hölldobler, 1976), *Formica rufa* (Skinner, 1980) and *Solenopsis saevissima* (Wilson *et al.*, 1971), evidence for territorial behaviour has been presented although no references to possible chemical marking of the territories were made.

N. Wilson *et al.* (1971) described foraging territories for *Solenopsis saevissima* colonies in the field. E. Wilson (1962) reported for laboratory colonies of the same species, that workers recruit nestmates to empty new areas, if they are given access to them. It is not known if the 2 types of territorial behaviour are related or if they are 2 different types of behaviour, related to 2 different situations, i.e. foraging and territorial expansion in space-limited laboratory colonies.

Solenopsis species use various different exocrine glands for chemical communication. The mandibular gland secretes an alarm pheromone (Wilson, 1962; 1965), and the Dufour's gland secretes a trail pheromone (Wilson, 1959; 1962; Barlin *et al.*, 1976). The function of the other glands in the workers is not known.

In this work, we describe the existence of a territorial pheromone, produced by the metapleural gland, in *Solenopsis geminata*.

MATERIALS AND METHODS

Eighteen colonies of *Solenopsis geminata* (Fabr.) were collected at the Universidad Simón Bolivar campus gardens, Estado Miranda, Venezuela. Each colony was placed into a 20–1 plastic container together with the earth and nest materials collected with the colony. The walls of the container were smeared with silicon oil in order to prevent the ants from escaping. The nest was connected to a foraging table with the help of a wooden bridge. The colonies were kept with natural illumination and were fed with

meat and honey (Puche, 1982). Each experiment was repeated in at least 10 different colonies.

In order to measure recognition of territories by the ants, two different bioassays were used. Bioassay I consisted of counting the number of ants on each of 2 cartons (15 × 15 cm). The carton cards were connected to a common point on the foraging table of a colony with 2 paper bridges. Ten min after connecting the cards to the foraging table of a colony, the number of ants on each card was counted simultaneously.

Bioassay II consisted of: Two workers, each from a different colony, were placed into a Petri dish (5 cm diameter) with a filter paper covering the bottom of the dish. The 2 ants were observed for up to 30 min and any attack, i.e. grasping the opponent with the mandibles while curving the abdomen towards the opponent, was recorded. For each assay, the occurence of attack and the ant which initiated it was noted. Three different observers repeated the experiment as "blind tests".

Cartons and filter papers for Bioassay I and II were prepared in the following way. They were either "naturally marked" or they were impregnated with different extracts. "Naturally marked" cartons or filter papers were those which were placed on the foraging table of the colony for more than 24 h, allowing the ants to explore them and eventually mark them. Extracts were prepared by dissecting the corresponding parts of 2 workers of the same colony. The parts were either the heads, the thoraces with their legs, the thoraces with their legs removed, the legs, or the gaster. Two worker parts were then placed in 0.5 ml of methanol (Fisher, P A). With thin glass rods, the parts were crushed. The solution was then applied onto 1 filter paper or carton using Pasteur pipette, trying to get the solution as homogeneous as possible on the substrate.

Extracts of metapleural glands were prepared in a similar way. The gland was not dissected, but the secretion was collected by introducing a fine glass capillary into an opening of the gland. This operation was made with the help of a dissecting microscope and a micromanipulator. The glass capillaries were then crushed in 0.5 ml of methanol as described above. The content of 2 capillaries, i.e. 2 glands, was used to impregnate either 1 carton or 1 filter paper.

With the exception of papers or cartons with no previous treatment (new papers or cartons), all substrates were used 10 min after removing them from the foraging table, or 10 min after the extract was applied on them. In some experiments shown in Table 1 and 2 the cartons were left at room temperature for 1, 6, 12 or 24 h before using them in Bioassay I.

All statistical tests used were taken from Siegel (1956).

RESULTS

When laboratory colonies, experimentally restricted in their foraging area, were given access to a new foraging field, such as a blank carton, the workers moved into the new area in large numbers in a similar fashion to that observed by Wilson (1962) in *S. saevissima*. They laid recruitment trails in order to attract nestmates to the new area. After the initial "land-rush", the number of workers on the card gradually declined and finally stabilized after about 2 h. When the carton, before connecting it to the foraging field, was placed on the foraging field for about 24 h, no recruitment was observed. In this case, some workers explored the carton in its new position but they did not lay recruitment trails back to the nest. Colonies in the field did not recruit nestmates to new cartons placed near their nest. Based on this behaviour of the laboratory colonies, Bioassay I was developed.

Table 1 shows the results of Bioassay I. Here the numbers of ants recruited to 2 different cartons were measured, after they had been connected to the foraging table. We observed that a new carton (*N*) induced a higher level of recruitment than a carton that formerly had been explored by the colony for 24 h (*C*) (*C* vs. *N*, Table 1). When two "naturally marked" cartons were compared, i.e. 2 cartons that had been explored by 2 different colonies (*C* vs. *D*, Table 1), where *D* had been explored by a foreign conspecific colony, no differences in the number of ants recruited to each carton was observed. If we

Table 1 Median and range of the number of ants recruited to two different carton cards, connected simultaneously to *S. geminata* colonies (Bioassay I)

Pairs of cards (*A* vs *B*)	Median (range) on: *A*	*B*	Wilcoxon matched pairs test (p)
C vs *N*	6 (1–23)	21 (2–87)	< 0.05
C vs *D*	13 (2–31)	14 (1–32)	> 0.1
D vs *N*	8 (1–19)	26 (3–52)	< 0.05
C after 1 h vs *N*	6 (2–23)	33 (5–66)	< 0.01
C after 1 h vs *N*	6 (2–30)	26 (2–70)	< 0.01
C after 12 h vs *N*	25 (3–72)	24 (2–69)	> 0.1
C after 24 h vs *N*	25 (4–72)	28 (4–81)	> 0.1

The cards had been explored previously by the same colony where the assay was performed (*C*), by another conspecific colony (*D*), or they were new cards (*N*). One replicate in each of 12 different colonies for each experimental situation were performed.

offered *D* and *N* cartons, again a large number of ants went to the new carton (*D* vs. *N*, Table 1). These experiments show that ants differentiate new territories from those which had formerly been explored by them. Thus, results of Bioassay I suggest the existence of a species-specific mark on the territory of this ant.

The data showed great intercolony variations in the number of ants recruited to each carton, probably due to differences in the activity level or to the "motivation" between the colonies. Therefore, in the experiments on Table 1, each colony was used as its own control, and the statistical tests were performed comparing the difference in the number of ants on the 2 cartons for each replicate colony, using the Wilcoxon's matched-pairs test.

In order to determine the duration of the territorial mark, experiments were performed in which the time lapse between the removal of the carton from the colony and the bioassay were varied. The results using Bioassay I (Table 1), show that the territorial cue which allows for the recognition of the "naturally marked" cartons, lasts for more than 6 h. These experiments suggest the presence of a chemical signal, deposited by the ants on the carton cards during exploration, which is used by the ants for territorial recognition. No other type of signal could explain the results on Table 1.

In order to locate the source of this chemical mark, different methanol extracts were tested with Bioassay I. Cartons with pure solvent on it behaved as *N* cartons (Table 2). Thus, the solvent does not seem to influence the outcome of the bioassay. Extracts of gasters, legs and thoraces with legs seemed to have an attractive effect, as they recruited significantly more workers than pure solvent on the cartons. Extracts from whole ants and from heads induced similar recruitment as the pure solvent. Only cards impregnated with legless thorax extracts and metapleural gland extracts did not induce recruitment of workers or induced significantly less recruitment as compared to cards with solvent. The effect of the metapleural gland extracts lasted for at least 6 h. If the whole ant extract was tested 6 h after impregnating the card, it induced less recruitment than similar cards with solvent. Extracts of heads, gasters and legs pooled together and tested 6 h after impregnating the cards induced similar recruitment as cards treated with solvent alone (Table 2). These results show that the territorial effect measured with Bioassay I is produced by the metapleural gland secretion from the thorax. No other body part is able to stop inducement of recruitment, even 6 h after application on the cards, such as "naturally marked" cards do (Table 1). If the metapleural gland extracts are compared with "naturally marked" cards from a conspecific colony, no differences in the number of ants recruited to the cards can be observed (Table 2). Thus, we can confidently assure that the metapleural gland secretion, if applied on carton cards, makes the cards indistinguishable to "naturally marked" cards as measured with Bioassay I.

Experiments in Table 2 also show that legs and the gaster contain attractive substances (and possibly the head also), as their extracts induced more recruitment to the cards than new cards with pure solvent alone. The fact that whole ant extracts behaved as the pure solvent, may be due to the fact that the addition of different pheromones cancels the effect of each of them. This may also explain the fact that thoraces with their legs produced a different effect in Bioassay I than did legless thoraces. Thus, from Table 2 we might conclude that legs and gasters contain attractive substances which exert their effect for less than 6 h. Experiments in which gaster extracts were presented in the form of a trail, induced trail following, but leg extracts did not (Puche, 1982).

The experiments with Bioassay I suggest that *S. geminata* uses a metapleural gland secretion to mark the foraging area with a specific mark. In order to see if this mark is also colony specific, a more sensitive bioassay was developed. Bioassay II measures the

Table 2. Median of the number of ants recruited to two different cartons, connected simultaneously to *S. geminata* colonies (Bioassay I)

Extract	*E*	*S*	*n*	*p* Wilcoxon's matched pairs test
No extract (new paper)	9	11	15	NS
Whole ant	11	10	15	NS
Head	16	10	10	NS
Thorax with legs	14	9	11	< 0..01
Legles thorax	15	21	13	< 0.01
Legs	26	11	13	< 0.01
Gaster	34	16	13	< 0.01
Metapleural gland	7	13	10	< 0.02
Metapleural gland after 6 h	7	16	10	< .01
Whole ant after 6 h	9	15	15	< 0.01
Head, gaster and legs after 6 h	7	7	15	NS
Metapleural gland extract vs. naturally marked control card	12	14	13	NS

NS indicates not significant differences ($\alpha = 0.1$).

The cards were impregnated with either an extract (*E*) or with pure solvent (*S*). *n* indicates the number of colonies tested.

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