Caste, colony, and species specificity of the trail pheromone in two sympatric Nasutitermitinae

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ABSTRACT

Using Y-choice bioassays for testing the preference for two artificial trails, made from sternal gland extracts from workers and/ or soldiers, we demonstrate the existence of caste, colony and species specificity of the pheromone trail following response of the sympatric termite species *Nasutitermes ephratae* and *N. corniger*. The results suggest that both quantitative and qualitative differences in the trail pheromone are used by the termites to discriminate among conspecific and non-specific trails. The ecological role of trail following specificity is discussed.

Termites live in colonies that exhibit a well-marked division of labor associated with presence of several distinctive castes. The presence and utilization of trail pheromones in termites for orientation and recruitment during foraging is widely acknowledged (Lüscher & Müller 1960, Stuart 1961, Traniello 1982; Reinhard & Kaib 1995). In many termite species, a short-lived recruitment and a durable orientation component of the sternal gland secretion serve as trail pheromone (Traniello & Robson 1995). This two-component system seems to have evolved several times among insect species, since it is also widely used by ants (Jaffe 1984).

Some studies propose that the sternal gland secretion is not caste-specific in its recruitment effect but induces recruitment of soldiers and workers depending upon pheromone concentration (Tschinkel & Close 1973; Traniello & Busher 1985). However, behavioral evidence for trail pheromone species specificity (Gessner & Leuthold, 2001) and even colony specificity (Oloo 1981) in trail discrimination experiments has been suggested. *Nasutitermes corniger* (Motchulsky) and *N. ephratae*

(Holmgren) (Termitidae: Nasutitermitinae) are sympatric and can be found in neotropical gallery forests, exploiting the same food sources. Thus, as part of a more extensive study of these species, we present here behavioral evidences for species, colony, and caste specificity of the trail pheromone of these species.

METHODS

Study species

Arboreal nests of *N. ephratae* and *N. corniger* were collected in gallery forests located in the Miranda State, Venezuela. The nests were taken to the laboratory at the Simón Bolívar University, where they were placed on an acrylic platform (1 x 1 m) in a room with ≈70% of relative humidity and ≈26^{o}C of temperature. The photoperiod was 12:12 L: D. Each nest was supplied with dry wood and water *ad libitum*.

Sternal Glands Extracts

Foraging workers and soldiers were immobilized by exposure to dry ice. Sternal gland extracts were prepared by excising the abdomen between the second and seventh sternites from 300 workers and 300 soldiers for each species. Each group of 40 sternites was immersed in 2 ml of hexane and stored at -15 ºC. Pure hexane was used as control. The concentration of the extracts used in the bioassays was 0.01 glands/µl of solvent, which induced trail following activity on the termites.

Activity and Bioassays

To determine preferences for the trail pheromone, a dual trail with a Y configuration was used. Two 10 cm long parallel trails were drawn on a glass platform (20 cm x 30 cm) using five µL of each of the sternal gland extract (0.0025 gl/cm) of the colony, species, or caste involved. At the bifurcation point on the Y, the trails diverged at a 45^{o} angle. For each bioassay, we placed 10 individuals inside a Petri dish that was modified to allow the exit of termites without disturbance. For each replicate, we evaluated the first individual exiting the Petri dish and following the trails drawn as a Y on the glass platform. Responses were considered positive when the termites walked through the end of one branch of the Y diagram in less than 5 min. After this period, a new platform and news individuals were used. Forty five replicates were made for each bioassays: caste from de same colony; castes from different colonies and between both species. A Sign test ($p< 0.01$) was used to analyze individual preferences of individuals (Sokal & Rolhf, 1995).

RESULTS

Differences between castes

The results of dual trail bioassays on the worker and soldier castes of *N. corniger* and *N. ephratae* are given in Table 1. Caste differences were also observed for the two termite species. Workers and soldiers of *N. corniger* showed preference by the trails made from sternal gland extracts from conspecific soldiers over that from workers. The opposite was true for *N. ephratae*, where both castes choose significantly trails made from sternal gland extract of conspecific workers when tested against trails from conspecific soldiers (Table 1).

Differences between colonies of same species

Workers and soldiers of either species preferentially chose the trails made from glands of their own colony nestmates (Sign test $P<0.05$; $N= 180$), showing that these extract were identified as colony specific.

Differences between species

The results of the dual trail bioassays given in table 1 show a certain species specificity in the trail following behavior elicited by sternal gland extracts. In most cases, termites preferred the trails made from extracts of conspecifics. The exception was *N. ephratae* workers that showed a balanced preference for soldier extracts of their own species and those of *N. corniger.*

DISCUSSION

Caste and colony specificity in trail discrimination experiments have not been observed for some termite species (Oloo & McDowell 1982). Our results clearly show that the sternal gland secretion of the sympatric termites *N. corniger* and *N. ephratae* carries sufficient information to provide nestmates with clues about the colony, the caste, and the species nature of the trail pheromone deposited on the substrate.

Studies of colony specificity in termites are scarce and fail to show discrimination among different colonies. At the early stages of foraging behavior of some *Nasutitermes* species, i.e. when foraging tunnels have not yet been built, agonistic interactions occur frequently between neighboring colonies (Traniello & Leuthold 2000). Therefore, it is possible that the colony specificity of the trail pheromone observed in the bioassays might function as an advertisement for foragers about the foraging territory of an alien colony or even as a signal used for competitive displacement (LePonce et al. 1996), leading to the colonization of territories occupied by other colonies. The nature of the colony specific components of the trail pheromone remains to be determined.

According to Bordereau et al. (1993) species specificity can be explained by quantitative differences of the trail pheromone. Furthermore, Kaib et al. (1982), working with several species of Rhinotermitidae, Hodotermitidae, and Termitidae found that when given no other choice, workers followed trails made from extracts from different species at approximately the same threshold levels as conspecific extracts. The balanced preference of *N. ephratae* workers by soldier extracts of their own species and those of *N. corniger* suggest the existence of a common component in the soldier gland, which is present in the trail-active extracts of both species.

However, behavioral evidence also suggests that additional compounds may act as cues for the termites to differentiate between conspecific and nonspecific trails (Peppuy et al. 2001). Howard et al. (1976), demonstrated that *Reticulitermes* spp. and *Coptotermes formosanus* (Rhinotermitidae), presented species-specific trail following behavior when the pheromone was present at just above threshold level, suggesting that secondary compounds may be responsible for the species-specific trail following behavior. Thus, a species-specific component of the trail pheromone of *N. ephratae* and *N. corniger* appears to attract the termites towards conspecific trails, suggesting a multicomponent nature of trail pheromones in these termite species. Up to date, only a

few compounds have been identified as trail pheromones in termites, however no species-specific components of the trail pheromone have yet been identified (Traniello & Leuthold 2000).

Chemically, the sternal gland secretion of *N. corniger* and *N. ephratae* is a complex blend constituted by numerous common compounds. For *N. ephratae*, these compounds were about 1.5 times more concentrated in glands of workers as compared from their soldiers. The opposite occurred in *N. corniger* where the worker/soldier concentration ratio was 0.2 times (Arab & Issa, unpublished results). Therefore, quantitative differences of the sternal gland secretion may explain why extracts *of N. ephratae* workers and *N. corniger* soldiers were highly preferred by conspecific workers and soldiers. Traniello (1982) reported for *N. costalis* that trails deposited by soldiers mainly recruit soldiers, whereas trails laid by workers recruit both castes. The differential caste recruitment dynamics observed in these species might be due to the quantitative differences of the trail pheromone between workers and soldiers, which may act as a mechanism to regulate the foraging behavior of these termites. However, chemical differences in the sternal gland secretion between castes, in addition to the concentration differences, cannot be discarded.

The recruitment system of *Nasutitermes* species is among the most sophisticated known for termites, resembling that of the autocratic decision making system described for some ants (Andara et al. 2004), and caste polyethism of workers and soldiers (Traniello & Buscher 1985) may be explained in part by the specific effect that the trail pheromone will induce on each caste, as reported in other termites (Gessner & Leuthold 2001). Nevertheless, the specific chemical cues allowing trail preferences and the social function of this caste specific trail pheromone in the context of the colony remains to be studied.

Our results show that in the case of the two *Nasutitermes* studied, the sternal gland carry sufficient information to provide clues as caste, colony and species. These results is congruent with the multicomponent nature of the trail pheromone and strongly suggest that workers and soldiers have different functions when trail laying, as their trail pheromone will elicit different responses from their colonies.

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Table 1. Summary of results from the bioassays testing preference for trails drawn with sternal extracts.

Nasutitermes individual tested	Trail with sternal gland of	Preference for	Preference in %	# of colonies tested x total # of replicate tests
Testing for caste specificity				
Ew	Ew-Es*	w	98	4 x 180
Es	Ew-Es*	s	93	4 x 180
Cw	Cw-Cs*	s	92	4 x 180
Cs	Cw-Cs*	s	98	4 x 180
Testing for colony specificity				
Ew	Ew_1-Ew_2	Own colony	70	4 x 180
Es	Es_1-Es_2	Own colony	70	4 x 180
Cw	Cw_1-Cw_2	Own colony	80	4 x 180
Cs	Cs_1-Cs_2	Own colony	80	4 x 180
Testing for species specificity of extracts from workers				
Ew	Ew-Cw	E	86	4 x 180
Es	Ew-Cw	E	79	4 x 180
Cw	Ew-Cw	C	82	4 x 180
Cs	Ew-Cw	C	75	4 x 180
Testing for species specificity of extracts from soldiers				
Ew	Es-Cs	C	53[#]	4 x 180
Es	Es-Cs	E	79	4 x 180
Cw	Es-Cs	C	89	4 x 180
Cs	Es-Cs	C	85	4 x 180

*all from the same colony
E = *N. ephratae*
C = *N. corniger*
w = worker
s = soldier
[#] Data no significantly different from random choice. All other data are significantly different from a random choice as tested with a Sign Test ($p < 0.01$)