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# LABORATORY INVESTIGATIONS OF THE TRAIL-FOLLOWING RESPONSES OF FOUR SPECIES OF LEAF-CUTTING ANTS WITH NOTES ON THE SPECIFICITY OF A TRAIL PHEROMONE OF *ATTA TEXANA* (BUCKLEY)

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## SUMMARY

Interspecific trail following activity of poison sac contents from four species leaf-cutting ants was investigated. With only one exception, all the species teste followed trails made from each others poison sac contents. However when the ant were given a choice of following one of two separate trails, clear differences were show in the poison sac contents of the three species tested.

The responses of 24 species of ants and one species of termite to an *Atta texan* (Buckley) trail pheromone were tested.

## RÉSUMÉ

### *Etude au laboratoire de réponses mutuelles aux pistes chez quatre espèces de Fourmis* Atta. *Spécificité d'une phéromone de piste de* Atta texana *(Buckley).*

Nous avons étudié comment quatre espèces de Fourmis *Atta* suivent mutuellemen les pistes tracées avec le contenu de leur sac à venin. Toutes les espèces étudiées on suivi les pistes des autres à une seule exception. Cependant, lorsque nous avons donn aux Fourmis le choix entre deux pistes distinctes, nous avons montré qu'il y ava des différences dans le contenu du sac à venin de trois espèces.

Nous avons étudié les réponses de 24 espèces de Fourmis et d'une espèce de Termit à une phéromone de piste de *Atta texana* (Buckley).

TABLE I. — *The numbers of « lost » ants (out of a total of 40 from four replicate experiments) that followed poison sac trails.*

TABLEAU I. — *Nombre de Fournis « perdues » (sur un total de 40 dans quatre expériences identiques) qui suivirent les pistes tracées avec le contenu du sac à venin.*

| Test species | Pheromone donor | | | |
|---|---|---|---|---|
| | *Acromyrmex octospinosus* | *Atta cephalotes* | *Atta sexdens* | *Atta texana* |
| *Acromyrmex octospinosus* | 28 | 23 | 34 | 39 |
| *Atta cephalotes* | 28 | 40 | 34 | 38 |
| *Atta sexdens* | 0 | 36 | 34 | 33 |
| *Atta texana* | 17 | 20 | 20 | 19 |

## II. — Response to a choice of trails.

A second experiment was done to determine if the ants could distinguish between trails made from poison sacs of their own species and trails made from poison sacs of other species that they would nonetheless follow. The results are shown in Table II.

TABLE II. — *The numbers of ants following « poison sac » trails when given a choice of two alternatives. All individual results are pooled from eight separate tests.*

TABLEAU II. — *Nombre de Fourmis qui ont suivi les pistes tracées avec le contenu du « sac à venin », lorsqu'elles ont eu le choix entre deux solutions possibles. Nous avons rassemblé les résultats de huit expériences.*

| Test species | Pheromone donors | | |
|---|---|---|---|
| | *A.o. : A.c.* | *A.s. : A.c.* | *A.o. : A.s.* |
| *Acromyrmex octospinosus* | 42 : 19<br>($p < 0.01$) | — | 57 : 11<br>($p < 0.001$) |
| *Atta cephalotes* | 40 : 98<br>($p < 0.001$) | 73 : 62<br>($p > 0.3$) * | — |
| *Atta sexdens* | — | 93 : 48<br>($p < 0.001$) | — |

— : not tested.
The probabilities shown are calculated from pooled Chi squared values assuming a 1 : 1 ratio.
* Replicates showed significant heterogeneity.

In all cases, but one, the test species followed trails from poison sacs of its own species in preference to those from sacs of other species, the exception being *Atta cephalotes* which showed no significant preference between its own poison sac contents and those of *Atta sexdens.*

*Atta cephalotes* was used for further experiments using a similar technique. Foraging workers were given a choice of following trails made from poison sacs of ants from the same colony (« own ») and trails made from sacs removed from ants of a different colony (« strange »). The pooled results from eight replicates gave an « own » followed to « strange » followed ratio of 96 : 91 (the pooled Chi squared value based on 8 replicates with an expected ratio of 1 : 1 =

0.13 with 1 d.f. $p > 0.7$ replicates were homogenous). A similar experiment was also done with natural trails by using string on which the ants had been recently foraging. The pooled results gave an « own » : « strange » ratio of 96 : 87 (the pooled Chi squared value based on 10 replicates with an expected ratio of 1 : 1 = 0.44 with 1 d.f. $p > 0.6$ replicates showed significant heterogeneity). To ensure that the foraging ants were making a choice at the « Y » junction a treated string was compared with an untreated string. The pooled results gave a « treated » : « untreated » ratio of 25 : 5 (the pooled Chi squared value based on 2 replicates with an expected ratio of 1 : 1 = 13.33 with 1 d.f. $p < 0.001$. Replicates were homogenous).

### III. — Bioassay of methyl-4-methylpyrrole-2-carboxylate.

M4MP2C was tested for trail following activity with a total of 24 species from several subfamilies of ants and on one species of termite. The responses of the ants are shown in Table III.

Table III shows that in general, Attines will follow trails of M4MP2C between the ranges of 4.0 pg to $4.0 \times 10^4$ pg spread around a 50 cm circular trail, the only exception was *Atta sexdens* which could not be made to follow a M4MP2C trail at any of the concentrations tested. Non-attine species did not follow trails streaked with M4MP2C. The termite *Reticulitermes flavipes* (Kollar) did not follow a trail of M4MP2C.

## DISCUSSION AND CONCLUSIONS

Several workers have shown in the laboratory that attine trails prepared from poison sac extracts are not species or even genera specific. However, responses of ants to artificial trails in the laboratory cannot always be correlated with field observations, and field studies indicate that the trails of leaf cutting ants appear to be specific. In field studies with *Atta cephalotes isthmicola* (Weber) and *Atta colombica tonsipes* (Santchi), Weber (1969) demonstrated that workers of both species when placed onto the trail of the other species, recognised the odour trail, became excited, and quickly left it. Blum *et al.* (1964) also noted in two field populations of leaf cutting ants that emit. *Acromyrmex octospinosus* workers when placed on an *Atta cephalotes* trail, would invariability leave the trail and resume trail following only when it crossed an *A. octospinosus* trail. They also recorded that although active field trails of *A. cephalotes* and *A. octospinosus* crossed in at least two places, in no instances were the ants seen to follow the trail of the other species. They could not duplicate this behaviour in the laboratory using poison sac extracts and attributed it to compounds other than trail substances (e.g. anal products). However Blum and Portocarrero (1966)

TABLE III. — *The numbers of ants responding to 10 μl of various concentrations M4MP2C trailed around a 50 cm circumference circular trail.*

TABLEAU III. — *Nombre de Fourmis ayant répondu à 10 μl de solutions de M4MP2 des concentrations variées, répandues selon une piste circulaire de 50 cm de diamè*

| Species tested | Number of ants tested at each concentration | Concentration of M4MP2C per μl | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Blank | 0.4 pg | 4.0 pg | 0.04 ng | 0.4 ng | 4.0 ng | 0.04 μg | 0.4 μg |
| FORMICIDAE : | | | | | | | | | |
| Attini : | | | | | | | | | |
| [d]*Atta texana* (Buckley) | 20 | 0 | 0 | 13 | 17 | 19 | 13 | 3 | 0 |
| [e]*Atta sexdens* (L.) | 30 | 0 | 0[g] | 0 | 0 | 0 | 0 | 0 | 0[g] |
| [f]*Atta sexdens* ssp. *rubropilosa* Forel | 15 | 0 | 0 | 0 | 3 | 1 | 1 | 0 | 1 |
| [e]*Atta cephalotes* (L.) | 30 | 0 | 1 | 11 | 16 | 10 | 5 | 4 | 4 |
| [a]*Atta colombica* Guerin | 30 | 0 | — | 1 | 13 | 6 | 4 | 0 | 0 |
| [f]*Atta laevigata* (F. Smith) | 15 | 0 | 0 | 4 | 0 | 5 | 7 | 0 | 0 |
| [e, b]*Acromyrmex octospinosus* (Reich) | 80 | 0 | 0 | 22 | 45 | 56 | 29 | 11 | 0 |
| [f]*Acromyrmex niger* (F. Smith) | 15 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| [d]*Acromyrmex versicolour* Pergande | 20 | 0 | 0 | 6 | 10 | 16 | 10 | 5 | 0 |
| [d]*Trachymyrmex septentrionalis* (McCook) | 20 | 0 | 0 | 11 | 11 | 14 | 7 | 0 | 0 |
| [b]*Trachymyrmex urichi* (Forel) | 30 | 0 | 0 | 14 | 17 | 20 | 13 | 1 | 0 |
| [b]*Apterostigma collare* (Emery) | 30 | 0 | 0 | 13 | 19 | 16 | 20 | 3 | 0 |
| [d]*Cyphomyrmex rimosus* (Spinola) | 20 | 0 | 0 | 6 | 10 | 16 | 10 | 7 | 0 |
| Non-attine species : | | | | | | | | | |
| [d]*Cardiocondyla venustula* W.M. Wheeler. | 6 | — | — | 0 | — | 0 | — | 0 | — |
| [d]*Pheidole dentata* Mayr | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [d]*Solenopsis invicta* Buren | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [d]*Pogonomyrmex barbatus* F. Smith | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| [d]*Crematogaster clara* Mayr | 7 | — | — | — | — | 0 | — | 0 | — |
| [c]*Neivamyrmex nigrescens* (Cresson) | 20 | 0 | — | 0 | — | 0 | — | 0[h] | — |
| [e]*Formica rufa* (L.) | 10 | 0 | — | — | 0 | — | 0 | — | 0 |
| [d]*Paratrechina parvula* (Mayr) | 5 | — | — | 0 | — | 0 | — | 0 | — |
| [d]*Camponotus pennsylvanicus* (DeGreer). | 4 | — | — | — | — | 0 | — | 0 | — |
| *Iridomyrmex pruinosus* (Roger) | 6 | — | — | — | — | 0 | — | 0 | — |
| [d]*Pseudomyrmex brunnee* (F. Smith) | 2 | — | — | 0 | — | 0[i] | — | 0[i] | — |
| ISOPTERA : | | | | | | | | | |
| [d]*Reticulitermes flavipes* (Kollar) | 7 | — | — | — | — | 0 | — | 0 | — |

a : Bioassayed by M. .M. MARTIN.
b : Bioassayed by M. S. BLUM and identified by N. A. WEBER.
c : Bioassayed by J. F. WATKINS II.
d : Bioassayed by J. C. MOSER.
e : Bioassayed by S. W. ROBINSON.
f : Bioassayed by E. AMANTE.
g : 20 ants tested.
h : 30 ants tested.
i : 1 ant tested.
— : not tested.

showed that not all Attine poison sac contents are identical by demonstrating t *Sericomyrmex urichi* will not follow an *Atta texana* trail and will only weal follow a trail prepared from a *Trachymyrmex septentrionalis* poison sac.

When « lost » ants from the four species were tested on a circular tr produced from poison sac extracts of the other species, most ants readily follow the trail, the only exception being *Atta sexdens* which would not follow a trail from an *Acromyrmex octospinosus* poison sac. This is somewhat surprising as the trail following response of *A. octospinosus* to *A. sexdens* poison sacs is strong.

Blum and Ross (1965) demonstrated that trails prepared from poison sac extracts of myrmicines in unrelated genera could be non specific even at the generic level. They suggested that it was not improbable that myrmicines might have a number of common chemical features in their venom and that even though ants from different genera might readily follow trails made from each others poison sacs extracts, they may each be employing different compounds for their odour trail pheromone. It seems that the compound that *Atta sexdens* utilizes for trail following is not found in *Acromyrmex octospinosus* poison sacs, although the compound by *A. octospinosus* is present in the *A. sexdens* poison sac but not used as a trail marker by *A. sexdens.* This would allow *A. octospinosus* to follow trails made from *A. sexdens* poison sacs but not vice-versa.

Watkins *et al.* (1967) using various species of army ants demonstrated that in nearly all cases, army ants would readily follow trails made by workers of other army ant species. When they were given a choice of either of two trails to follow, all the species tested (with one possible exception) chose to follow a trail laid by a member of its own species. Similar results were obtained in the present trials with the attines. Although in general attines will readily follow trails made from poison sacs of other attines, the results of the trail preference tests (table II) show that differences in poison sac contents do exist both between and within genera. In only one experiment, when *A. cephalotes* was given a choice of a trail made from its own or an *A. sexdens* poison sac, was there evidence of a species being unable to distinguish between the two trails. However when the same choice was tested with *A. sexdens* a clear preference was shown. The results for *A. cephalotes* do not preclude the possibility of *A. cephalotes* being able to recognise these differences in the field where both these species may be found in the same habitat.

The abilities of the ants to distinguish between the poison sac contents of various species in the same genus can only be demonstrated by the more sensitive trail choice experiments described.

As table III demonstrates the volatile component of the trail secretion of the texas leaf cutting ant *Atta texana* (M4MP2C) was tested on 24 species of ants from several tribes and on one trail laying termite. No non-attines followed a M4MP2C trail, although all the attines tested, with the exception of *Atta sexdens,* did. As table I shows, *Atta sexdens* together with *A. cephalotes* and *Acromyrmex octospinosus* will readily follow trails made from whole *A. texana* poison sacs, and this again suggests that the Attines are not all using the same compounds for trail marking. *Atta sexdens* is clearly either responding to some other compounds in the *A. texana* poison sac or will only follow trails of M4MP2C when some other compounds are also present. Although *Atta sexdens* could not be made to follow a M4MP2C trail, Robinson and Cherrett (1973) have shown that M4MP2C is an effective attractant in baits for *A. sexdens* as well as for *A. cephalotes* and *A. octospinosus.*

Thus it appears that at least some of the Attines utilize, for the purpos trail following, different trace constituents in their venom.

Tumlinson *et al.* (1972), whilst assaying fractions of the volatile compon of an *Atta texana* extract, found that more than one fraction was active promoting trail following although larger quantities of the fractions witl M4MP2C were needed. It is these minor active components that may al leaf-cutting ants to make « choices » when given an option of two trails possibly one of these that *Atta sexdens* relies on particularly for marking following trails.

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## REFERENCES

1. Blum (M. S.), Moser (J. C.) and Cordero (A. D.), 1964. — Chemical releasers of so behaviour. II. Source and specificity of the odour trail substance in four at genera (Hym. Form.). *Psyche. Camb., 71,* 1-7.
2. Blum (M. S.) and Portocarrero (C. A.), 1966. — Chemical releasers of so behaviour. X. An attine trail laying substance in the venom of a non t laying myrmicine, *Daceton armigerum* (Latreille). *Psyche. Camb., 73,* 150-
3. Blum (M. S.) and Ross (G. N.), 1965. — Chemical releasers of social behaviour. Source, specificity and properties of the odour trail pheromone of *Tetramor guineense* (F.) (Formicidae Myrmicinae). *J. Insect Physiol., 11,* 857-868.
4. Hangartner (von W.), 1969. — Orientierung von *Lasius fuliginosus* (Latr.) an ei gabelung der geruchsspur. *Ins. Soc., 16,* 55-60.
5. Moser (J. C.) and Blum (M. S.), 1963. — The trail marking substance of the Te leaf cutting ant : Source and potency. *Science N.Y., 140,* 1228.
6. Robinson (S. W.) and Cherrett (J. M.), 1973. — Studies on the use of leaf cutt ant scent trail pheromones as attractants in baits. *Proc. Int. Union for St of Soc. Insects VII Congr.,* 332-338.
7. Tumlinson (J. H.), Moser (J. C.), Silverstein (R. M.), Brownlee (R. G.) and R (J. M.), 1972. — A volatile trail component of the leaf-cutting ant, *Atta texc J. Insect Physiol., 18,* 809-814.
8. Tumlinson (J. H.), Silverstein (R. M.), Moser (J. C.), Brownlee (R. G.) and R (J. M.), 1971. — Identification of the trail pheromone of the leaf-cutting *Atta texana. Nature. Lond., 234,* 348-349.
9. Watkins (J. F.), 1964. — Laboratory experiments on the trail following of ar ants of the genus *Neivamyrmex* (Form. Doryl.). *J. Kansas Ent. Soc., 37,* 22-2
10. Watkins (J. F.), Cole (T. W.) and Balbridge (R. S.), 1967. — Laboratory studies the interspecies trail following and trail preference of army ants (Dorylin *J. Kansas Ent. Soc., 40,* 146-151.
11. Weber (N. A.), 1969. — Ecological relationships of three *Atta* species in Pana *Ecology, 50,* 141-147.
12. Wilson (E. O.), 1962. — Chemical communications amongst workers of the fire *Solenopsis saevissima* (F. Smith). II. An informational analysis of the od trail. *Anim. Behav., 10,* 148-158.
13. Wilson (E. O.) and Pavan (M.), 1959. — Glandular source and specificity of sc chemical releasers of social behaviour in dolichoderine ants. *Psyche. Camb.,* 70-76.