SITUATION AND LOCATION-SPECIFIC FACTORS IN THE COMPATIBILITY RESPONSE IN *RHYTIDOPONERA METALLICA* (HYMENOPTERA: FORMICIDAE: PONERINAE)*

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Introduction

Hangartner, Reichson, and Wilson (1970) reported some years ago that individual communities of harvester ants of the genus *Pogonomyrmex* are able to distinguish the scent of their own nesting material from that of other conspecific colonies. Holldobler and Wilson (1977) were able to show that the African weaver ants, *Oecophylla longinoda*, mark and advertise individual community territories by means of colony-specific pheromones deposited in the rectal fluids. And Traniello (1980) has recently demonstrated that, in the typically densely packed aggregations of colonies of *Lasius neoniger,* persistent trunk trails are maintained which arise from recruitment trails marked, again, with hindgut material. Here we describe what we believe to be nest-area marking with hindgut material in the primitive Ponerine ant *Rhytidoponera metallica.*

Experiments and results

The tests reported here were a continuation of a series carried on for some years, and earlier reported in part (Haskins and Haskins, 1979). Material and methods were essentially as described there, and need only be briefly reviewed. The specific population used in this work was collected as a single, rather small colony taken at Montville, in the Blackall Range of northern Queensland, Australia, on December 23, 1963. It was maintained as a closed inbreeding unit in the laboratory until the fall of 1979, at which time it had greatly increased in numbers, was active and vigorous, and contained

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numerous "worker", female and male brood.[1] Other things being equal, it might have been expected to have attained considerable genetic homogeneity, since new generations of "workers" and young queens were fathered exclusively by males reared within the colony.

On November 4, 1979 this population was divided into two roughly equal halves and placed in separate arenas standing side by side on the same laboratory bench. All conditions were kept constant for the two moieties, designated *A* and *B*, except that they were maintained on differing diets, comprising crickets and dilute honey water for *A* and mealworm larvae and dilute sugar water for *B*. Two years later, on November 7, 1981, a series of compatibility tests were run between pairs of individuals taken one each from the two halves and allowed to encounter one another in fingerbowls, as described earlier. These demonstrated only very limited incompatibility, as reported earlier (Haskins and Haskins, 1979), and suggested that diet, though possibly a measurable influence, was almost certainly not a critical factor in mediating compatibility as characterized in this test procedure.

Individual pair-tests after isolation on the same diets

On November 11, 1981 a further separation of the population was made by dividing Moiety *B* into two, designated *B′* and *B″*, and continuing to maintain both on the identical diets of mealworms and sugar water, and continuing with no worker interchange or communication between them. They were held in this manner for a further year. Then, on November 15, 1982, fifty pair-tests were run between Moieties *B′* and *B″*. In all but two of these pairs, full compatibility was exhibited in the fingerbowl trials. The same tests run the next day, November 16, between members of one of the pair of moieties maintained on the same diets (*B′* and *B″*), and the first moiety, *A*, still maintained on crickets and sugar water, showed

[1] In *R. metallica* reproduction occurs exclusively through fertilized ergatogynes which may make up from 5% to as much as 15% of the colony population and are morphologically indistinguishable from unfertilized sister workers. Thus reproduction is continuous and self-sustaining. Colonies are thus characteristically highly polygynous, and may persist nearly indefinitely under laboratory conditions. "True" females, fully winged and otherwise morphologically typical, can also be produced (and frequently were in the present population) but they seem to be without reproductive function, soon dealating themselves, functioning briefly as workers, and dying in a short time.

results generally confirmatory of those reported earlier, though with somewhat higher levels of aggression than the year before. Thus, of fifty-one pairs tested, 37 showed full compatibility, 8 exhibited "startle" reactions, in 2 cases there was momentary seizure with immediate release, and in 4 cases there was violent attack. Thus noncompatibility between the members of Moieties *B′* and *B″* maintained for a year separately on the same diets, was virtually negligible, while that of moiety *A* and the other pair, maintained on different diets, was generally confirmatory of earlier findings: significantly higher but still, after two years of separation, not nearly comparable with reactions toward the members of another, widely separated population taken near Sutherland, N.S.W., as earlier reported. From all these tests it might have been concluded that, as indicated by pair compatibility encounters, genetic factors were significant but were overlain by a measureable diet factor. In fact, the situation now appears more complex.

Tests with whole nests

On June 27, 1982, a single nest, housing 70–100 workers of the second moiety (*B′*) (nests consisted of earth-filled Lubbock-type glass "sandwiches" stacked) was transferred to the arena of the first moiety (*A*). The introduced nest was placed as far away as possible from the stacked *A* nests in the arena. Arenas used throughout were fabricated from 5/8 cm. thick transparent polyster sheets glued together to form lidless boxes of dimensions 59.5 cm. × 44.5 cm. × 18.5 cm. covered with screening set in wooden frames, and lined with white paper.

The reaction was immediate, violent, and virtually universal. Massed workers from *A* entered the introduced *B′* nest in force, showing unequivocal hostility, seized and dragged out almost the entire *B* population, ultimately killing a large fraction of them. The struggle went on for two days, and resulted in the apparent total occupation of the *B′* nest by *A* workers. Subsequently, this nest was fully incorporated into the *A* colony. Thus the reaction in this experiment was in dramatic contrast to the very limited aggression shown in the pair-tests.

It remained to determine whether similar behavior would occur between moieties *B′* and *B″*, which had been maintained on the same diets and, as described, had exhibited nearly complete compatibility in the pair-tests.

At 2:03 p.m. on November 15, 1982 a nest of moiety *B′* was transferred to the arena of moiety *B″* immediately following the *B′*-*B″* pair-tests described above (workers of *B′* and *B″* which had been used in the pair-tests were not returned to their respective arenas until after the nest-transfer experiment was complete). Again, in the most conspicuous contrast to the experience in *B′*-*B″* pair-tests, but in the same pattern as the reaction when the nest of *B′* was introduced to *A*, immediate mass hostility was exhibited between the two fragments. Eight minutes after introduction it had become general, with many interlocked pairs. By 6:55 p.m. pairs "clinched" and stinging were still present within the introduced *B′* nest, and disturbed young males present in that nest were emerging prematurely. This condition persisted until the following day, by which time it appeared that occupation of the *B′* colony by members of the *B″* moiety had been completed, and things settled down, leaving many dead workers in the arena.

It therefore became clear that previous dietary history was not a dominant factor in mediating the mass hostility so conspicuous between *A* and *B* on the one hand and *B′* and *B″* on the other. It remained to test whether it was in fact the presence of the "foreign" nest with its soil that triggered the mass incompatibility or simply the introduction of many alien workers at one time near the home "site"—a "mass" effect of numbers on the one hand or the possible influence of a familiar site for the test, rather than fingerbowls, on the other. To check this, at 8:00 a.m. on November 18, 1982 ten workers of *B′* were introduced together into the *B″* arena, being placed close to the entrances of the *B″* stack of nests. Reactions were completely compatible until 8:25, when two of the introduced workers were seen being dragged about. This continued for the next five minutes, when one was released, the other being freed by 8:30. There was then entire quiet and apparent compatibility until 3:15 p.m., with no further aggression except that a single worker (living and uninjured) was being dragged about the arena at 12:00 noon of the following day. The remaining nine were apparently "adopted".

Simultaneously with this experiment, the reciprocal transfer was carried out. (10 workers of *B″* introduced into the *B′* arena, near the entrances to the *B′* nests). The experiment was begun at 8:10 a.m. Here also there was complete compatibility, except for two workers seen dragged out of a nest entrance at 3:15 p.m., as observations

TABLE 1

A. 10 WORKERS OF GROUP B′ INTRODUCED CLOSE TO THE NEST ENTRANCE OF GROUP B″

	Introduced at 8:00 a.m.		
	Totally amicable reception		
8:05	″ ″ ″	8:30	2 ☿ ☿ being dragged
8:10	″ ″ ″	8:35	1 ☿ ″ ″
8:20	″ ″ ″	8:40	All quiet. No dragging seen.
8:25	2 ☿ ☿ dragged (after introduction of *A*)	8:55	All quiet. No dragging seen.
		12:00M	1 ☿ seen still being dragged
		2:00 pm	All quiet.
		3:15 pm	All quiet.

B. 10 WORKERS OF GROUP B″ INTRODUCED CLOSE TO NEST ENTRANCE OF GROUP B′

	Introduced at 8:05 a.m.		
	Totally amicable reception		
8:10	″ ″ ″	8:30	All quiet. Most ☿ ☿ inside nest
8:20	″ ″ ″	8:35	″ ″ ″ ″
8:25	″ ″ ″	8:40	″ ″ ″ ″
		8:55	″ ″ ″ ″
		12:00M	″ ″ ″ ″
		2:00 pm	″ ″ ″ ″
		3:15 pm	2 ☿ ☿ seen dragged out of nest entrance by 2 ☿ ☿ each, released unharmed.

C. 10 WORKERS OF GROUP A INTRODUCED CLOSE TO NEST ENTRANCE OF GROUP B″

	Introduced at 8:15 a.m.
8:20	No attacks whatever. Slight suspicion once or twice. Two or three ☿ ☿ bit briefly at nesting material.
8:23	1 ☿ being dragged about
8:25	2 ☿ ☿ ″ ″ ″
8:35	1 ☿ ″ ″ ″
8:40	All quiet. No further dragging seen.
8:55	″ ″ ″ ″ ″ ″
3:15 pm	″ ″ ″ ″ ″ ″

were closed. They were shortly released unharmed. The contrast with the *B'*-*B"* and *B"*-*B'* nest introductions could hardly have been more vivid.

The same experiment was also carried out between the *A* and *B"* moieties. At 8:15 a.m. on November 18, 10 workers of *A* were introduced into the *B"* arena, again close to the nest entraces of *B"*. Five minutes later there seemed complete compatibility. At 8:23 one worker was seen being dragged about, and at 8:25 two were being so treated. By 8:35 only one such pair was seen, and nothing further developed through the cessation of observations at 9:15 p.m. The results of all three of these experiments are summarized in Table I.

One further confirmation of these results was required. Only three days had elapsed between the confrontation of nest *B'* with that of *B"* in the *B"* arena, (when the *B'* colony was apparently occupied by the *B"* moiety) and the test introduction of ten *B'* workers into the *B"* arena. If (as seemed likely) the introduced *B'* nest had been occupied by *B"* workers, could not the passive reception of the new *B'* workers be attributed either to the presence of other *B'* workers in the arena or, alternatively (or in addition) might not *B"* workers have become somewhat adapted to *B'* odors, modifying their reaction? Though the introduced *B'* nest in the *B"* arena was removed after the "nest experiment" and before the new experiment with the ten *B'* workers, since but three days had elapsed between experiments, both factors might well have been involved.

To check this, a longer time interval was allowed to intervene before the 10-worker test was repeated. On February 15, 1983, 92 days after the preceding tests (all colonies having been left undisturbed in the meantime) 10 workers of *B"* were again introduced to the *B'* arena, close to the stacked nests of *B'*. Introduction was made at 3:45 p.m. At 4:10 two workers were "clinched" and mutually stinging near a nest entrance. Five minutes later activity at the nest entrance was much diminished, and the stinging pair was not seen. At 4:12, and again at 4:30 p.m., general activity was much diminished but two workers presumed "alien" were being dragged about the arena. At 4:35 p.m. no further hostility had developed, but one or two males had emerged from a *B'* nest. At 5:00 p.m. the arena was entirely quiet, with only two workers outside the nests. An hour later the situation was similarly quiet, but one "alien" worker was being dragged about the arena and two freshly killed workers were

in a corner. Except for these three, no further attacks were witnessed. The other seven workers appeared to have been "adopted". It is possible that the attacked workers were in fact egglaying individuals, which may have stimulated the hostile attacks, as found by Holldobler (in litt.) for *Novomessor* in similar situations.

Simultaneously the reciprocal introduction was performed. Ten workers of *B'* were introduced into the *B"* arena in similar fashion, at 3:45 p.m. Here the reaction was even more passive. Observations made at five minute intervals until 5:00 p.m. revealed no conflict whatever. At 6:00 p.m. the same observation was repeated and at 8:00 a.m. the following day the situation remained the same. (Table 2.)

Thus these later tests seemed entirely to confirm the earlier ones: the introduction of a "mass" of ten workers simultaneously provoked reactions not essentially different from those observed in the pair-tests on the one hand, and, on the other, in conspicuous contrast to the situation when whole nests were introduced. This was true with moieties which had been maintained since isolation both on the same and on differing diets.

Discussion

Experiments testing compatibilities between workers from three moieties of an originally single nest population of *Rhytidoponera metallica* after mutual isolation for a period of two years under conditions identical except for diet on the one hand, and for another year between halves of one of these moieties isolated and maintained under entirely identical conditions (including diet) led to some interesting conclusions. Pair-tests in fingerbowls indicated that some incompatibility, with accompanying suspicion or aggression, could occur between individuals from isolated moieties maintained on identical diets for a year, but it was infrequent. Both the frequency and vigor of aggression were somewhat greater when the tests were made between workers drawn from moieties isolated on differing diets but under otherwise identical environmental conditions. Thus it seemed possible that previous dietary history could have a minor role in mediating compatibility, but not an important—much less a decisive—one. Similar tests using ten-worker samples introduced between the moieties in all combinations yielded results essentially the same as the pair-tests, indicating that "mass

TABLE 2

FINAL RECIPROCAL TESTS OF TEN WORKERS BETWEEN COLONY FRAGMENTS B′ AND B″

February 15-16, 1983: 92-93 days after first reciprocal tests 11/15/82

February 15:

Workers *B*□ into *B*#

3:45 p.m. 10 workers introduced from B□

Many workers clustered inside nest entrance, but no hostility, *until*

4:10 p.m. Two workers "clinched" and stinging near nest entrance.

4:15 p.m. Activity much diminished at nest entrance. The "clinched" pair not seen.

4:25 p.m. Generally quiet but two "alien" workers seen being dragged in arena.

4:30 p.m. Generally very quiet, but the two "alien" workers still being dragged in arena.

4:35 p.m. *Some activity around nest entrance, and one or two males emerging.* No hostility observed.

5:00 p.m. Entirely quiet in arena with only two workers out. Some activity about nest entrance. No conflict.

6:00 p.m. One "alien" worker seen being dragged by two others. Otherwise all normal and quiet.

February 16:

8:00 a.m. Arena quiet with one or two males emerging from nest. However, 1 dead worker (presumably alien) being dragged about arena, and two freshly killed workers in corner. These three presumably *B*□ aliens.

Thus the general picture was one of *no general arousal* (as before) but ultimate individual hostility to three out of ten workers, with eventual killing. Entirely confirmatory of earlier results.

February 15:

Workers *B*# into *B*□:

3:45 p.m. *10 workers introduced from B.* All introduced workers immediately disappeared into *B*□ nests, without causing any sign of disturbance.

4:20 p.m. Only 5 workers seen outside nests. No conflict and no signs of disturbance.

4:25 p.m. All very quiet in arena. Only 2 workers out. No conflict.

4:30 p.m. All entirely quiet. 1 worker only seen in arena. No conflict.

4:35 p.m. Completely quiet. One worker seen in arena. No conflict.

5:00 p.m. Completely quiet. One worker seen in arena. No conflict.

6:00 p.m. All completely quiet. 1 worker seen in arena. No conflict.

TABLE 2 (CONTINUED)

FINAL RECIPROCAL TESTS OF TEN WORKERS BETWEEN COLONY FRAGMENTS B′ AND B″

February 16:

8:00 a.m. Arena entirely quiet. Only 2 workers seen in arena. No hostility, and no "alien" bodies found.

Thus, throughout this run, there was no hostility of any kind between host and introduced individual workers. It should be noted that *B*□ was markedly less numerous and strong than *B*#, and while *B*# contained considerable regenerating brood, none was found in *B*□.

These test, therefore, were confirmatory of the earlier ones run on November 15, 1982. Like them, they emphasize the important role played by site nest marking, as opposed to individual odor characteristics—an interesting convergence to the Traniello findings (Naturwissenschaften 67, S. 361 (1980).

effects" were not demonstrable and almost certainly not significantly involved.

In sharp contrast, the introduction of long-occupied earth-containing Lubbock nests of one moiety into the arena of another, whether the moieties had been maintained on identical or non-identical diets, was very different, resulting in vigorous mass attacks and the invasion and occupation of the introduced nest.

This dramatic contrast suggests that, as in the cases of *Pogonomyrmex, Oecophylla,* and *Lasius*, colony-specific nest-site marking with gut contents (perhaps containing colony-specific pheromones) is important and regularly employed even in so primitive an ant, and one with so diffuse and vagile a colony structure, as *Rhytidoponera metallica*. This conclusion is reinforced by the extensive (though apparently random) marking of the substrate with fecal droplets that we have found general in arenas containing long-occupied *metallica* nests, a typical example of which is illustrated in Figure I. It strongly supports the recent findings of Holldobler (unpublished ms.) that in the Ponerine ants *Paltothyreus tarsatus*, a species of *Leptogenys* and in two species of *Hypoponera* fecal droplets deposited at the nest entrances can serve as orientation cues in homing, while in the last genus colony-specific preferences for these markings could be demonstrated.

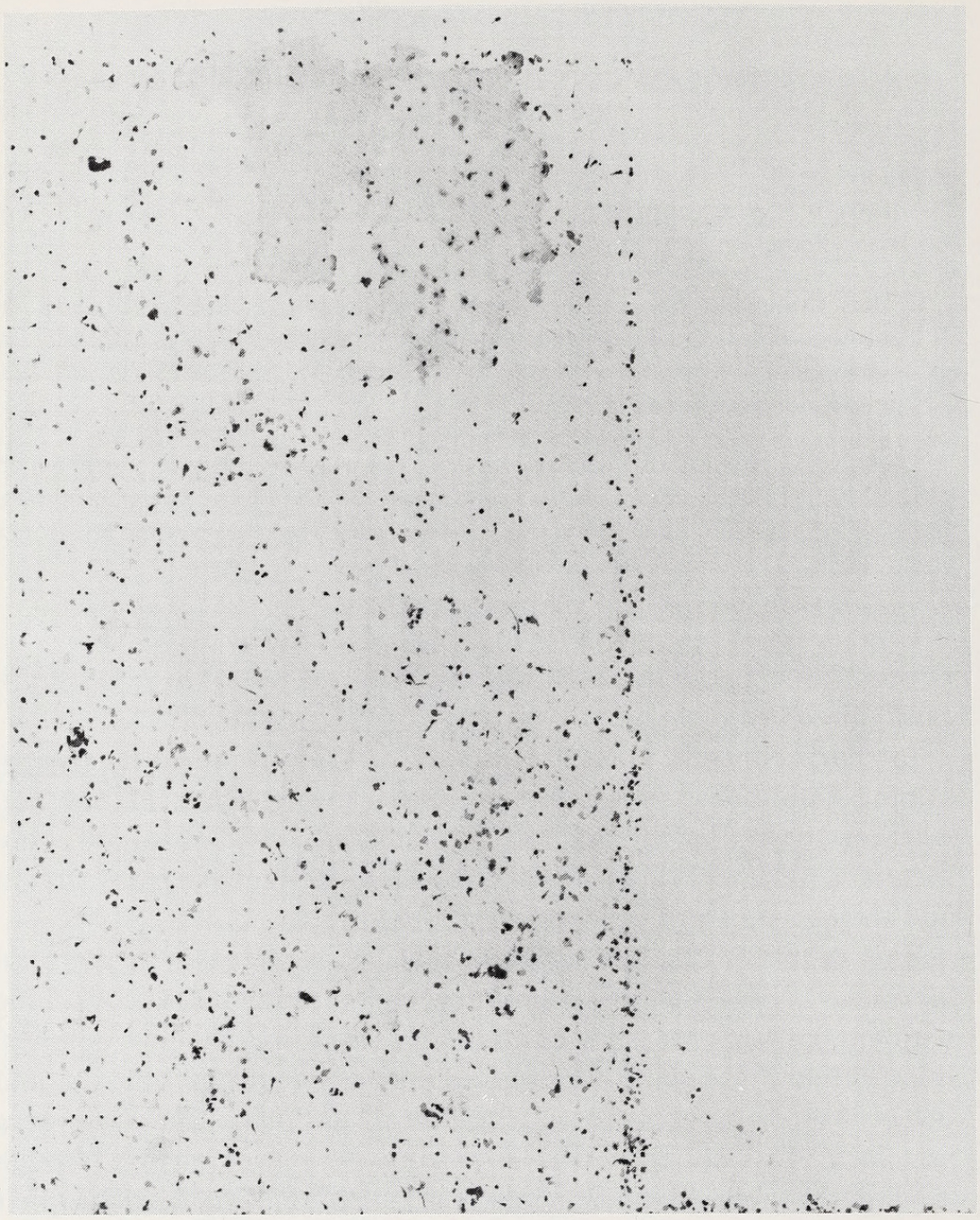

Figure 1. Random marking with fecal droplets of territory surrounding nest in *Rhytidoponera metallica* (Straight edge corresponds to margin of Lubbock nest.)

Summary and Conclusions

The following conclusions seem probable from the present work: (1) As suggested in a previous paper (Haskins and Haskins, 1979) "recognition" between the members of fragments of a single population separated for a year or more appears to remain on the whole stable through several "generations" of workers which have not

been in direct contact during their ontogeny, when those workers are pair-tested in fingerbowls on an individual basis. This compatibility is not universal, however. Incompatibility was observed in a few cases even between workers of two halves of a population separated for a year or more but maintained under identical environmental conditions, including diet, whether tested in pairs or in groups of ten. When the diet had consistently differed markedly throughout the period of separation, the numbers of workers exhibiting incompatibility appeared somewhat increased, but was still a minor proportion. It is possible that such individuals eliciting attack were in fact laying workers, as found by Holldobler in *Novomessor.* (2) When earth-containing Lubbock nests occupied by one fraction of the divided population throughout the periods of separation were introduced into the arena of another, the situation was dramatically altered. Mass hositility and mass raiding of the introduced nest by the recipient moiety regularly followed, regardless of whether the preceding dietary history was the same or different. We conclude that, as reported by other investigators in a number of higher ant genera (*Pogonomyrmex, Oecophylla, Lasius*) and in the Ponerine genus *Hypoponera*) colony-specific nest site marking is important also in *Rhytidoponera metallica*, despite its relative primitiveness and the typical diffuseness and vagility of its colonies. Typical random markings of the floors of arenas about earth-containing Lubbock nests long occupied by colonies of *metallica*, as illustrated, indicate that, as with at least some higher ants, and in several Ponerine genera including *Paltothyreus, Leptogenys* and *Hypoponera*, fecal contents are the characteristic marking "vehicle", perhaps including, as in the higher ants, colony-specific pheromones. If this is true of *R. metallica,* as suggested in the experiments reported, it becomes interesting to consider the factors involved in mediating this specific reaction between two halves of a single population separated for less than two years and maintained on identical diets and in identical arenas placed side by side on the same laboratory bench during that period. No evidence has been found of trail marking, or indeed of trail laying, in *R. metallica.*

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