

Research article

Nestmate recognition in the absence of a pheromone

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Summary. Individual recognition cues were manipulated to elucidate the system of nestmate recognition employed by the introduced European paper wasp, *Polistes dominulus*. Tests involved exposing *P. dominulus* individuals to their natal nest material, to which we added a novel hydrocarbon, pentacosanoic acid methyl ester, or exposing them to the artificial odor alone. Results show that *P. dominulus* (1) performs nestmate discrimination, (2) can use artificial exogenous chemicals as recognition odors, and (3) can distinguish single differences in hydrocarbon profiles. Taken individually, these experiments provide evidence revealing the nature of the production and action components of recognition. Taken together, these results establish that recognition takes place outside the classical definition of a pheromone-based system.

Key words: Nestmate recognition, *Polistes dominulus*, pheromones.

Introduction

The social Hymenoptera have been an historical focus of many conceptual investigations of sociality in general. The genus *Polistes*, a cosmopolitan paper wasp, has played a pinnacle role in these investigations due to the ease by which investigators can observe colony life (i.e., the nest is not surrounded by an envelope), the ease at which colonies can be located (often on human constructions), and the small size of colonies (usually <100 individuals) which allows for detailed behavioral observation (Pardi, 1996). Knowledge obtained from these studies has had profound effects on the understanding of sociality. Recently, investigations using *Polistes* as a model organism have included a central question of social organization: How do social animals recognize nestmates and kin?

The importance of nestmate recognition and discrimination in social insects and vertebrates is widely accepted (Holmes and Sherman, 1983; Fletcher and Michener, 1987; Hepper, 1991). Rau (1939) and Pardi (1947) both thought that nestmate recognition could be due to colony odor, and West-Eberhard (1969) commented that perhaps approach behavior at the nest, other visual cues, or colony odor could be employed by the wasps. Since these speculations, it has been discovered that polistine wasps (including *Polistes* spp.) reinforce their nest carton with many different secretions (Downing, 1991; Wenzel, 1991; Kudô et al., 1998), and many studies reveal that these are used as recognition odors (see review in Gamboa, 1996).

Newly emerged adult *Polistes* learn their natal colony odor from the nest itself, rather than from the cuticles of nestmates (Shellman and Gamboa, 1982; Pfennig et al. 1983 b; Gamboa et al., 1986 b; Singer and Espelie, 1992). Pfennig et al. (1983 a) showed that prior exposure to nest material was necessary and sufficient for nestmate discrimination to proceed in *P. fuscatus*. In the absence of nest material, newly emerged *Polistes metricus* workers neither discriminate their natal nest from other nests (Espelie et al., 1990) nor nestmates from non-nestmates (Singer and Espelie, 1992). However, the presence of natal nest material at the time of emergence allows for both of these discrimination processes (Espelie et al., 1990; Singer and Espelie, 1992). Additionally, *P. metricus* workers that are allowed to emerge in the presence of natal nest material from which cuticular hydrocarbons were extracted do not show recognition of their natal nest (Espelie et al., 1990) or their nestmates (Singer and Espelie, 1992). When the hydrocarbons are reapplied to the nest material, workers which emerge in the presence of the reapplied compounds are capable of nest (Espelie et al., 1990) and nestmate (Singer and Espelie, 1992) recognition.

The work of Espelie and his co-authors has led to the belief that because the majority of the cuticular chemicals are

hydrocarbons, the hydrocarbons alone serve as the recognition pheromones (Gamboa, 1996). Although these studies have shown conclusively that cuticular hydrocarbons present on the nest are involved in nestmate recognition, their data do not exclude the possibility that other chemicals are involved (Howard, 1993). Particularly, the role of exogenous odors remains unclear.

Gamboa et al. (1986b) performed experiments to attempt to determine if odors are exclusively acquired in the adult stage and if so, if those acquired odors are exogenous or endogenous. The authors compared the tolerance levels of laboratory colonies to the tolerance levels of field colonies using triplet test protocols (Pfennig et al., 1983a). Observing that lab colonies were generally more tolerant overall, the authors concluded that because the laboratory colonies were maintained under a homogenous environment, the increased tolerance is explained by the inference that environmentally acquired odors are used by the wasps (Gamboa et al., 1986b). Although this conclusion is logical, other phenomena, including the confinement of the laboratory setting itself, can explain the data. Because laboratory wasps only encounter close relatives, and are not allowed to forage where they would normally encounter non-relatives, universal tolerance is predictable (Reeve, 1989). Also, endogenous odor production may be uniformly influenced by uniform environmental variables, especially food. Because of these reasons, the ability of *Polistes* to use exogenous odors as recognition cues remains undemonstrated.

Although several members of the genus *Polistes* have been used as behavioral models for recognition investigations in the USA, the majority of work in Europe has focused on chemical analysis of cuticular and nest chemicals, and some results are contradictory (see review in Lorenzi et al., 1996). Accordingly, the recognition ability of *P. dominulus*, the predominant European paper wasp, was obscure for some time (Gamboa, 1996; but see Starks et al., 1998). Pratte (1982) performed behavioral investigations on *P. dominulus* (cited as *P. gallicus*) to determine if previous cohabitation of related and unrelated foundresses would affect how the foundresses associated. Pratte found no evidence that cohabitation in hibernacula affects spring colony association. Dani et al. (1996) used *P. dominulus* to test the hypothesis that Dufour's gland secretions are the recognition chemicals. Dufour's gland products were smeared onto the cuticle of dead wasps from which epicuticular hydrocarbons had been removed. These results show that Dufour's gland product can be adequate for recognition.

Cuticular hydrocarbon analyses of *P. dominulus* colonies have revealed little variance between colonies (Bonavita-Cougourdan et al., 1991), and some have speculated whether the difference in colony chemical profiles may be too small for *P. dominulus* to detect or use for discrimination (Lorenzi et al., 1996, p. 184, 187–88; see review in Howard, 1993). This idea rests on the assumption that *Polistes* spp. are unable to detect small (or single) differences in colony profiles, and they therefore need larger differences in order to make decisions.

Starks et al. (1998) demonstrate that *P. dominulus*, like its North American relatives, expresses nestmate recognition.

But in using a novel approach, that study did not use the protocol of previous North American studies (i.e., the use of naive subject wasps, and resting in contact or mounting scored as tolerance) and therefore may not be comparable to North American studies.

Given the current ambiguities regarding the potential influence of exogenous odors and the ability of *Polistes* to detect "small" or single differences in colony odor, we designed three experiments to address these issues. Experiment 1 was designed to determine if *P. dominulus* performs nestmate recognition in the paradigm of the better-known North American *P. metricus*. Experiment 2 investigates if *P. dominulus* can use an artificial ester for the purposes of nestmate discrimination, and if it can detect single differences in colony chemical profiles. Experiment 3 asks if *P. dominulus* can learn only the ester as a recognition odor, and then use that one odor for recognition when all other odors encountered are unlearned.

Methods

We collected twenty five gynes (colony founding females) of *P. dominulus* in late April, May and June, 1997 from Columbus, Ohio, USA and the surrounding area; Wooster, Ohio, USA; and Upper Sandusky Ohio, USA. A few of the gynes had not yet constructed nests, but most were collected with nests with fewer than 7 cells. We placed each gyne in a separate plastic container (27 cm × 16.5 cm × 19 cm) to the roof of which we had previously glued a wooden popsicle stick. If the gyne had already begun nest construction, the nest was glued to the wooden stick. All of the colonies were housed in the OSU greenhouse under natural light and outside ambient temperature. Each gyne was supplied with water, rock sugar, and filter paper for nest construction. If the nestless gynes began construction on a surface of the chamber other than the wooden stick, we moved the nest to the stick after she had built two or three cells (usually one day). After brood were present, we supplied the wasps (now queens) with *Galleria melonella* caterpillars each day of the experiment.

Pupae were individually removed approximately 5 days before eclosion so that they could learn odors in isolation. For experiments 1 and 2, a nest fragment of seven cells was cut from the outer surface of the natal colony, and then subdivided into two smaller fragments: one of 5 cells, and the other 2 cells. For experiment 3, 1 piece of filter paper was subdivided into two smaller pieces equal in size to the two pieces of nest fragments from experiments 1 and 2. The larger nest fragment (or piece of filter paper in experiment 3) was glued to the top of a plastic isolation chamber placed atop an inverted petri dish. The top of the isolation chamber was formed using a sheet of Teflon mesh. The pupae were placed on the inner surface of one of the cells of the smaller fragment (or on the smaller piece of filter paper in experiment 3) at the bottom of the isolation chamber. Because the pupae had no enclosure from which to "emerge," adulthood was determined by mobility and coloration. As has been performed in many well-known experiments on *Polistes* recognition, after "emergence" (actively walking about), the adult subjects were maintained in isolation for 4 days prior to testing. The triplet test protocol (Pfennig et al., 1983a; Singer and Espelie, 1992) was used, in which a subject wasp is allowed to emerge from its pupal case in isolation, and is then presented with two other wasps, one usually a nestmate, the other a non-nestmate; the behaviors of the subject wasp toward the other wasps were recorded as either tolerant or intolerant. Nest fragments (or filter paper) were differentially treated for the three experiments, as were nestmate and non-nestmate combinations during the triplet testing. Subjects were not marked, but the nestmates and non-nestmates with which the subjects were tested were all marked in a blind design. No subject was used for more than one triplet test, and subjects

were randomly selected for testing from one of the twenty five colonies. When non-nestmates were used in triplet testing, we used members of colonies collected from cities different from the subjects' colonies to ensure that the wasps were not close relatives.

After Singer and Espelie (1992), the following behaviors were considered tolerant for all experiments: brief (<2 s) unilateral antennation; brief (<2 s) mutual head-to-head antennation; prolonged or repeated (>2 s) unilateral antennation; prolonged or repeated (>2 s) mutual antennation; stepping or resting on another wasp; solicitation of trophallaxis; trophallaxis; standing stationary (>1 min) within 2.5 cm of wasp. The following behaviors were considered intolerant: chasing; fighting while falling from the top of the test chamber; grappling on the floor of the test chamber; biting; lunging; avoiding or detouring. For experiment 1, tolerance values were calculated using the formula proposed by Singer and Espelie (1992), $T = [(t_n + i_{nn}) - (t_{nn} + i_n)]$, where T is the overall tolerance, t_n is the tolerance toward the nestmate, i_n is the intolerance toward the nestmate, t_{nn} is tolerance toward the non-nestmate, and i_{nn} is the intolerance toward the non-nestmate. This formula contrasts overall values from nestmates against those of non-nestmates. As in Singer and Espelie (1992), tolerance values of between -2 and 2 were considered neither tolerant nor intolerant. In experiment 2, the formula was modified: instead of tolerance and intolerance toward the non-nestmate, the calculation involved tolerance (t_{nc}) and intolerance (i_{nc}) toward the nestmate that had acquired the ester ($T = [(t_n + i_{nc}) - (t_{nc} + i_n)]$). In experiment 3, the formula was essentially the same as in experiment 1, except that we recorded tolerant and intolerant behaviors toward the nestmate (t_n , i_n) and the non-nestmate (t_{nnc} , i_{nnc}) which had acquired the ester ($T = [(t_n + i_{nnc}) - (t_{nnc} + i_n)]$); the distribution of intolerant behaviors from experiment three was compared to the distribution of intolerant behaviors from experiment 1 using the Mann-Whitney U-test.

Can P. dominulus perform nestmate discrimination?

Experiment 1 was designed to test if *P. dominulus* performs nestmate discrimination using common experimental design. Sixteen subjects were exposed to untreated fragments of their natal nest material. After four days of isolation with the nest fragments, subjects were placed in a 27 cm × 16.5 cm × 19 cm plastic testing chamber with a nestmate and a non-nestmate for one hour. Tolerant and intolerant behaviors toward the nestmate and non-nestmate were recorded.

Can P. dominulus detect single differences in colony odor profiles?

Experiment 2 was designed to test if *P. dominulus* can detect single differences in colony odor profiles. Fifteen subjects were exposed to natal nest fragments to which we had applied 100 µl of 250 millimolar pentacosanoic acid methyl ester (in hexane), a chemical not found in *Polistes* nests, but similar in carbon-length to naturally occurring chemicals. This chemical was selected because the carbon-chain length (C-25) is well within the range of normally occurring *Polistes* cuticular hydrocarbons, but the ester group provides a distinctiveness to the chemical that is novel to *Polistes* nests. Therefore, the chemical is not too long or too short to be detected by the wasps but is different enough to be an appropriate assay. The amount of chemical used was applied to the larger (7 cell) nest fragment before subdivision to ensure uniformity of the applied ester. This concentration was calculated such that nanogram quantities of ester were applied to the nest fragment – a concentration comparable to the individual concentrations of many other cuticular hydrocarbons (see Fig. 3). Experiment 2 was identical to experiment 1 in other respects, except that two nestmates of the subject were used rather than one nestmate and one non-nestmate. Prior to testing, one of the nestmates was placed in a petri dish (9.2 cm in diameter) lined with filter paper to which we applied 500 µl of the ester solution. This nestmate was allowed to walk on the filter paper overnight, thus acquiring the ester on its cuticle in the process (Bowden et al., 1998). The other nestmate was untreated. This allowed us to compare how nestmate recognition is affected by a single chemical.

Can P. dominulus learn one odor and use it for discrimination when many other odors are present?

Experiment 3 was designed to test if after exposure to only one chemical, *P. dominulus* can detect that chemical from a milieu of others chemicals on the cuticle of another wasp, and use that one chemical as the basis of discrimination. Thirteen subjects were exposed to filter paper, to which we added the same amount of ester (100 µl of 250 millimolar solution in hexane) as we applied to the nest fragments in experiment 2. The piece of filter paper was subdivided into two smaller pieces equal in size to the nest fragments from experiments 1 and 2 after the application of the ester solution to ensure uniformity of the chemical. The larger sub-fragment of filter paper was attached to the top of the isolation cup as if it were a nest fragment, and pupae were placed on the smaller sub-fragment of ester-laden filter paper at the bottom of the isolation cup. Subjects were triplet-tested with a nestmate, and a non-nestmate that had been exposed to the ester as in experiment 2.

Results

Table 1 displays the average tolerant and intolerant scored behaviors for all three experiments.

Can P. dominulus perform nestmate discrimination?

Figure 1 shows the distribution of tolerance values (T) for experiment 1. Ten of twelve subjects who make discrimination decisions preferentially associated with nestmates ($G = 5.822$, $N = 16$, $P < 0.025$). Four subject wasps were not clearly tolerant or intolerant toward nestmates, but the tolerance scores of all four, though not significant, were toward nestmates.

Can P. dominulus detect single differences in colony odor profiles?

Figure 2 shows the distribution of tolerance values for experiment 2. With one exception, the subjects preferentially associated with the nestmate that was allowed to acquire the novel ester on its cuticle ($G = 6.859$, $N = 15$, $P < 0.01$). In many cases, the tolerance toward the chemical-treated nestmate was much higher than that for the nestmate in experiment 1. Gas chromatography mass spectrometry readings of the cuticular hydrocarbons extracted from a sample nest after application of the ester are shown in Figure 3.

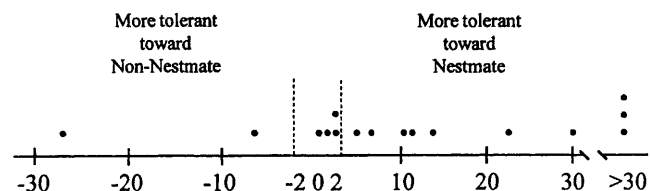
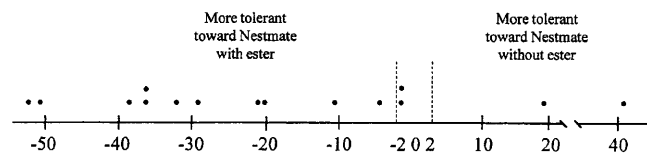


Figure 1. Distribution of tolerance behaviours of subjects in Experiment 1. Values between -2 and 2 were not considered. The subjects showed preferential tolerance toward nestmates ($n = 16$, $G = 5.822$, $P < 0.025$)

Table 1. Average number of interactions per behavior category. Experiment 1: behaviours toward nestmates (N) and nonnestmates (NN); experiment 2: behaviours toward nestmates (N) and nestmates with ester applied to cuticle (NE); experiment 3: behaviours toward nestmates (N) and nonnestmates with ester applied to cuticle (NNE)

Behaviors	Experiment 1		Experiment 2		Experiment 3	
	N	NN	N	NE	N	NNE
<i>Tolerant</i>						
Brief (<2 s) unilateral antennation	15.31	8.06	14.93	25.93	16.85	17.62
Brief (<2 s) mutual head-to-head antennation	4.19	2.00	3.13	4.73	1.77	5.54
Prolonged or repeated (>2 s) unilateral antennation	1.19	0.19	5.93	7.00	4.38	2.23
Prolonged or repeated (>2 s) mutual antennation	1.50	1.00	1.60	3.87	0.69	2.08
Stepping or resting on another wasp	1.06	1.06	1.80	2.87	1.38	0.04
Solicitation of trophallaxis	1.00	0.31	–	0.53	0.38	0.38
Trophallaxis	0.03	–	–	0.07	–	0.69
Standing stationary (>1 min) within 2.5 cm of wasp	11.44	6.13	5.50	10.87	1.21	2.05
Total	35.94	18.75	27.40	45.00	25.46	28.92
<i>Intolerant</i>						
Chasing	–	0.06	–	–	0.15	4.38
Fighting while falling from the top of the test chamber	–	–	–	–	–	–
Grappling on the floor of the test chamber	–	–	–	–	–	–
Biting	0.06	0.063	2.27	1.00	6.38	5.31
Lunging	0.25	–	0.20	0.07	–	0.15
Avoiding or detouring	–	0.38	0.33	0.87	0.92	1.15
Total	0.01	1.06	2.62	1.81	7.46	11.00

**Figure 2.** Distribution of tolerance behaviours of subjects in Experiment 2. Values between -2 and 2 were not considered. Subjects learned the odor of their natal nest material to which we added pentacosanoic acid methyl ester. When presented with two nestmates, one of which had been allowed to acquire the novel ester, the subjects showed preferential tolerance toward nestmates which had acquired the ester ($n = 15$, $G = 6.859$, $P < 0.01$)

Can *P. dominulus* learn one odor and use it for discrimination when many other odors are present?

The subjects of experiment 3 showed no greater tolerance for nestmate or non-nestmate (Fig. 4: $G = 0.076$, $N = 13$, NS). However, using experiment 1 as a model, experiment 3 showed a significantly greater number of intolerant behaviors per triplet test (Fig. 5A: Median = 10.00, Mann-Whitney U-test: $U = 173.5$, $N_1 = 16$, $N_2 = 13$, $P < 0.0025$) and significantly greater percent intolerance per triplet test (Fig. 5B: Mann-Whitney U-test: $U = 261.5$, $N_1 = 16$, $N_2 = 13$, $P < 0.005$).

Discussion

Many arthropods and vertebrates use chemical-based recognition systems. Many of these systems rely on genetic

endogenous odors, but many are a combination of genetic and environmental odors, and some may be mediated exclusively by environmental cues (see review in Sherman et al., 1997). Writing of the difficulty in developing behavioral assays that demonstrate the relative importance of genetic and environmental cues, Sherman et al. (1997) state “The issue that must be addressed is whether such [genetic] cues would be supplemented, or even supplanted, in nature by variable environmental cues.” We have addressed this question using *Polistes dominulus* as a model system. After demonstrating that *P. dominulus* performs nestmate discrimination, we show that the addition of a single artificial odor drastically alters recognition decisions. Next, we demonstrate that while the presence of one learned odor is not sufficient to skew acceptance toward a non-nestmate (that is, supplant the natural cues) when many other unlearned odors are present, the presence of the unlearned odors shifts the subject’s behavior from virtually universal tolerance to a significantly increased intolerance. These data reveal important information regarding the production and action components of recognition (Holmes and Sherman, 1983), and demonstrate that an explanation involving the presence of a pheromone need not be invoked to explain the data.

The first step is to demonstrate recognition in naive subject wasps. We did this in experiment 1 using standard laboratory protocols, namely the widely used “triplet test” (Pfennig, 1983a; Singer and Espelie, 1992). Pratte’s (1982) work, although cited as a refutation of nestmate recognition in *P. dominulus* (Gamboa et al., 1986a; Gamboa, 1996; Starks et al., 1998), was presented as a study of prior association and nest founding, and was not a study of nestmate

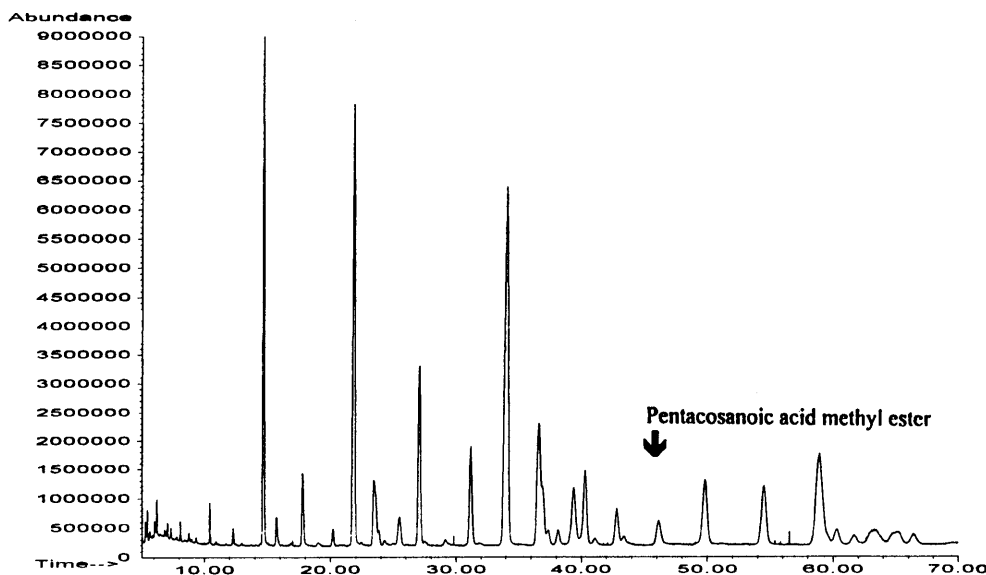


Figure 3. Gas-chromatograph of a hexane washing of a nest of *Polistes dominulus* to which 100 μ l of 250 millimolar pentacosanoic acid methyl ester was previously added. The extract exhibits pentacosanoic acid methyl ester (Time = 46 minutes) in comparable abundance to the majority of other naturally occurring cuticular hydrocarbons; nests which were not experimentally augmented lacked the ester. Subjects in experiment 2 were exposed to nests exhibiting the applied ester after emergence for four days before triplet testing

recognition (*contra* Gamboa et al., 1986a; Gamboa, 1996; Starks et al., 1998).

The demonstration that naive *P. dominulus* wasps perform nestmate recognition runs counter to previous predictions based on the demonstration that hydrocarbon profile variance between *P. dominulus* colonies in Europe is low (Bonavita-Cougourdan et al., 1991). Our behavioral assay demonstrating nestmate recognition implies that the wasps can use small or perhaps even single hydrocarbon differences in colony odors for discrimination. Even if North American populations do not possess the low variance of European populations, we demonstrate that under such conditions, nestmate recognition can occur. We tested this prediction in experiment 2. In this experiment, a subject wasp learned its natal colony odor from a nest fragment to which we added an ester not previously recovered from the nests of *Polistes*. Figure 3 shows that the concentration of ester that we added to nest fragments was comparable to the abundance of the majority of naturally occurring nest hydrocarbons. Subject wasps in experiment 2 made discrimination decisions between an untreated nestmate, and one which was allowed to walk on ester-laden filter paper overnight, thus acquiring the ester on its cuticle. The subject wasps were significantly more tolerant of their nestmates which had acquired the ester. This experiment demonstrated that while *P. dominulus* colonies may differ only slightly in odor, even single differences in colony hydrocarbon profiles are sufficient for nestmate discrimination. Apparently, *Polistes* is capable of detecting some chemical differences with high fidelity, and the "small" differences in *P. dominulus* colonies' chemical profiles (Bonavita-Cougourdan et al., 1991) are sufficient for discrimination in the behavioral assay widely used by American authors. Also, as shown in Figure 3, the abundance of the applied ester, while well within the range of many other chemicals present on the nest, is not as abundant as a few. Many authors have argued that chemicals which are in great

abundance must serve as the primary recognition odors (Espelie et al. 1994), but our data contradict this idea. The neural ability to make such reliable distinctions was likely coopted from some other function, perhaps the recognition of the nest itself in solitary ancestors (Hölldobler and Michener, 1980; Wcislo, 1992). Because the variance in colony odor necessary for nest recognition existed prior to nestmate recognition, and because many wasps and bees learn and perhaps acquire odors on their cuticles from the nest, there need not be specific pressure for nestmate recognition in order for it to evolve.

Experiment 2 also reveals information regarding the production component of recognition. The single ester that we applied to the nests, which the subjects in experiment 2 learned, was completely novel to the wasps. Pentacosanoic acid methyl ester has never been identified from the nests of *Polistes* wasps. The fact that the wasps used this odor for discrimination demonstrates empirically that exogenous odors can be used by *Polistes* for recognition.

In experiment 3, we attempted to determine the relative importance of an exogenous component of recognition. In this experiment, we tested if a wasp could learn one exo-

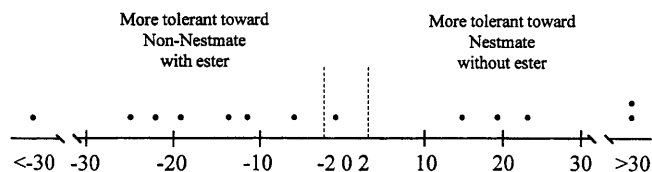


Figure 4. Distribution of tolerance behaviours of subjects in Experiment 3. Values between -2 and 2 were not considered. Subjects were exposed to the odor of the novel ester alone. The subjects were presented with untreated nestmates and non-nestmates that had acquired the novel ester. The subjects showed no preference for nestmate or non-nestmate ($n = 13$, $G = 0.076$, $0.9 < P < 0.5$).

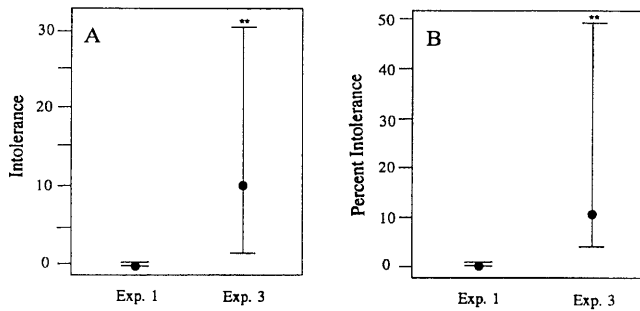


Figure 5. Median intolerance across trials for experiments 1 and 3. A: The median number of intolerant behaviors (•) for experiment 1 was 0.00 (95% CI = 0.00–1.24); the median number of intolerant behaviors for experiment 3 was 10.00 (95% CI = 1.70–30.36) (Mann-Whitney U-test: $U = 173.5$, $N_1 = 16$, $N_2 = 13$, $P < 0.0025$). B: The median percent intolerance (•) for experiment 1 was 0.00 (95% CI = 0.00–1.73); the median percent intolerance for experiment 3 was 11.49 (95% CI = 2.79–48.12) (Mann-Whitney U-test: $U = 261.5$, $N_1 = 16$, $N_2 = 13$, $P < 0.005$).

genous odor, and then use that odor alone for recognition while many other unlearned odors were present on the cuticles of the other wasps. Here, subject wasps learned only the odor of the ester after emergence, and were then tested against one untreated nestmate and a non-nestmate that was allowed to acquire the odor of the ester as in experiment 2. The subject wasps were unable to discriminate between the two test wasps, perhaps because the suite of unlearned odors detected by the subject wasps provided too much interference; in other words, the subject wasps could not tease out the one chemical it learned from all the other unlearned chemicals.

Experiment 3 also demonstrates that the action component of recognition in *Polistes* relies on many odors. In experiment 1, intolerance across trials was low (Fig. 5A) as was percent intolerance across trials (Fig. 5B). In fact, median intolerance for experiment 1 was 0.00 (Fig. 5A). This is expected, as colony odor differences are small between colonies of *P. dominulus* (Bonavita-Cougourdan et al., 1991). In experiment 3 subjects learned only the odor of the ester, and all other chemicals were unlearned. Although the wasps could not discriminate nestmates from non-nestmates (Fig. 4), intolerance per trial was high (Fig. 5A) as was the percent intolerance across all trials (Fig. 5B). When compared to the median intolerance from experiment 1, the intolerance per trial from experiment 3 was much higher (Fig. 5A) as was the percent intolerance per trial (Fig. 5B). Stated in another way, a single learned chemical is enough for discrimination when all other chemicals are known (as in experiment 2), but one learned chemical is not sufficient for recognition when all other chemicals are unlearned. This strongly suggests that the one artificial chemical in experiment 2 was augmenting existing recognition cues, supporting the “gestalt model” of kin recognition (Crozier and Dix, 1979).

Our interpretation is also supported by behavioral data regarding the recognition decisions of other social Hymenoptera. In *Apis*, Breed et al. (1988) found that anise oil,

linalool, and geraniol can be used as recognition cues which, when applied experimentally, augment existing recognition cues. Breed and Stiller (1992) have found that *Apis* can also use tetracosanoic acid as an augmenting recognition cue. Similar results have been reported from the perhaps more similar system of the primitively eusocial halictine bees. Greenberg (1981) found that when one colony of *Lasioglossum zephyrum* is fed only pollen and another is fed pollen plus a pollen substitute, guard bees that were fed the pollen and pollen substitute mixture were more aggressive toward bees fed on pollen alone, demonstrating a clear influence of environmental odors.

Although Gamboa et al. (1986b) demonstrated that individuals from laboratory colonies of *P. fuscatus* are more tolerant of non-nestmates than field colonies, this observation may not be due to the acquisition and use of homogenous environmental odors. The mere fact that laboratory wasps rarely encounter non-nestmates can also account for the lowered intolerance. Mathematical models also support our interpretation. Reeve (1989) postulated that acceptance (i.e., tolerance) should increase towards universal in a situation where, given other fitness and cost parameters, the frequency at which the subject encounters “desirable” individuals (i.e., kin) increases; rejection will increase to universal when encounters with “undesirable” individuals (i.e., non-kin) increase. Therefore, this is the first empirical demonstration that *Polistes* spp. can use environmentally acquired odors as recognition cues.

Is Polistes using a pheromone?

Research programs concerning kin recognition generally rely on the assumption that chemical cues can indicate kinship. This postulate includes two different avenues. One is that wasps generate pheromonal cues that vary genetically and indicate genotype; another is that wasps are prone to recognize cues associated with kinship and ignore others. The latter path does not require cues to be determined genetically, for animals may use any cue opportunistically.

Hölldobler and Michener (1980) define recognition pheromones as, “Odor signals that differ among individuals of a population, when not of extrinsic origin” and distinguish them from discriminating substances, “used when it is not clear whether identification of an individual is based on secreted odor substances which are genetically controlled (discriminators) or on environmental materials that differ among individuals or colonies.” Although the data have previously been inconclusive, most workers studying the discrimination ability of *Polistes* believe that *Polistes* uses both endogenous and extrinsic chemical cues as recognition chemicals (see review in Gamboa, 1996). Many of these same researchers continue to use the term pheromone to describe these chemicals (Layton et al., 1994; Espelie et al., 1994; Gamboa, 1996; Lorenzi et al., 1996). Part of the tendency to use the term pheromone likely stems from the desire to demonstrate that nestmate recognition is kin recognition. But the ability to detect relatedness directly (i.e., kin recognition

sensu Halpin, 1991) may not be equivalent to nestmate recognition as colonies may consist of relatives and non-relatives (Fletcher, 1987; but see Gamboa et al., 1991, and Sherman et al., 1997). However, for a system of nestmate recognition to function effectively as kin recognition, nestmates need only be probable relatives (Gamboa et al., 1986a; Fletcher, 1987), and chemicals which confer information about the likelihood of relatedness need not be endogenous as the chemicals are not direct reflections of genotype. We have demonstrated that *P. dominulus* can use exogenous chemicals as the basis of nestmate recognition. And although our experiments do not preclude the possibility of a pheromone-based system, they demonstrate that nestmate recognition occurs in the absence of such.

According to Wilson (1971, 1987), Hölldobler and Michener (1980), and Blum (1985), recognition pheromones are endogenous and, once learned, these odors release a defined change in behavior. Yet, our studies show tolerance toward nestmates in *Polistes* does not satisfy these criteria. The use of exogenous odors by basal groups of polistines may reflect the retention of a primitive system of nestmate recognition, in contrast with a more derived system of endogenously produced odors that may be found in more apical vespids. Accordingly, acknowledgement of this distinction is necessary if we are to understand the underlying natural history of paper wasp societies. We show that the production component of recognition in *Polistes dominulus* is heavily influenced by environmental odors, but functions nevertheless. Also, the action component of recognition is apparently mediated by the presence of many different odors which need not be endogenous. Strictly speaking, these data show that nestmate recognition in *Polistes* can function in the absence of a pheromone.

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