Individual Recognition in Ant Queens

Patrizia D'Ettorre[1,2,*] and Jürgen Heinze[1]
[1] Biologie I
Universität Regensburg
Universitätsstrasse 31
D-93040 Regensburg
Germany

Summary

Personal relationships are the cornerstone of vertebrate societies, but insect societies are either too large for individual recognition, or their members were assumed to lack the necessary cognitive abilities [1, 2]. This paradigm has been challenged by the recent discovery that paper wasps recognize each other's unique facial color patterns [3]. Individual recognition is advantageous when dominance hierarchies control the partitioning of work and reproduction [2, 4]. Here, we show that unrelated founding queens of the ant *Pachycondyla villosa* use chemical cues to recognize each other individually. Aggression was significantly lower in pairs of queens that had previously interacted than in pairs with similar social history but no experience with one another. Moreover, subordinates discriminated familiar and unfamiliar dominants in choice experiments in which physical contact, but not odor perception, was prevented and in tests with anaesthetized queens. The cuticular chemical profiles of queens were neither associated with dominance nor fertility and, therefore, do not represent status badges [5, 6], and nestmate queens did not share a common odor. Personal recognition facilitates the maintenance of stable dominance hierarchies in these small societies. This suggests that the ability to discriminate between individual traits is selected for when it incurs net benefits for the resolution of conflict.

Results and Discussion

The efficient organization of group-living vertebrates, including humans, is based on individual recognition, i.e., the discrimination of distinctive cues specific to given individuals [1, 7–9]. Most social contexts, such as mate selection, territorial defense, dominance, and social competition require the ability to recognize individual identity [10]. Well-known examples come from mammals [7], birds [11], fishes [12], and some invertebrates [13, 14]. Nevertheless, in some circumstances, selection may have favored those individuals that conceal their identity in social interactions because the costs of revealing identity could be greater than the benefits [15]. Colonies of social insects can consist of more than one genetically distinct lineage (e.g. when there are multiple queens or

*Correspondence: pdettorre@bi.ku.dk
[2] Present address: Institute of Biology, Department of Population Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark.

when the queen mates with more than one male). In these circumstances, nepotistic behavior may decrease colony efficiency and productivity, thus kin discrimination rarely occurs [16] and indiscriminate altruism is the rule [17]. Ants, the largest group of social insects, are traditionally believed to be incapable of recognizing each other as individuals [18]. But in some particular situations it may pay to reveal and recognize identity. Where colonies are structured by dominance hierarchies, as in many species of ants, wasps, and bumblebees, individual distinctive cues could minimize the costs of repeated status testing and role assessment [2]. Indeed, besides one example in the mating behavior of sweat bees [14], the only evidence for individual recognition in social insects involves visual cues in the paper wasp *Polistes fuscatus* [3], a social insect with dominance hierarchies [19] and small colony size. However, such distinctive facial color patterns might also serve as status badges in other species of paper wasps [6].

Colonies of the ponerine ant *Pachycondyla villosa* are usually started cooperatively by several unrelated queens, which establish a stable dominance hierarchy with a division of labor [20]. Although in most ant species only one queen survives cooperative founding [21], *P. villosa* queens stay together after worker emergence [22], and the original hierarchy might therefore affect the social structure of mature colonies, which usually consist of few dozens of individuals. We tested whether founding queens recognize individual identity or social status (dominant/subordinate) of other queens in two rounds of binary interactions. In the first, two queens were housed together for 24 hr, which was long enough to establish a clear dominance relationship; the second was between a subordinate queen and a "familiar" or "unfamiliar" dominant (experiment 1, Figure 1). Here "familiar" means an individual that had been met prior and not an individual that was recognized as belonging to a known, familiar group of individuals. In this experiment, queen hostility should not have differed between the treatments if they were only capable of status recognition. However, familiar pairs were much less aggressive (Figure 1), which suggests individual recognition. We can exclude that these results were the effect of a difference in the agonistic level between pairs that were subject to one of the two experimental treatments. Queens confronted with unfamiliar individuals ("tests," Figure 1), were significantly less aggressive when again encountering the familiar partner in a third round of binary interactions (Wilcoxon matched pairs test: Z = 2.8, p < 0.01). But there was no significant difference in the aggression level of these queens confronted with the previously familiar partner and the pairs of queens involved in the "sham" treatment (Mann-Whitney U test: U = 27.5, p = 0.08).

P. villosa nests in cavities in rotting wood, where visual cues are unimportant. Recognition might instead be based on behavioral or chemical cues, especially hydrocarbons present on the cuticle, which are important in social insect communication [23]. The following experiments allowed for differentiation between these
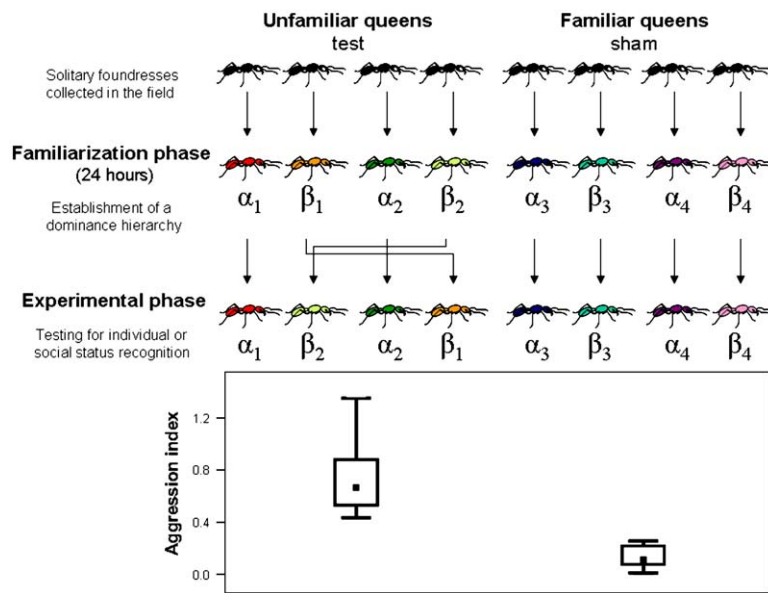
Figure 1. Schematic Set-Up and Results of Experiment 1 to Discriminate between Individual and Social Status Recognition

Arrows indicate the movements of the queens from phase to phase, and α represents the dominant and β the subordinate individual. Box plots represent median, 25%, and 75% percentiles and nonoutlier range. Aggression was significantly higher in unfamiliar (n = 10) than in familiar pairs (n = 10) (Mann-Whitney U test: U = 0.00, p < 0.001).

two cues. Subordinates, which could chose between a familiar and an unfamiliar dominant queen in the unknown environment of a Y maze, spent significantly more time with the familiar than with the unfamiliar dominant queen (experiment 2, Figure 2A). Moreover, subordinates confronted in their own nest with an anaesthetized familiar or unfamiliar dominant (experiment 3, Figure 2B) investigated the unfamiliar queen longer, as it represented an intruder (median, 25% and 75% quartiles for investigation; unfamiliar: 95, 89 and 115 s; familiar: 31, 23 and 40 s; Mann-Whitney U test: U = 0.0, p = 0.003). Both results suggest that chemical cues are involved. It is unlikely that the discrimination between familiar and unfamiliar individuals might depend upon habitat- and/or diet-based cues (e.g., [24]) because queens in our experiments came from the same population and the habitat is uniform (cocoa plantation, see Experimental Procedures).

Social insects share a common colony odor, and there is strong evidence that cuticular hydrocarbons are responsible for nestmate recognition. This has been shown by many studies, both indirectly (for review, see [25–27]) and directly (e.g., [28, 29]). Moreover, rather small differences in the relative proportion of the cuticular hydrocarbon blend are also important in intracolony recognition, such as reproductive status (cf. [5, 30, 31]). We analyzed the chemical profile of *P. villosa* founding queens, which is characterized by a complex mixture of hydrocarbons in variable relative proportions (cf. [32] and Figure S1). We tested whether familiar cofounding queens share a common odor and whether cuticular hydrocarbons are associated with the social or reproductive status of queens. We will first deal with the social and reproductive status and then with the possible common odor. A factorial analysis of the profiles of young queens after one week of joint nesting produced four principal components (explaining 91.8% of the variance). The first two principal components (explaining 74.4% of the variance) were neither correlated with fecundity (total egg length, factor 1: $r_s = -0.33$, p = 0.3; factor 2: $r_s = 0.16$, p = 0.6), nor with body size

(head width, factor 1: $r_s = 0.13$, p = 0.67; factor 2: $r_s = -0.22$, p = 0.49). We did not detect chemical similarities between queens with identical social status: the groups of dominants and subordinates were not differentiated by a discriminant analysis (Wilks' Lambda = 0.473, $F_{4,7} = 1.947$, p = 0.207). Although the behavior of queens clearly indicates their social rank (dominant/subordinate) after few hours of cohabitation, to exclude that the lack of discrimination based on chemical profiles
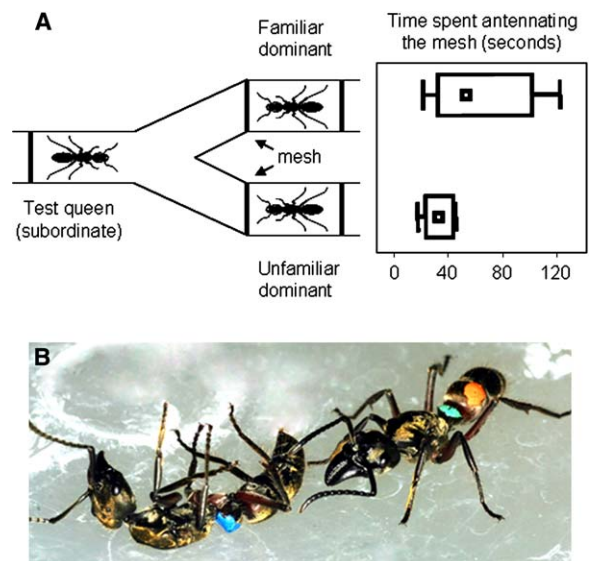

Figure 2. Experiments to Discriminate between Behavioral and Chemical Cues

(A) Experiment 2: schematic set-up and results of the choice experiment with the Y maze. Box plots represent median, 25%, and 75% percentiles and nonoutlier range. Subordinate queens spent significantly more time antennating the mesh of the familiar dominant queen (n = 8) than the one of the unfamiliar dominant (n = 8) (Wilcoxon matched pairs test: Z = 3.0, p = 0.035).
(B) Experiment 3: photograph of a *P. villosa* subordinate queen investigating an unfamiliar anesthetized dominant queen.
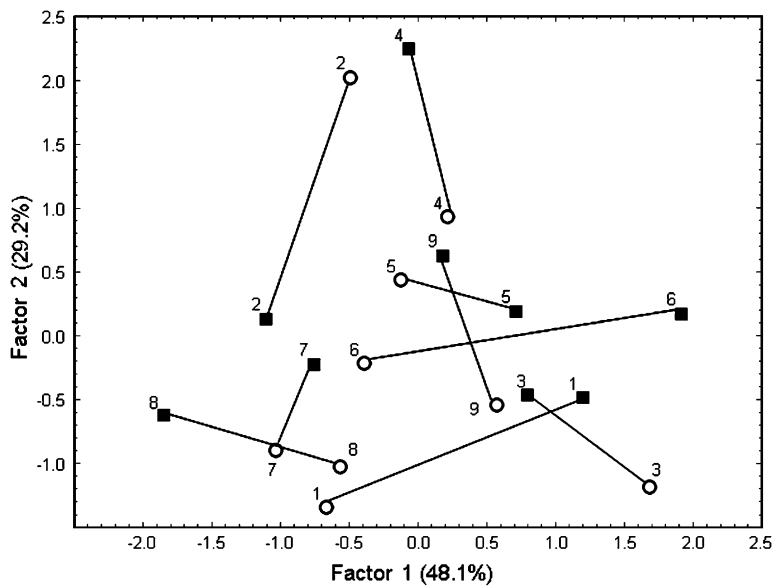
Figure 3. Plot of the First Two Factors of the Principal Component Analysis Based on the Proportion of Cuticular Hydrocarbons of Founding Queens

See also Experimental Procedures and Figure S1. Closed squares represent dominant queens and open circles subordinate queens. Queens belonging to the same founding association are given the same number and are connected by lines. The percentage of explained variance is given in parenthesis.

might be due to the short time these queens had spent together (1 week), we analyzed additional foundresses after 10 weeks of joint nesting. Results were confirmed: queens of different social status did not form separate clusters in a factorial analysis (Figure 3) and were not separated by a discriminant analysis based on the social rank (Wilks' Lambda = 0.643, $F_{7,10}$ = 0.793, p = 0.609).

Finally, we show that mutual recognition, in the particular case of cofounding *P. villosa* queens, is most likely not based on a common odor. Queens, after 1 week of joint nesting, could not be significantly discriminated as belonging to the same founding association on the basis of their chemical profile (Wilks' Lambda = 0.001, $F_{25,8}$ = 1.923, p = 0.153). Similarly, cofounding queens did not cluster together even after 10 weeks of join nesting (Figure 3), and a discriminant analysis failed in distinguishing them according to the nest origin (Wilks' Lambda = 0.0002, $F_{56,21}$ = 1.419, p = 0.189). This is supported by the lack of physical contact, grooming, and food exchange between queens, behaviors that facilitate odor transfer [33]. During our observation sessions, we did not record any occurrence of these behaviors in two-queen founding associations.

We do not claim that *P. villosa* ants do not have a colony odor, but we suggest that the common odor emerges once there is a colony and not at the founding stage in which only young queens, and not workers, are present. Indeed, adult workers from different mature colonies could be significantly discriminated at the colony level on the basis of their cuticular hydrocarbon profile (Wilks' Lambda = 0.047, $F_{12,13}$ = 7, 365 p < 0.001, Figure S2). Nevertheless, workers might rely on individual chemical distinctiveness in different contexts, such as scouting and foraging behavior. In the related species *Pachycondyla tesserinoda*, workers search individually for food and new nest sites and lay down individual-specific trail markers [34].

Interestingly, *P. villosa* founding queens do not show yet a cuticular profile typical of fertile queens in mature colonies, which is characteristic of many ant queens and different from a worker profile. When the first

workers appear, the chemical profile of queens undergoes a significant modification with the acquisition of a typical queen-bouquet (P.D., unpublished data).

Aggression among nestmates, as during hierarchy establishment, has been thought to be based on badges of status by which individuals can crudely recognize who is higher or lower in rank or fecundity [5]. Instead, our results suggest that cofounding *P. villosa* queens recognize each other through individual chemical signatures, which bear neither information on their quality [35] nor group membership. The life history of *P. villosa* may promote individual recognition because, unlike other ant species, female sexuals are produced year-round and new queens may seek adoption into established associations. Founding queens are therefore repeatedly confronted with unfamiliar and unrelated queens and must resolve possible destructive conflict.

Similarly to visual cues in paper wasps, highly variable chemical cues in ants favor the evolution of individual distinctiveness, which may increase group productivity by permitting a relatively inexpensive maintenance of dominance hierarchies in small societies [4, 36]. In large societies, such distinctiveness is likely to be selected against at the group level because it would allow the expression of selfish traits by fractions of the colony, with disrupting consequences on social cohesion and colony productivity [37]. This previously undocumented ability of ants suggests a novel synthesis of the evolutionary mechanisms maintaining order in small animal societies and the generalization of principles of individual and colony selection that maintain these distinctiveness traits.

Experimental Procedures

Study Organisms

Pachycondyla villosa founding queens were collected from knotholes in a cocoa plantation near Ilhéus, Bahia, Brazil. Experiments were carried out on site. Queens were individually marked and housed in plastic dishes (10 cm diameter) with a glass tube with moistened cotton serving as nest site. Food (honey and dead insects) was provided daily. Rank orders were determined by continuous observation for 15 min and then for 1 min every hour until

the relationships had become clear. Usually such a dominance relationship is established very quickly: queens are very aggressive toward each other only during the first 30–60 min.

Experiment 1: Familiar versus Unfamiliar Queens

We followed a standard set up (cf. [38] and Figure 1). Forty randomly chosen solitary founding queens were used to form 20 pairs and allowed to establish dominance relationships over a 24 hr period (familiarization phase). In the experimental phase, a dominant and a subordinate queen were moved to a clean plastic box. Queens were either unfamiliar (test) or familiar with each other (sham). The frequency of all interactions was noted for 10 min after transfer to the new box and aggression was scored as follows: 0, inspection and antennal contact; 1, threat with opened mandibles; 2, biting; and 3, biting with stinging attempts. The directionality of aggression was not noted since each different behavior was always shown simultaneously by both queens. The overall aggression was calculated as (modified from [39]):

$$AI = \frac{\sum_{i=1}^{n} AI_i * f_i}{F}$$

where AI_i is the aggression score, f_i the frequency of each act, and F the total number of interactions. In this second round, queens were housed together for about 60 min.

Even if the pairs of queens subject to the two experimental treatments were chosen randomly, a possible difference in the aggression index between "test" and "sham" might be the effect of a difference in the agonistic level between pairs that were subject to one of the two experimental treatments. To exclude this possibility, queens that were confronted to unfamiliar individuals (test) were subject to a third round of interactions. One hour after the second round, they were moved to a clean plastic box together with the previous partner (the same of the first round "familiarization phase" in Figure 1). We expected no difference in the aggression level of these pairs of queens which encountered again the previously familiar partner and the pairs of queens involved in the "sham" treatment.

Experiment 2: Choice Tests

Sixteen experimental two-queen associations were formed in the lab using queens unfamiliar with one another. After 48 hr of familiarization, eight subordinates were tested in a Y maze in which the familiar and an unfamiliar dominant were confined at the extremities behind a mesh that allowed odor perception but prevented physical contact (Figure 2). The test lasted 5 min, and the time the subordinate investigated by antennation each mesh was recorded on a PC with the software EthoLog [40].

Experiment 3: Discrimination of Anaesthetized Queens

Twelve experimental two-queen associations were formed in the lab using unfamiliar queens. After 1 week, the new associations had adapted to their artificial nest sites. Dominant queens were then removed from their nests, kept for 1 min at 0°C, which immobilized them for about 4 min, and either returned to their own (n = 6) or the nest of an unfamiliar subordinate (n = 6). The behavior of subordinate queens toward the anaesthetized dominants and in particular, the duration of investigative antennation over 3 min was recorded.

Analysis of Chemical Cues

Twelve queens from six experimental two-queen associations were killed by freezing after 1 week in the laboratory. Ovaries were dissected, all developing eggs were counted and their length was measured and summed to estimate current fecundity (total egg length). Head width was measured under a stereomicroscope as an indication of body size.

Cuticular hydrocarbons were extracted by washing queens' heads in 50 μl of pentane for 10 min. Of this extract, 2 μl were injected into an Agilent Technologies 6890N gas chromatograph with a flame ionization detector and a capillary column (Rtx-5, 30 min × 0.25 mm × 0.50 μm, Restek, Bellefonte, PA). The injector was a split-splitless type, the carrying gas helium at 1 ml/min, and the temperature rose from 70°C to 200°C at 20°C/min, from 200°C to 320°C at 4°C/min, and finally was kept at 320°C for 5 min. Compounds were identified by comparison with published results and by their mass spectra produced by electron ionization mass spectrometry using a Hewlett Packard (Palo Alto, Ca) 5890A gas chromatograph coupled to an HP 5917A mass selective detector (70eV electron impact ionization). Nine additional two-queen associations were kept in the laboratory for 10 weeks. Cuticular chemicals were extracted by rubbing the queens' gasters for 3 min with a 7 μm polymethylsiloxane fiber (Supelco; Solid Phase Micro Extraction) and analyzed by gas chromatography as above. Both extraction methods gave similar results, i.e., a variable mixture of linear and branched hydrocarbons (Figure S1).

To compare the queens' profiles, the relative proportions of 20 identified hydrocarbons were analyzed by factorial analysis (principal components analysis). In a discriminant analysis, based on the PCA factor scores, we determined whether dominant and subordinate queens or queens belonging to the same association could be discriminated on the basis of their chemical profiles (Statistica 6, StatSoft). Factor scores were also used to test whether correlations exist between chemical profile, ovarian development, and body size in the 12 dissected queens.

Behavioral Observations

To quantify the occurrence of behaviors allowing a possible exchange of chemical cues between queens, six two-queen founding associations were observed continuously in 20 three-minute sessions (total observation time, 360 min), during which the frequency of all interactions was recorded, with special attention to the occurrence of physical contact and grooming between the two queens. *P. villosa* do not exchange liquid food.

Supplemental Data

Supplemental Data include Experimental Procedures and two figures and are available with this article online at http://www.current-biology.com/cgi/content/full/15/23/2170/DC1/.

References

1. Wilson, E.O. (1975). Sociobiology (Cambridge, MA: Harvard University Press).
2. Hölldobler, B., and Wilson, E.O. (1990). The Ants (Cambridge, MA: Harvard University Press).
3. Tibbetts, E.A. (2002). Visual signals of individual identity in the wasp *Polistes fuscatus*. Proc. R. Soc. Lond. B. Biol. Sci. *269*, 1423–1428.
4. Chapuisat, M. (2004). Social selection for eccentricity. Curr. Biol. *14*, R1003–R1004.
5. Cuvillier-Hot, V., Lenoir, A., Crewe, R., Malosse, C., and Peeters, C. (2004). Fertility signalling and reproductive skew in queenless ants. Anim. Behav. *68*, 1209–1219.
6. Tibbetts, E.A., and Dale, J. (2004). A socially enforced signal of quality in a paper wasp. Nature *432*, 218–222.
7. Halpin, Z.T. (1980). Individual odors and individual recognition: review and commentary. Biol. Behav. *5*, 233–248.
8. Hurst, J.L., Payne, C.E., Nevison, C.M., Marie, A.D., Humphries, R.E., Robertson, D.H.L., Cavaggioni, A., and Beynon, R.J. (2001). Individual recognition in mice mediated by major urinary proteins. Nature *414*, 631–634.

9. Posamentier, M.T., and Abdi, H. (2003). Processing faces and facial expressions. Neuropsychol. Rev. *13*, 113–143.

10. Thom, M., and Hurst, J.L. (2004). Individual recognition by scent. Ann. Zool. Fennici *41*, 765–787.

11. Bonadonna, F., and Nevitt, G.A. (2004). Partner-specific odor recognition in an antarctic seabird. Science *306*, 835.

12. Hojesjo, J., Johnsson, J., Petersson, E., and Jarvi, T. (1998). The importance of being familiar: individual recognition and social behavior in sea trout (*Salmo trutta*). Behav. Ecol. *9*, 445–451.

13. Gherardi, F., and Tiedemann, J. (2004). Binary individual recognition in hermit crabs. Behav. Ecol. Sociobiol. *55*, 524–530.

14. Barrows, E.M. (1975). Individually distinctive odors in an invertebrate. Behav. Biol. *15*, 57–64.

15. Johnstone, R.A. (1997). Recognition and the evolution of distinctive signatures: when does it pay to reveal identity? Proc. R. Soc. Lond. B. Biol. Sci. *264*, 1547–1553.

16. Hannonen, M., and Sundström, L. (2003). Worker nepotism among polygynous ants. Nature *421*, 910.

17. Keller, L. (1997). Indiscriminate altruism: unduly nice parents and siblings. Trends Ecol. Evol. *12*, 99–103.

18. Wilson, E.O. (1971). The Insect Societies (Cambridge, MA: Harvard University Press).

19. Pardi, L. (1948). Dominance order in *Polistes* wasps. Physiol. Zool. *21*, 1–13.

20. Kolmer, K., and Heinze, J. (2000). Rank orders and division of labour among unrelated cofounding ant queens. Proc. R. Soc. Lond. B. Biol. Sci. *267*, 1729–1734.

21. Bernasconi, B., and Strassmann, J.E. (1999). Cooperation among unrelated individuals: the ant foundress case. Trends Ecol. Evol. *14*, 477–482.

22. Heinze, J., Trunzer, B., Hölldobler, B., and Delabie, J.H.C. (2001). Reproductive skew and queen relatedness in an ant with primary polygyny. Insectes Soc. *48*, 149–153.

23. Howard, R.W., and Blomquist, G.J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu. Rev. Entomol. *50*, 371–393.

24. Ward, A.J.W., Holbrook, R.I., Krause, J., and Hart, P. (2005). Social recognition in sticklebacks: the role of direct experience and habitat cues. Behav. Ecol. Sociobiol. *57*, 575–583.

25. Singer, T.L. (1998). Role of hydrocarbons in the recignition system of insects. Am. Zool. *38*, 394–405.

26. Vander Meer, R.K., and Morel, L. (1998). Nestmate recognition in ants. In Pheromone communication in social insects. Ants, wasps, bees and termites, R.K. Vander Meer, M.D. Breed, K. Espelie, and M.L. Winston, eds. (Boulder: Westview Press), pp. 79–103.

27. Lenoir, A., D'Ettorre, P., Errard, C., and Hefetz, A. (2001). Chemical ecology and social parasitism in ants. Annu. Rev. Entomol. *46*, 573–599.

28. Lahav, S., Soroker, V., and Hefetz, A. (1999). Direct behavioral evidence for hydrocarbons as ant recognition dicriminators. Naturwissenschaften *86*, 246–249.

29. Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., Nisimura, T., and Yamaoka, R. (2005). Ant Nestmate and Non-Nestmate Discrimination by a Chemosensory Sensillum. Science *309*, 311–314.

30. D'Ettorre, P., Heinze, J., Schulz, C., Francke, W., and Ayasse, M. (2004). Does she smell like a queen? Chemoreception of a cuticular hydrocarbon signal in the ant *Pachycondyla inversa*. J. Exp. Biol. *207*, 1085–1091.

31. Sledge, M.F., Boscaro, F., and Turillazzi, S. (2001). Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. Behav. Ecol. Sociobiol. *49*, 401–409.

32. Lucas, C., Fresneau, D., Kolmer, K., Heinze, J., Delabie, J.H.C., and Pho, D.B. (2002). A multidisciplinary approach to discriminating different taxa in the species complex *Pachycondyla villosa* (Formicidae). Biol. J. Linn. Soc. *75*, 249–259.

33. Soroker, V., Fresneau, D., and Hefetz, A. (1998). Formation of colony odor in ponerine ant *Pachycondyla apicalis*. J. Chem. Ecol. *24*, 1077–1090.

34. Jessen, K., and Maschwitz, U. (1986). Orientation and recruitment behavior in the ponerine ant Pachycondyla tesserinoda (Emery): laying of individual-specific trails during tandem running. Behav. Ecol. Sociobiol. *19*, 151–155.

35. Dale, J., Lank, D.B., and Reeve, H.K. (2001). Signalling individual identity versus quality: a model and case studies with ruffs, queleas, and house finches. Am. Nat. *158*, 75–86.

36. Strassmann, J.E. (2004). Rank, crime and punishment. Nature *432*, 160–162.

37. Boomsma, J.J., Nielsen, J., Sundström, L., Oldham, N.J., Tentschert, J., Petersen, H.C., and Morgan, E.D. (2003). Informational constraints on optimal sex allocation in ants. Proc. Natl. Acad. Sci. USA *100*, 8799–8804.

38. Zulandt-Schneider, R.A., Huber, R., and Moore, P.A. (2001). Individual and Status Recognition in the Crayfish, *Orconectes rusticus*: The Effects of Urine Release on Fight Dynamics. Behaviour *138*, 137–153.

39. Errard, C., and Hefetz, A. (1997). Label familiarity and discriminatory ability of ants reared in mixed groups. Insectes Soc. *44*, 189–198.

40. Ottoni, E.B. (2000). EthoLog 2.2 - a tool for the transcription and timing of behavior observation sessions. Behav. Res. Methods Instrum. Comput. *32*, 446–449.