



Status discrimination through fertility signalling allows ants to regulate reproductive conflicts



Boris Yagound^{a,*}, Pierre Blacher^a, Dominique Fresneau^a, Chantal Poteaux^a,
Nicolas Châline^{a,b}

^a Laboratoire d'Éthologie Expérimentale et Comparée, E.A. 4443, Université Paris 13, Sorbonne Paris Cité, Villetaneuse, France

^b Department of Experimental Psychology, Institute of Psychology, University of São Paulo, São Paulo, Brazil

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Dominance hierarchies allow group-living animals to regulate the partitioning of reproduction, but the recognition systems underlying dominance interactions remain equivocal. Individual recognition, a cognitively complex recognition system, is often posited as an important mechanism for the regulation of linear dominance hierarchies because of its high level of precision. However, providing it actually allows a fine-scale discrimination of the individuals' statuses, status discrimination may offer an alternative, simpler, recognition system allowing the same level of precision while saving the memory-related costs associated with individual recognition. With the aim of disentangling the cognitive mechanisms underlying the formation and maintenance of hierarchies, we here studied the within-group recognition systems in the ant *Neoponera apicalis*, where orphaned workers compete over male parentage in a linear hierarchical structure. Overall, we found that status discrimination abilities were in fact sufficient for the establishment and stabilization of linear hierarchies. The observed level of accuracy allowed fine-scale discrimination of all top rankers' hierarchical status, and thus translated into a functional individual discrimination of all competing workers at the top of the hierarchy. Low-ranking workers did not exhibit such fine-scale status discrimination. We moreover showed that a putative signal of fertility, 13-methylpentacosane, precisely labelled the workers' position in the hierarchy, thereby providing the recognition cue likely to explain the individuals' discrimination abilities. This signal could therefore play a key role in the regulation of the reproductive conflict in this species. In contrast with the traditional view, our study shows the implication of a cognitively simple but equivalently efficient recognition system during the emergence and stabilization of a linear dominance hierarchy.

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The existence of recognition systems is a central feature of group living. Recognition is used in a wide range of social interactions, thereby allowing group members to adapt their behaviour according to the age, sex, kinship, group membership, hierarchical status, reproductive status, species and neighbourhood of the individuals with which they interact (Sherman, Reeve, & Pfennig, 1997; Thom & Hurst, 2004; Tibbetts & Dale, 2007). Understanding the exact nature of the recognition mechanisms across taxa, their contexts and associated costs and benefits is therefore a major challenge in the biological sciences (Wiley, 2013).

Dominance hierarchies are widespread throughout the animal kingdom. These hierarchies are characterized by asymmetries among group members in the partitioning of resources (Zanette & Field, 2009), and can induce important fitness consequences by mediating access to reproduction, food resources or susceptibility to diseases (Ellis, 1995). Nevertheless, the overt aggression often associated with these hierarchies can also bear important costs in terms of time, energy, physical injuries or vulnerability to predators (Hsu, Earley, & Wolf, 2006; Rutte, Taborsky, & Brinkhof, 2006). Reducing these costs may imply the use of ritualization mechanisms, as is frequently observed in hierarchical contests (Hemelrijk, 2000; Hsu et al., 2006; Tibbetts & Dale, 2007). These mechanisms allow the individuals to adapt their behaviour towards encountered nestmates without the need for overt aggressive interactions, and therefore play a key role in the stabilization of dominance hierarchies.

* Correspondence: B. Yagound, Laboratoire d'Éthologie Expérimentale et Comparée, E.A. 4443, Université Paris 13, Sorbonne Paris Cité, 99 avenue J.-B. Clément, 93430 Villetaneuse, France.

E-mail address: boris.yagound@leec.univ-paris13.fr (B. Yagound).

Numerous empirical and theoretical studies have proposed a variety of intrinsic and extrinsic factors that may be responsible for the formation and maintaining of dominance hierarchies (Dugatkin & Earley, 2004; Hsu et al., 2006). These factors include pre-existing differences between competing individuals (Parker, 1974), the value of the contested resource (Maynard Smith & Parker, 1976) and the influence of previous experiences on the outcome of future encounters (Dugatkin & Earley, 2004; Hsu et al., 2006; Rutte et al., 2006). Game-theoretical studies have shown that hierarchy formation could rely on self-organizing processes, such as winner and loser effects (Dugatkin & Earley, 2004; Hsu et al., 2006; Rutte et al., 2006), without the need for any particular recognition mechanism. In this case the outcome of past encounters influences the chance of winning or losing in future interactions in a self-reinforcing manner, i.e. regardless of the identity or rank of the opponent. However, dominance interactions are often highly directed (Chase & Seitz, 2011; Hsu et al., 2006; Tibbetts & Dale, 2007), indicating that individuals actually recognize the status of their opponents, through either direct or indirect (i.e. memory-based) rank perception (Hemelrijk, 2000; Tibbetts & Dale, 2007). Recognition systems are therefore an important feature of dominance interactions, although not mutually exclusive with self-organizing processes. However, the recognition systems underlying dominance interactions remain equivocal (Hsu et al., 2006), particularly since they very often translate into a linear hierarchical structure.

Individual recognition has often been posited as an important mechanism for the regulation and stabilization of linear dominance hierarchies (d'Ettorre & Heinze, 2005; Dale, Lank, & Reeve, 2001; Thom & Hurst, 2004; Tibbetts, 2002; Tibbetts & Dale, 2007). In this indirect rank perception system (Hemelrijk, 2000), individuals remember earlier interactions with specific group members and adjust their dominance behaviour in subsequent encounters with these same individuals (Dale et al., 2001; Tibbetts, 2002). Despite the complexity of this cognitive mechanism, recognizing individual identity is therefore supposed to provide high benefits by matching the level of precision required for the maintenance of linear hierarchies (Thom & Hurst, 2004).

However, linear hierarchies can also theoretically emerge and be maintained through direct rank perception (i.e. status recognition; Hemelrijk, 2000). Individuals in this case base their decisions on the characteristics signalling an opponent's absolute fighting abilities (resource-holding potential; Parker, 1974), such as age, size, weight or dominance badge (Chase & Seitz, 2011). In contrast to individual recognition, there is thus no need for the opponents to be familiar (Tibbetts & Dale, 2007). Status recognition could therefore save the costs of memory characterizing individual recognition (Thom & Hurst, 2004). However, a critical assumption for the involvement of such a recognition system in the formation and stabilization of linear hierarchies is that it allows a fine-scale discrimination of ranks, but this has never been demonstrated.

Dominance hierarchies are commonly found in social insects (e.g. ants: Cuvillier-Hot, Lenoir, Crewe, Malosse, & Peeters, 2004; Heinze, Hölldobler, & Peeters, 1994; Heinze, Stengl, & Sledge, 2002; Liebig, Peeters, Oldham, Markstädter, & Hölldobler, 2000; Monnin & Peeters, 1999; bees: Ayasse, Marlovits, Tengö, Taghizadeh, & Francke, 1995; Bull, Mibus, Norimatsu, Jarmyn, & Schwarz, 1998; wasps: Sledge, Boscaro, & Turillazzi, 2001; Tibbetts, 2002), and this is particularly true when the colonies comprise several individuals with equivalent reproductive potentials competing to gain access to reproduction. Workers in hopelessly queenless colonies thus typically compete with one another over male parentage (Bourke, 1988; Ratnieks, Foster, &

Wenseleers, 2006), with a resulting linear or near-linear hierarchical structure of dominance relationships regulating the partitioning of reproduction (Heinze et al., 1994; Heinze et al., 2002; Peeters & Liebig, 2009), as in the Neotropical ant *Neoponera* (formerly *Pachycondyla*; Schmidt & Shattuck, in press) *apicalis* (Oliveira & Hölldobler, 1990). This species shares all the traits typically characterizing Ponerinae ants, i.e. small societies, a limited queen–worker dimorphism and a high potential for worker reproduction (Fresneau, 1994), and is therefore a good model system for studying the recognition mechanisms involved in the formation and maintenance of dominance hierarchies.

A previous study has shown that low-ranking individuals are able to discriminate top-ranking from low-ranking workers, suggesting a capacity to recognize the social status of their nestmates (Blacher, Lecoutey, Fresneau, & Nowbahari, 2010). However, these recognition abilities have never been investigated in top-ranking workers. Since they are the individuals actually involved in the reproductive competition, the costs of mistaking ranks for those of adjacent-ranking nestmates are, in contrast to low rankers, potentially high. We could hypothesize that a more precise recognition system (e.g. individual recognition) is necessary for an efficient discrimination among top-ranking individuals (Tibbetts & Dale, 2007), but this could also be achieved without a necessarily greater level of cognitive complexity in the eventuality of fine-scale status discrimination. Assessing top rankers' cognitive abilities therefore remains a crucial step in understanding the recognition mechanisms underlying the formation and stabilization of the hierarchical structure in these social groups (Elwood & Arnott, 2012; Wiley, 2013). Here we tested the possibility of fine-scale status discrimination without the need for individual recognition by studying the cognitive abilities of *N. apicalis* top- and low-ranking workers. Furthermore, the nature of the recognition cues involved in these dominance interactions remains unknown, but they probably involve chemical communication. Chemical signals, mainly cuticular hydrocarbons, are widely acknowledged to be of primary importance in the communication of dominance and especially reproductive status in social insects (Liebig, 2010; Monnin, 2006). We therefore also analysed the individuals' chemical profile to investigate the nature of the putative recognition cues at the basis of these dominance interactions.

METHODS

Ants

Colonies of *N. apicalis* were collected in the Kérenroch forest, Petit Saut (5° 04'15.8" N, 53° 02'36.3" W), French Guiana in March 2007 and have been kept in the laboratory in France ever since. Ants were housed in plaster nests (18 × 14 cm) connected to a foraging area of the same dimensions, where food (crickets and honey/apple mixture) was provided twice a week and water ad libitum. Each colony had a queen, more than 70 workers and brood at every developmental stage. Nests were maintained at a temperature of 27 ± 2 °C, a relative humidity of 60 ± 5% and a 12:12 h light:dark cycle. Ant collection, husbandry and experimental procedures used in this study fulfilled all the legal requirements concerning insect experimentation of France.

Dominance Hierarchy

From our stock colonies, we created six experimental colonies by isolating 40 randomly chosen workers and placing them in a

new nest. Taking the workers away from the influence of the queen induces the formation of a dominance hierarchy by means of ritualized agonistic behaviours (Blacher et al., 2010; Oliveira & Hölldobler, 1990). All ants were individually labelled with numbered tags and dots of paint to allow the individual monitoring of their behaviour. Housing and feeding conditions were the same as above.

Each experimental colony was then observed 1 h a day during a 14-day period, during which we recorded all behavioural acts linked to the establishment of the dominance hierarchy, i.e. ritualized biting and antennal boxing (antennal strokes on another ant's body) (Cuvillier-Hot et al., 2004; Heinze et al., 2002; Oliveira & Hölldobler, 1990). All observations started the same day the workers were isolated from the queen, and were performed through a red plastic film to avoid disturbances that may affect the ants' behaviour. All agonistic interactions (performed and received) were then compiled in a matrix and arranged in an order minimizing the number of inconsistencies (i.e. when an individual is given a lower rank than an individual it dominates). This allowed us to reconstruct the dominance hierarchy and to assign a hierarchical rank to each individual (see Blacher et al. (2010) and references therein for a detailed description of the method used). Ants at the top of the hierarchy that collectively performed more than 75% of the agonistic acts (mean \pm SE: 11.5 ± 0.2 individuals, $N = 6$ colonies; Table 1) were considered high-ranking individuals. Two other classes of individuals were additionally determined, namely middle-ranking individuals (remaining ants performing up to 95% of the agonistic acts with the exclusion of high-ranking workers, 11.8 ± 1.6 individuals) and low-ranking individuals (remaining ants at the bottom of the hierarchy, 13.8 ± 1.6 individuals).

Habituation–Discrimination Procedure

To test the cognitive abilities of high- and low-ranking workers, we used a habituation–discrimination paradigm, a classical procedure in cognitive studies (Ferguson, Young, & Insel, 2002) consisting of two consecutive phases. The habituation phase consists of four consecutive exposures (4 min each with a 5 min interval) of a stimulus (a nestmate ant) to a tested individual. The tested ant thus becomes familiar with the proposed stimulus. The stimulus ant is CO₂-anaesthetized to avoid any influence of its behaviour on the tested ant's response. Following a 5 min interval, the discrimination phase consists of a single test

(4 min) in which the tested ant is confronted with two stimuli: a familiar stimulus (the anaesthetized ant previously used during the habituation phase) and an unfamiliar stimulus (another anaesthetized nestmate). Discrimination is typically manifested by a longer duration of the behavioural response towards the unfamiliar stimulus compared with the familiar stimulus (Wiley, 2013). Note that both stimuli are nestmates of the tested ant, and therefore both have potentially already interacted with it. The use of 'familiar' and 'unfamiliar' thus refers to the habituation–discrimination procedure only, since these terms are classically used in these experiments.

Each test was performed in a neutral arena (diameter = 5.3 cm) with externally black-covered walls and Fluon-coated sides to prevent the ant from escaping. To enable familiarization with the device, the tested ant was gently placed in it 30 s before the test began. The stimulus ant was then introduced into the centre of the arena, in which a filter paper had been placed as a substrate (the paper was replaced between each test to avoid any odour residues). All tests were videorecorded for subsequent analyses of the tested ants' behaviour. During these analyses, we measured the duration of antennal contacts with the stimulus (commonly taken as a measure of an ant's interest towards a social stimulus; Boulay, Hefetz, Soroker, & Lenoir, 2000) with EthoLog 2.2 software (Ottoni, 2000). In addition, we investigated the possibility that the behavioural response of high- and low-ranking workers could be influenced by differences in their overall reaction or motivation in the experimental device by quantifying their mobility pattern in 58 randomly selected habituation tests derived from all four habituation tests and all colonies using EthoVision 3.1.16 (Noldus Information Technology, Wageningen, Netherlands). Three behavioural variables were quantified: total distance moved, mean angular velocity and duration of mobility. We also quantified the duration close to the stimulus ant, which reflects the general attraction/avoidance of the tested ant towards the proposed stimulus. Observations were performed twice and blind.

On day 15, four different experiments were carried out using this procedure in which tested and stimuli ants were either low- or high-ranking individuals as previously defined. All tested and stimuli ants were only used in a single habituation–discrimination test.

Experiment 1: status discrimination by high rankers

In the first experiment, the tested ant ($N = 21$, two for colony B, three for colony A, four for colonies C, D, E and F) was a high ranker and was confronted during the discrimination phase with stimuli belonging to different rank classes, i.e. a low- and a high-ranking individual (mean gap in their respective rank of 22.5 ± 0.6). This experiment was aimed at verifying whether high-ranking workers are capable of status discrimination, an ability that has already been shown in low-ranking workers (Blacher et al., 2010). To avoid the stimulus rank class affecting the tested ant's behaviour, the familiar stimulus was a low-ranking individual for half of the tested ants ($N = 11$) and a high-ranking individual for the other half ($N = 10$).

Experiment 2: fine-scale discrimination of high-ranking individuals by high rankers

The second experiment was aimed at testing the possibility of fine-scale discrimination among high-ranking workers. High rankers (tested ants, $N = 19$, two for colony E, three for colonies B, C and D, four for colonies A and F) were confronted during the discrimination phase with two high-ranking workers (stimuli ants)

Table 1
Dominance hierarchy characteristics

Colony	Number of top rankers	Linearity*	Correlation with dominance index	Correlation with ovarian index
A	11	$K=0.85$ $P<0.0001$	$r_s=-0.68$ $P<0.0001$	$r_s=-0.75$ $P<0.0001$
B	12	$K=0.91$ $P<0.0001$	$r_s=-0.80$ $P<0.0001$	$r_s=-0.85$ $P<0.0001$
C	11	$K=0.45$ $P=0.031$	$r_s=-0.40$ $P=0.014$	$r_s=-0.69$ $P<0.0001$
D	12	$K=0.69$ $P<0.0001$	$r_s=-0.69$ $P<0.0001$	$r_s=-0.75$ $P<0.0001$
E	12	$K=0.94$ $P<0.0001$	$r_s=-0.89$ $P<0.0001$	$r_s=-0.61$ $P<0.0001$
F	11	$K=0.92$ $P<0.0001$	$r_s=-0.86$ $P<0.0001$	$r_s=-0.81$ $P<0.0001$

* See Appleby (1983). Note that the smaller values of K for colony C and to a lesser extent colony D are mainly due to more missing values for some dyads (11 and 3 for colonies C and D respectively) compared with the other colonies (0 or 1) (de Vries, 1995).

located higher in the hierarchy and separated by a single rank (mean gap in their respective rank of 1.0 ± 0.0).

Experiment 3: fine-scale discrimination of low-ranking individuals by high rankers

In the third experiment, high rankers (tested ants, $N = 21$, three for colonies B, E and F, four for colonies A, C and D) were confronted during the discrimination phase with two low-ranking workers (stimuli ants) with again very similar ranks (mean gap in their respective rank of 1.7 ± 0.3), but this time located at the bottom of the hierarchy.

Experiment 4: fine-scale discrimination of high-ranking individuals by low rankers

Finally, the fourth experiment was done in order to compare the behavioural discrimination of high- and low-ranking workers. Low rankers have already been shown to discriminate high- and low-ranking individuals, but not low-ranking individuals (Blacher et al., 2010). However, their ability to discriminate high-ranking workers has never been investigated. Low rankers (tested ants, $N = 24$, four in each colony) were therefore confronted with two high-ranking workers (stimuli ants, mean gap in their respective rank of 1.1 ± 0.1) in the discrimination phase.

Fertility Measurement

To link the ants' hierarchical rank with their reproductive dominance, all workers were frozen for dissection at the end of the habituation–discrimination procedure, and their fertility was determined. As an ovarian index, we measured the total size of the six basal oocytes. We then determined three classes of individuals depending on their number of developed oocytes (i.e. size > 0.5 mm; Fresneau, 1994): highly fertile individuals (five to six developed oocytes, 11.8 ± 2.4 individuals, $N = 6$ colonies), moderately fertile individuals (one to four developed oocytes, 13.3 ± 2.7 individuals) and infertile individuals (no developed oocytes, 12.0 ± 2.3 individuals). Some individuals (two in colonies A, D and F, three in colonies C and E, five in colony B) died before the experiments and were therefore not included in the analyses.

Chemical Profiles

We finally investigated the nature of the putative recognition cues involved in the dominance interactions of the reproductive hierarchy by analysing the cuticular hydrocarbon profile of all individuals. This procedure allowed us to study how ants diverged in their chemical signature according to their social rank and fertility state. We sampled the cuticular hydrocarbons of a total of 223 ants. Extraction was performed by placing an ant in 400 μ l of pentane containing 8 ng/ μ l of an internal standard (*n*-C₁₇) for 20 min. We then transferred 100 μ l into a 200 μ l glass insert. Following evaporation, 20 μ l of pentane were added to the 200 μ l glass insert. We then manually injected 2 μ l of the extract into an Agilent 7890A gas chromatograph, equipped with an HP-5MS capillary column (30 m \times 25 μ m \times 0.25 μ m) and a split–splitless injector, coupled to an Agilent 5975c mass spectrometer with 70 eV electron impact ionization. The carrier gas was helium at 1 ml/min. The temperature program was as follows: an initial hold at 70 °C for 1 min, then 70–180 °C at 30 °C/min, then 180–320 °C at 5 °C/min, then hold at 320 °C for 5 min. The areas of 34 peaks present in all ant cuticular extracts (Appendix Fig. A1) were integrated with the Agilent ChemStation

software. Hydrocarbons were identified on the basis of their mass spectra and retention times, and compared with known standards.

Statistical Analyses

Following hierarchy reconstruction, we calculated the *K* index of linearity varying from 0 (no linearity) to 1 (linear hierarchy) and tested the statistical significance of linearity according to Appleby (1983). The test of linearity was performed on high-ranking workers only, because middle- and particularly low-ranking workers performed far fewer agonistic interactions, thus creating incomplete information, which is known to underestimate the values of linearity (de Vries, 1995). Using the Spearman rank correlation test, we calculated the correlation between the hierarchical rank and (1) the dominance index (proportion of agonistic acts performed) and (2) the ovarian index. We compared the proportion of agonistic acts that high rankers performed towards other high-ranking workers of consecutive ranks with the Friedman test followed by post hoc exact permutation tests with the Bonferroni–Holm method (Holm, 1979).

For each experiment, we compared the duration of antennal contacts with the stimulus between the first and the fourth habituation test, and the antennation duration towards the familiar and the unfamiliar stimulus in the discrimination test using exact permutation tests for paired samples. Each mobility variable was compared between high and low rankers confronted with high-ranking nestmates, and between high rankers confronted with high- and low-ranking nestmates using exact permutation tests for independent samples.

For chemical analyses, we arcsine-transformed (Sokal & Rohlf, 2012) the relative quantities of 34 compounds common to all individuals. We then performed a discriminant function analysis to investigate how individuals diverge in their chemical profile according to their hierarchical rank (i.e. high-, middle- and low-ranking individuals), and their fertility level (i.e. highly fertile, moderately fertile and infertile individuals). We finally investigated the existence of a putative fertility signal (Monnin, 2006) by comparing the absolute quantity of all compounds between the three fertility groups using one-way ANOVAs followed by post hoc exact permutation tests with the Bonferroni–Holm method. Absolute quantities of the compounds with the highest contribution to the discrimination were also correlated with the individuals' fertility and social rank for all individuals using the Spearman rank correlation test. The Monte Carlo procedure was used when appropriate to deal with large sample sizes (Metropolis & Ulam, 1949).

All statistical analyses were performed with StatXact 8.0 (Cytel Software Corporation, Cambridge, MA, U.S.A.) and Statistica 8.0 (StatSoft, Tulsa, OK, U.S.A.). Statistical significance was set at $P < 0.05$.

RESULTS

Dominance Hierarchy

During the 14-day observation period of dominance/subordinate relationships, we recorded a total of 11808 agonistic acts (1968.0 ± 248.2 per colony), which allowed us to determine the hierarchical rank of the ants successfully. The linearity or near-linearity of the hierarchy was significant in all colonies (Table 1). Furthermore, the individuals' hierarchical rank was highly correlated with their dominance index (Table 1). Among high rankers,

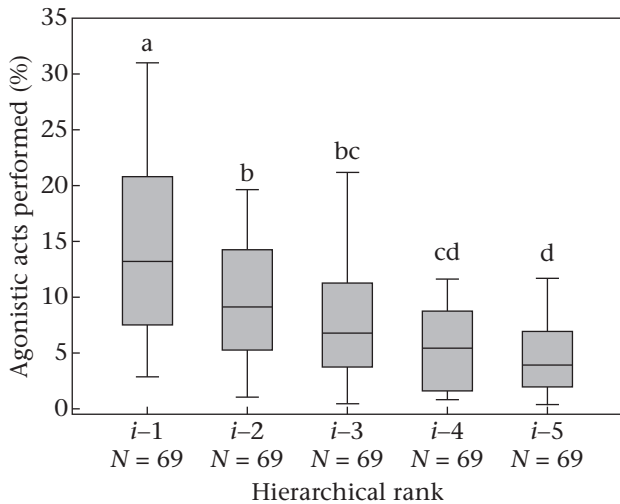


Figure 1. Percentage of agonistic acts performed by high rankers (rank i) towards the individuals with the closest lower ranks in the hierarchy (rank $i-1$ to rank $i-5$). Box plots represent 10th, 25th, 50th (median), 75th and 90th percentiles. Sample sizes of individuals are indicated below each box plot. Different letters denote statistical differences.

agonistic interactions were not randomly directed towards other high-ranking workers, but instead were highly biased towards the individuals with the closest lower ranks in the hierarchy (Friedman test on the proportion of agonistic acts that high rankers performed towards individuals with immediate consecutive ranks: $T_{F, 4} = 53.73$, $N = 69$, $P < 0.0001$; Fig. 1).

Agonistic interactions directed towards dominant individuals (inconsistencies) were very rare among high rankers ($2.56 \pm 0.84\%$ per colony). In addition, the formation of a linear hierarchy was very quick, since the proportion of inconsistencies was already small during the first 24 h of isolation ($12.76 \pm 8.09\%$ per colony).

Habituation–Discrimination Procedure

Habituation, manifested by a decrease in the duration of antennal contacts with the stimulus ant between the first and the last habituation test (Wiley, 2013), occurred in the four experiments (permutation tests: all $P < 0.021$; Appendix Fig. A2). In the first experiment, habituation occurred whether the tested ant was familiarized with a high- or a low-ranking nestmate ($N = 10$, $P = 0.041$ and $N = 11$, $P = 0.003$, respectively; Appendix Fig. A2a). High and low rankers showed similar mobility patterns in the experimental device ($N = 39$, all $P > 0.18$; Appendix Table A1), as did high rankers towards high- and low-ranking individuals ($N = 39$, all $P > 0.18$; Appendix Table A1). Furthermore, duration of contacts with the stimulus ant was similar for each of the four habituation tests and all tests combined whether the stimulus was a high- or a low-ranking individual ($N = 21$, all $P > 0.22$). Tested ants were thus able to familiarize themselves with a nestmate, the social status of this nestmate having no influence on this process.

In the discrimination test of the first experiment, tested ants spent more time antennating unfamiliar than familiar ants ($N = 21$, $P = 0.0004$; Fig. 2), irrespective of the status of the habituation stimulus (high-ranking nestmates: $N = 10$, $P = 0.020$; low-ranking nestmates: $N = 11$, $P = 0.018$). There was no significant difference in the duration of antennal contacts between high- and low-ranking familiar stimuli ($N = 21$, $P = 0.96$) and between high- and

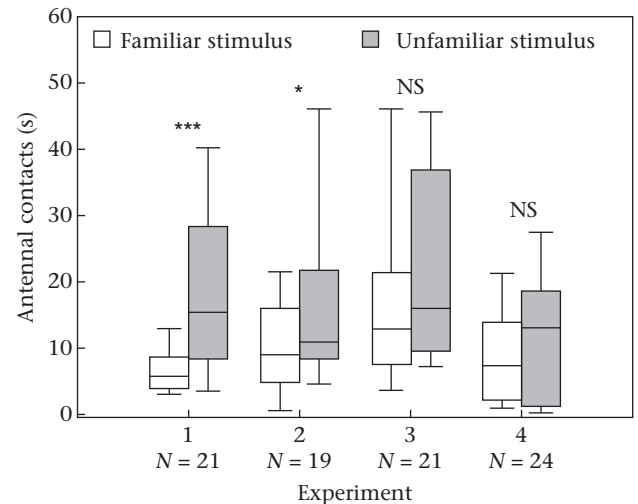


Figure 2. Duration of antennal contacts (s) towards the familiar and unfamiliar stimuli during discrimination tests in all experiments. In experiments 1–3, tested ants were high rankers and were confronted with a low- and a high-ranking nestmate (experiment 1), two high-ranking nestmates (experiment 2) or two low-ranking nestmates (experiment 3). In experiment 4, tested ants were low rankers and both stimuli were high-ranking nestmates. Box plots represent 10th, 25th, 50th (median), 75th and 90th percentiles. Sample sizes of individuals are indicated below each box plot. *** $P < 0.001$; * $P < 0.05$.

low-ranking unfamiliar stimuli ($N = 21$, $P = 0.97$). High rankers were thus well able to discriminate the social status of their nestmates, as has already been shown in low rankers (Blacher et al., 2010).

In the second experiment, duration of antennal contacts towards unfamiliar ants was higher than towards familiar ants ($N = 19$, $P = 0.037$; Fig. 2). Since stimuli ants were separated by a single rank, this clearly demonstrates that high rankers were capable of fine-scale status discrimination of other high-ranking workers.

In the third experiment, duration of antennation towards unfamiliar and familiar nestmates was not significantly different ($N = 21$, $P = 0.42$; Fig. 2). High rankers therefore did not show a behavioural discrimination of low-ranking nestmates with very similar statuses. This result further confirms that the differential response of the tested ants towards unfamiliar stimuli compared with familiar stimuli in the first and second experiments was based on status discrimination.

In the fourth experiment, low-ranking tested ants did not spend significantly more time antennating unfamiliar or familiar nestmates ($N = 24$, $P = 0.15$; Fig. 2). Furthermore, there was no significant difference between low- (experiment 4) and high-ranking tested ants (experiment 2) in the duration of antennal contacts towards high-ranking familiar stimuli ($N = 43$, $P = 0.79$) and in the duration of antennal contacts towards high-ranking unfamiliar stimuli ($N = 43$, $P = 0.36$). In contrast to high rankers, low rankers thus did not exhibit fine-scale status discrimination of high-ranking nestmates.

Fertility Measurement

Hierarchical rank was strongly correlated with the ovarian index in all colonies (Table 1). This corroborates the well-known relationship between fertility and social status in insect societies (Blacher et al., 2010; Cuvillier-Hot et al., 2004; Heinze et al., 2002; Monnin & Peeters, 1999).

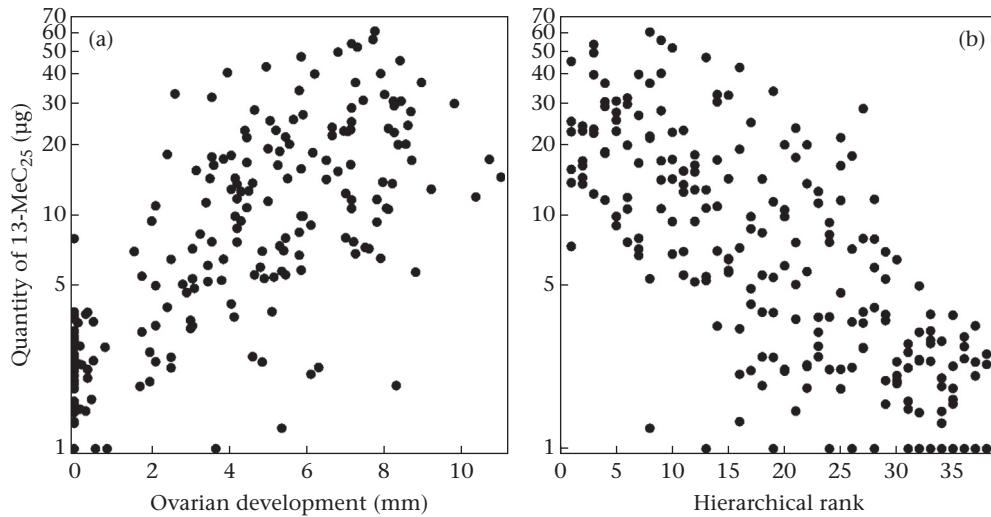


Figure 3. Relationship between quantity of 13-MeC₂₅ (µg) and (a) ovarian development (mm) and (b) hierarchical rank. Note log scale on ordinates.

Chemical Profiles

Workers could clearly be separated on the basis of their cuticular hydrocarbon profiles according to their hierarchical rank (Wilks's $\lambda = 0.304$, $F_{40,402} = 8.19$, $P < 0.0001$; Appendix Fig. A3a) and even more strongly to their ovarian development (Wilks's $\lambda = 0.130$, $F_{52,390} = 13.33$, $P < 0.0001$; Appendix Fig. A3b). This indicates that the chemical signature provides a reliable cue for an ant to discriminate the fertility and social rank of its nestmates.

From the 34 compounds constituting the shared chemical profile of *N. apicalis* workers (Appendix Fig. A1), 14 displayed significant differences in their amounts between individuals of varying fertility (Appendix Table A2). Among these compounds, 13-methylpentacosane (13-MeC₂₅) had the highest contribution to the discrimination of all individuals according to their ovarian development (partial Wilks's $\lambda = 0.799$, $F_{2,195} = 24.39$, $P < 0.0001$) and hierarchical rank (partial Wilks's $\lambda = 0.867$, $F_{2,201} = 15.47$, $P < 0.0001$) in the discriminant function analyses. Quantity of 13-MeC₂₅ was further strongly correlated with both fertility (Spearman rank correlation: $r_s = 0.77$, $N = 223$, $P < 0.0001$; Fig. 3a; Appendix Table A2), and hierarchical rank ($r_s = -0.73$, $N = 223$, $P < 0.0001$; Fig. 3b).

DISCUSSION

Our results clearly show that *N. apicalis* workers in hopelessly queenless colonies establish a linear dominance hierarchy in which agonistic behaviour, fertility, cuticular hydrocarbon profile and social rank are all closely correlated. Dominance interactions were highly directed, with top-ranking individuals performing most agonistic acts towards other high rankers with immediate consecutive ranks. The vast majority of hierarchical relationships were clearly and quickly established. Furthermore, it has been shown in *N. apicalis* and several other species that once the hierarchy has emerged, dominance relationships can be maintained for extended periods of time, but with a dramatic decrease in agonistic behaviour (Blacher et al., 2010; Cuvillier-Hot et al., 2004; Monnin & Peeters, 1999). Although this does not exclude the influence of self-organizing processes, at least in the beginning (Dugatkin & Earley, 2004; Hsu et al., 2006; Rutte et al., 2006), it unambiguously indicates the implication of recognition

mechanisms in the formation and maintenance of the hierarchical structure.

All ants were able to discriminate top-ranking from low-ranking nestmates, thus confirming and expanding the existence of status discrimination abilities in this species (Blacher et al., 2010). Furthermore, top rankers were able to discriminate two high-ranking nestmates separated by a single rank in the hierarchy. This ability of fine-scale status discrimination among top rankers is beneficial for the regulation of reproductive dominance. It allows each competing ant to adapt its behaviour according to the social status of all encountered nestmates, i.e. dominantly towards a lower ranker and submissively towards a higher ranker. Avoiding recognition errors decreases the costs associated with dominance interactions in terms of colony productivity, and therefore enhances the individuals' inclusive fitness (Gobin, Heinze, Strätz, & Roces, 2003). Note that both stimuli in the discrimination phase were dominant for the tested ant, meaning that individuals did not merely discriminate an ant higher than itself from a lower ant in the hierarchy. This emphasizes that recognizing all the competing individuals' statuses can be adaptive in the eventuality of a hierarchy disruption (Hart & Monnin, 2006), as has been shown to occur (Oliveira & Hölldobler, 1990).

It has been shown in two related species, *N. villosa* and *N. inversa*, that unrelated co-founding queens establishing dominance hierarchies seem capable of individual recognition (d'Etterre & Heinze, 2005; Dreier, van Zweden, & d'Etterre, 2007). In our study, top rankers did not behaviourally discriminate low rankers of virtually identical status. This could partly stem from a low motivation for accomplishing this task, as low-ranking nestmates are not involved in the reproductive competition. The habituation–discrimination procedure was, however, used to reduce the influence of motivation on the tested ants' response. Although the context of hierarchy formation is different in *N. apicalis*, and unambiguously demonstrating individual recognition abilities is particularly challenging (Wiley, 2013), our results nevertheless suggest an absence of identity-based discrimination in workers. Within the scope of status discrimination abilities, and as we discuss in more depth below, this absence of behavioural discrimination between two low rankers could most likely be explained by an absence of recognition cues allowing an unequivocal discrimination.

If our results fail to provide any strong evidence in favour of individual recognition, they, however, clearly indicate that linear hierarchies can arise without such a recognition system. Status discrimination, a cognitively simpler mechanism (Wiley, 2013), has in this species at least the level of accuracy enabling a fine-scale discrimination of the individuals' statuses without the necessity of recognizing their identity. This recognition system thus appears suitable for precise regulation of dominance interactions without the need for aggressive behaviours, and more importantly avoids the cognitive costs linked to individual recognition. Indeed, individual recognition relies on the memory of each opponent's distinctive features, and of their history of encounters in the context of dominance interactions. It thus requires active learning and an accurate memory, both of which are costly processes in terms of time and energy expenditure (Burns, Foucaud, & Mery, 2011; Dukas, 2008). By contrast, status discrimination is a cognitively less demanding task, as it relies on the direct perception of each encountered individual's rank. Carefully investigating alternative hypotheses thus remains a crucial step when studying animal cognition (Elwood & Arnott, 2012). Overall, these results show that direct rank perception is probably a critical factor in the establishment and stabilization of the hierarchical structure.

Whereas high and low rankers showed very similar behavioural reactions in the experimental device in terms of their proximity and duration of antennal contacts towards stimuli in both the habituation and the discrimination phase, low rankers failed to discriminate at a fine scale ants belonging to the same rank class, be they high- or low-ranking nestmates (this study; Blacher et al., 2010). This difference with top rankers could be due to an absence of motivation since all high-ranking nestmates are by definition much higher in the hierarchy and could induce the same submissive behaviours from low rankers. In contrast to other species (Hart & Monnin, 2006), low rankers indeed play no role in top ranker replacements (Oliveira & Hölldobler, 1990), and thus in this context they have no benefits from discriminating two high rankers. Alternatively, the difference between high- and low-ranking workers could also be due to differences in their cognitive abilities. Indeed, top and low rankers can differ in a number of physiological characteristics. For example, neuroendocrine activities (levels of brain biogenic amines) can vary depending on the dominance and reproductive status. Top rankers have higher levels of octopamine than low rankers in the bumble bee *Bombus terrestris* (Bloch, Simon, Robinson, & Hefetz, 2000), and octopamine levels are correlated with reproductive activity in the queenless ant *Streblognathus peetersi* (Cuvillier-Hot & Lenoir, 2006). Octopamine further acts as a neuromodulator which is known to affect cognitive processes such as learning and memory (Farooqui, 2007; Verlinden et al., 2010). Different internal states according to the individuals' hierarchical status could thus theoretically mediate various levels of recognition abilities. Intraspecific variation in recognition abilities has recently begun to be explored (e.g. Injaian & Tibbetts, 2014), and future investigations are therefore required to examine the existence of actual differences in cognitive abilities depending on the individual's social status.

Cuticular hydrocarbon profiles diverged between reproductive and nonreproductive individuals, and probably constitute the recognition cues used in dominance interactions. The chemical nature of cuticular hydrocarbons signalling fertility can differ markedly according to species, but the occurrence of fertility signals seems to be a general phenomenon in social insects (Cuvillier-Hot et al., 2004; Heinze et al., 2002; Liebig, 2010; Liebig et al., 2000; Monnin, 2006; Sledge et al., 2001). This can be

explained by the fact that being permanently informed about the fertility state of the egg-layer(s) provides inclusive fitness benefits to all colony members (Keller & Nonacs, 1993). Here, amounts of 13-methylpentacosane (13-MeC₂₅) were highly correlated with the individuals' ovarian activity. This compound may therefore have the role of a putative fertility signal (Liebig, 2010; Monnin, 2006). Hydrocarbons are synthesized in the oenocytes, cells associated with the epidermis and the fat bodies (Martins & Ramalho-Ortigão, 2012). It is generally assumed that common endocrinological mechanisms (e.g. gonadotropic hormones) underlie the biosynthesis of cuticular hydrocarbons and the activity of the ovaries (Cuvillier-Hot et al., 2004; Liebig, 2010; Peeters & Liebig, 2009), thus explaining the close link between reproductive activity and chemical signals. Such an intrinsic causal link would mean the recognition system could not be faked, and fertility signals would be evolutionarily stable (i.e. honest; Laidre & Johnstone, 2013; Maynard Smith & Harper, 1995).

The quantity of the putative fertility signal was also highly correlated with the individuals' social rank, because of the close link between fertility and hierarchical status. The exponential form of the relationship between amounts of 13-MeC₂₅ and hierarchical rank means that two individuals with very different social statuses (i.e. a high- and a low-ranking worker) would have a large difference in their amounts of 13-MeC₂₅, therefore allowing status discrimination. Two nestmates having close but none the less different hierarchical statuses (i.e. two top rankers in this case) would have a lower but yet significant difference in their amounts of 13-MeC₂₅. In this case, status discrimination becomes precise enough to allow a fine-scale discrimination of their ranks, corresponding to a functional individual discrimination. In contrast, two individuals having a very similar social status (i.e. two low rankers) would have a very small difference in their amounts of 13-MeC₂₅, thus making any discrimination probably difficult, possibly beyond the workers' sensory and information-processing capabilities. According to this hypothesis, 13-MeC₂₅ is likely to form the proximate signal at the basis of the individuals' status discrimination, and could in this respect constitute a chemical badge of status (Guilford & Dawkins, 1995). Such signals, traditionally linked with the individuals' resource-holding potential (Johnstone & Norris, 1993), could allow individuals in this context to select the best egg-layer in the colony. Similar mechanisms have already been suggested (Cuvillier-Hot et al., 2004), and could form a general rule in the regulation of dominance hierarchies in insect societies. The dynamics of fertility signalling during the establishment of the hierarchical structure remains unknown, but it is conceivable that preferential differences at the onset of orphaning could strongly influence dominance interactions and therefore the determination of hierarchical ranks. Such investigations should thus be conducted in future studies.

In conclusion, we have shown here that status discrimination based on a putative fertility signal is able to generate a linear dominance hierarchy in *N. apicalis* ants. This single cuticular hydrocarbon appears to act as a badge of status by precisely labelling the individual's position in the hierarchy, and therefore regulates the conflict over male parentage in this species. Whereas the formation of linear hierarchies is often assumed to rely on complex cognitive processes such as individual recognition, our results suggest on the contrary that simpler recognition mechanisms can be sufficient to regulate dominance interactions efficiently. By mutually benefiting all members of the nest, this recognition system is thus very likely to have been selected for by both individual- and colony-level selection pressures.

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Appendix

Table A1
Mobility pattern of tested ants in the experimental device

	Total distance moved (cm)	Mean angular velocity (rad/s)	Duration of mobility (s)	Duration close to the stimulus ant (s)
High-ranking tested ants confronted with high-ranking stimuli (1, N=20)	28.72±5.48	18.14±3.62	28.14±5.28	8.12±1.73
Low-ranking tested ants confronted with high-ranking stimuli (2, N=19)	39.67±5.78	14.62±2.22	35.86±4.97	11.87±3.01
High-ranking tested ants confronted with low-ranking stimuli (3, N=19)	22.21±4.76	12.19±2.22	22.09±4.29	8.80±1.89
P (1) vs (2)	0.18	0.42	0.29	0.31
P (1) vs (3)	0.38	0.18	0.38	0.79

Data are presented as mean ± SE.

Table A2
Chemical differences between workers of varying fertility

Compound	Highly fertile individuals (N=72)	Moderately fertile individuals (N=80)	Infertile individuals (N=71)
Unidentified	23.57±6.38	24.70±8.11	28.03±3.82
10-MeC ₁₉	2.68±0.23 a	2.70±0.20 a	3.65±0.29 b
n-C ₂₀	24.17±1.50	20.10±1.21	23.27±1.57
Unidentified	3.10±1.04	2.97±1.26	1.87±0.23
C _{21:1}	132.14±34.10	45.90±11.84	108.62±47.33
C _{21:1}	42.12±33.76 a	183.59±79.26 a	802.62±178.15 b
C _{21:1}	12.50±0.63	10.75±0.57	69.30±59.80
C _{21:1}	79.31±9.56	67.66±8.42	67.56±9.76
n-C ₂₁	2400.67±106.70 a	2215.76±79.44 ab	2023.28±113.08 b
11-MeC ₂₁	3.21±0.50 a	4.64±2.15 a	0.65±0.12 b
9-MeC ₂₁	3.92±0.52 a	2.77±0.33 a	1.56±0.28 b
C _{22:2}	189.62±14.08	184.60±10.75	202.48±14.92
n-C ₂₂	214.87±9.78	203.73±6.85	199.56±8.37
11-MeC ₂₂	10.76±1.09	9.18±0.74	8.53±0.97
9-MeC ₂₂	1.80±0.19	2.02±0.23	2.42±0.46
C _{23:2}	7333.15±388.67 ab	7548.17±286.85 a	6265.83±444.56 b
C _{23:1}	261.48±33.84	220.02±27.47	277.65±65.43
n-C ₂₃	2300.92±72.74	2272.40±70.29	2409.52±82.63
11-MeC ₂₃	75.45±6.59 a	61.35±4.79 a	28.63±3.98 b
C _{24:2}	38.40±3.12	37.92±1.81	36.29±2.38
n-C ₂₄	19.02±0.62	17.73±0.56	19.24±0.62
C _{25:2}	485.87±41.50 a	395.56±27.64 a	219.15±27.79 b
n-C ₂₅	288.14±12.40	258.25±9.96	263.71±13.81
13-MeC ₂₅	19.50±1.61 a	9.96±1.06 b	1.25±0.14 c
11-MeC ₂₅	4.42±0.55 a	4.12±0.42 a	2.67±0.32 b
n-C ₂₆	22.49±1.13	20.13±0.93	19.13±1.23
C _{27:2}	31.19±4.13 a	20.19±2.05 b	5.90±0.60 c
n-C ₂₇	444.30±25.12 a	378.11±18.45 ab	354.58±21.98 b
n-C ₂₈	27.25±1.51	26.61±1.10	29.15±1.87
2-MeC ₂₈	88.35±4.64	87.93±3.86	98.24±6.52
C _{29:2}	18.55±1.47 a	14.27±0.85 b	11.13±0.72 c
C _{29:1}	12.91±0.71	12.17±0.62	11.47±0.73
n-C ₂₉	338.79±23.86	340.40±17.10	380.66±22.18
n-C ₃₁	43.92±3.82 a	59.54±4.64 b	86.39±5.70 c

Data correspond to absolute quantities (µg) of 34 cuticular hydrocarbons common to all individuals, and are presented as mean ± SE. Different letters (a, b, c) denote statistical differences.

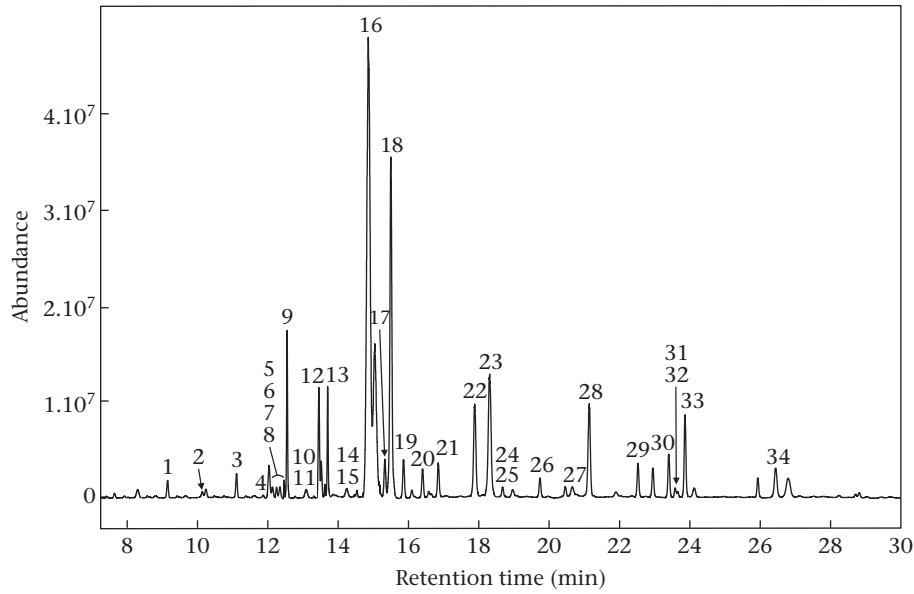


Figure A1. Chromatogram of the cuticular hydrocarbon profile of a moderately fertile *N. apicalis* worker. Peaks used in the statistical analysis are indicated by numbers: 1 = unidentified; 2 = 10-MeC₁₉; 3 = *n*-C₂₀; 4 = unidentified; 5 = C_{21:1}; 6 = C_{21:1}; 7 = C_{21:1}; 8 = C_{21:1}; 9 = *n*-C₂₁; 10 = 11-MeC₂₁; 11 = 9-MeC₂₁; 12 = C_{22:2}; 13 = *n*-C₂₂; 14 = 11-MeC₂₂; 15 = 9-MeC₂₂; 16 = C_{23:2}; 17 = C_{23:1}; 18 = *n*-C₂₃; 19 = 11-MeC₂₃; 20 = C_{24:2}; 21 = *n*-C₂₄; 22 = C_{25:2}; 23 = *n*-C₂₅; 24 = 13-MeC₂₅; 25 = 11-MeC₂₅; 26 = *n*-C₂₆; 27 = C_{27:2}; 28 = *n*-C₂₇; 29 = *n*-C₂₈; 30 = 2-MeC₂₈; 31 = C_{29:2}; 32 = C_{29:1}; 33 = *n*-C₂₉; 34 = *n*-C₃₁.

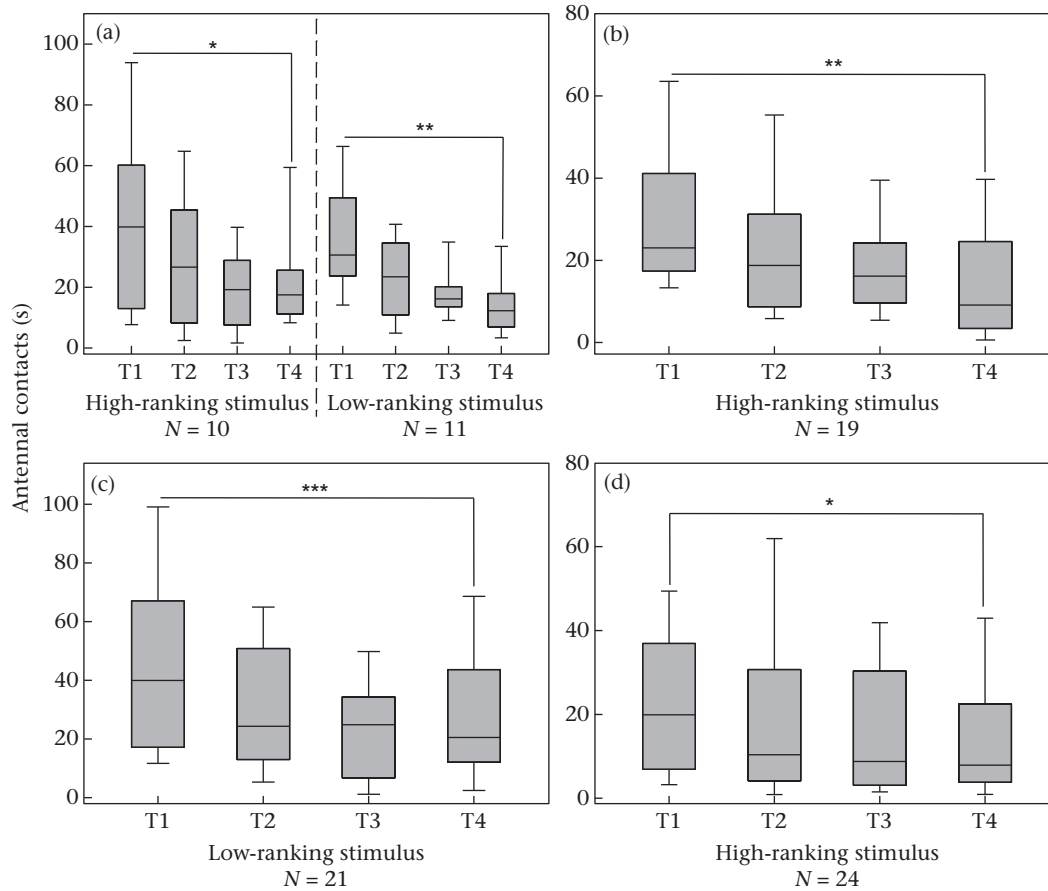


Figure A2. Duration of antennal contacts (s) towards the stimulus nestmate during habituation tests 1–4 (4 min each with a 5 min interval) in all experiments (see text). (a) Experiment 1: a high ranker was confronted with either a high- or a low-ranking nestmate. (b) Experiment 2: a high ranker was confronted with a high-ranking nestmate. (c) Experiment 3: a high ranker was confronted with a low-ranking nestmate. (d) Experiment 4: a low ranker was confronted with a high-ranking nestmate. Box plots represent 10th, 25th, 50th (median), 75th and 90th percentiles. Sample sizes of individuals are indicated for each experiment. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

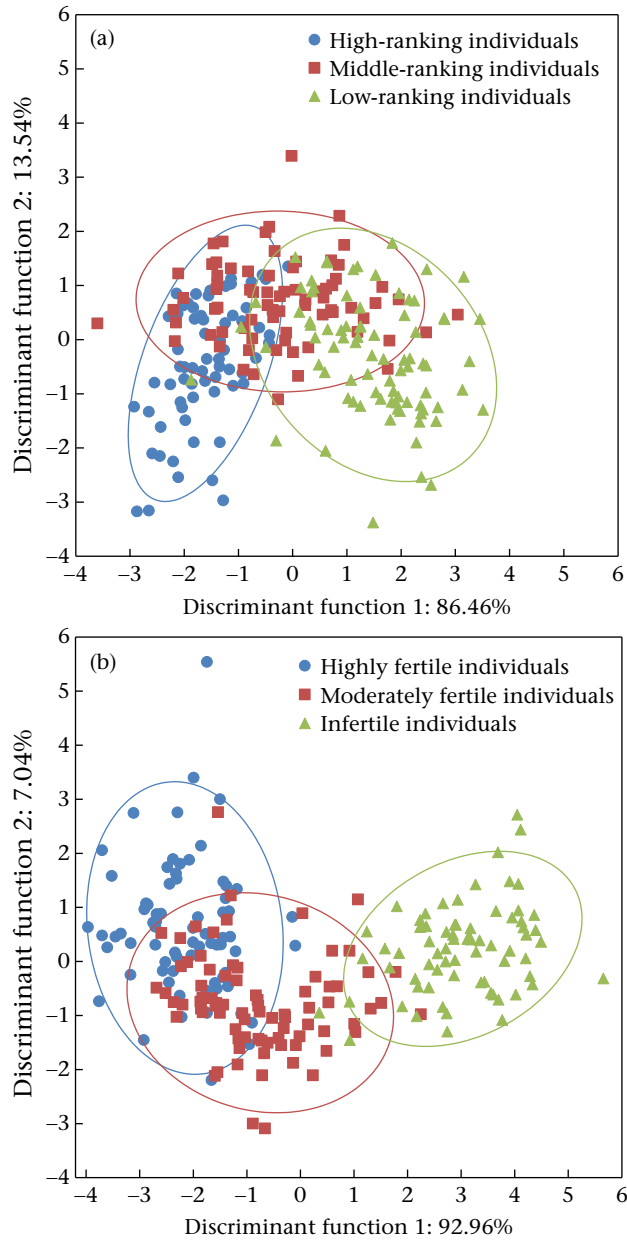


Figure A3. Discriminant function analyses showing the differences in the chemical profiles of 223 workers according to their (a) hierarchical rank and (b) ovarian development. Ellipses represent 90% confidence intervals around centroids. The percentage of variance explained is depicted on each axis.