

Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant

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In insect societies lacking morphologically specialized breeders and helpers, reproduction is often restricted to behaviorally dominant individuals. Such societies occur in about 100 species of ants that have secondarily lost the queen caste. All females, who are morphologically workers, can potentially mate and lay eggs but only a few do so, and we demonstrate in *Dinoponera quadriciceps* that this is regulated by a dominance hierarchy. Six types of agonistic interactions allowed the ranking of 5–10 workers in the hierarchy ($n = 15$ colonies). In particular, alpha and beta had characteristic behavioral profiles and were easily recognized. Only alpha mated, and workers ranking beta to delta sometimes produced unfertilized, male-destined eggs. Natural replacements ($n = 19$) and experimental removals ($n = 15$) of alpha demonstrated that beta was the individual most likely to replace alpha, although gamma and more rarely delta sometimes did, and we discuss the conflict that occurs among high-ranking individuals over who should replace alpha. After such replacements, the new alpha behaved more aggressively than the overthrown alpha. Newly emerged workers tended to reach high ranks and displaced older high-ranking individuals down the hierarchy. Low-ranking subordinates often prevented high-ranking individuals from replacing alpha by biting and holding their appendages (worker policing), which is consistent with the pattern of relatedness associated with monogyny and monandry in *D. quadriciceps*. We investigated the relative importance of chemical communication and dominance interactions to regulate reproduction. Alpha, beta, and sterile workers have different signatures of cuticular hydrocarbons, and these may provide honest information which underpins worker policing by low-ranking individuals. *Key words*: dominance, gamergate, hierarchy, Ponerinae, pheromone, relatedness, reproduction. [*Behav Ecol* 10:323–332 (1999)]

Reproductive skew is characteristic of many animal societies, and it is often regulated by dominance hierarchies. Typically, only one or a few individuals of both sexes behave dominantly and reproduce in cooperative breeding birds (Stacey and Koenig, 1990) and mammals (Solomon and French, 1997) where all individuals have equal potential to reproduce. Similarly, in polistine wasps (Reeve, 1991; Röseler, 1991), halictid bees (Breed and Gamboa, 1977), and some honeybees (Röseler and van Honk, 1990), all adult females have the potential to mate and lay fertilized eggs, but only dominants reproduce (Wilson, 1971). This is also the case in about 100 species of ants in subfamily Ponerinae where the queen caste has been lost permanently and where mated workers reproduce instead (called “gamergates”; Peeters, 1993). The majority of nest-mate workers remain sterile, and this is mediated by behavioral interactions (e.g., *Amblyopone* sp., Ito, 1993b; *Diacamma australe*, Peeters and Higashi, 1989; *D. vagans*, Peeters et al., 1992; *Dinoponera australis*, Paiva and Brandão, 1995; *Pachycondyla sublaevis*, Ito and Higashi, 1991; *Pachycondyla* sp., Ito, 1993a). This regulation contrasts with social insects that have a morphologically specialized queen caste where workers cannot mate and are restricted to laying unfertilized eggs (ants, Bourke, 1988b; honeybees, Winston, 1987). Typically, such workers oviposit only when the queen is absent, and this information is communicated by pheromones (Fletcher and Ross, 1985; Keller and Nonacs, 1993).

Dinoponera quadriciceps is a queenless ant, with colonies con-

taining 89 ± 50 workers (mean \pm SD, range 37–238, $n = 27$) and one gamergate only (Monnin and Peeters, 1998). Although a few workers occasionally lay unfertilized, male-destined eggs, the gamergate produces all diploid eggs (Monnin and Peeters, 1997). The gamergate yields higher amounts of the long-chain hydrocarbon 9-hentriacontene (9-C₃₁:1) on her cuticle than her nest mates (Monnin et al., 1998). This chemical difference is a consequence of ovarian activity, as indicated by repeated measurements of the individual acceding to breeding status after removal of the gamergate (Peeters et al., in press). Therefore, 9-C₃₁ seems to be an honest marker of fertility, providing an olfactory means of recognizing the principal egg layer from workers laying eggs infrequently. The hydrocarbon 9-C₃₁ is also present on the surface of eggs just laid by the gamergate but not on the eggs of other workers, and this may explain how the gamergate can recognize and destroy eggs that are not her own (Monnin and Peeters, 1997). Unlike ant queens with a relatively long life span (Keller, 1998), the gamergate probably lives 1–2 years and is then replaced by a nest-mate worker. Colony fission, by which queenless ants always produce new colonies (Peeters, 1997), creates additional opportunities for the differentiation of a new gamergate. Because males use their genitalia as a plug preventing a second copulation by workers (Monnin and Peeters, 1998), *D. quadriciceps* is monandrous as well as monogynous. In an established colony the gamergate is therefore the mother of all workers, who are full-sisters. However, after either replacement of the gamergate or colony fission, the new gamergate is initially a full-sister of nest-mate workers.

We demonstrate that reproduction is regulated by a dominance hierarchy in the queenless ant *D. quadriciceps* and discuss the mechanism and the dynamics of such regulation. Workers with different dominance ranks have distinct behavioral profiles, and rank correlates with egg-laying activity. Natural re-

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placement and experimental removal of the top-ranking worker ("alpha") show that the hierarchy is near-linear. We compare the pattern of agonistic interactions following the replacement of alpha with the pattern observed in undisturbed colonies and describe how the new alpha behaves more aggressively than an established alpha. Her rate of egg-laying increases only after a few weeks (irrespective of whether she has mated yet), and thus a new alpha initially lacks the level of 9-C₃₁ characteristic of a fully fertile alpha. We discuss the implication of olfactory information in the behavioral regulation of reproduction. Studying the replacement of alpha and the interactions in disturbed hierarchies is important because these are natural events, occurring when the gamergate dies or when colony fission occurs. We also investigated the fate of many young workers eclosed in the laboratory and their effect on the dynamics of the hierarchy. Finally, we use information on relatedness among workers to discuss reproductive conflicts among high-ranking individuals on the one hand, and between high-ranking versus low-ranking individuals on the other hand. We observed that low-ranking workers, which are no longer competing for reproduction, can interfere in the hierarchical relationships among high-ranking workers (worker policing; Ratnieks, 1988).

MATERIALS AND METHODS

Fifteen colonies of *Dinoponera quadriceps* were collected near Sambaiba, Bahia state, Brazil, in October, 1994 and January, 1996 (81 ± 31 workers, mean \pm SD, range 39–141). They were maintained in plaster nests consisting of various preformed chambers with a glass roof, allowing observations. Live insects were deposited daily as food in a foraging arena connected to the nest. All workers were identified with numbers glued onto the thorax (these ants are 3 cm long). We observed each colony for 59 ± 11 h over 86 ± 8 days (mean \pm SE; total = 885 h), with each observation bout lasting at least 30 min.

Description of the agonistic interactions

Six types of fairly ritualized agonistic behaviors were regularly observed: (1) blocking: an active worker stretched her antennae on either side of the head of a target worker, which generally stood more or less crouched (Figure 1a). When the target worker moved, the active worker often repositioned herself in front of her and hit her head with an antenna. The target worker then crouched, or if she still tried to move away, the active worker frequently performed gaster rubbing. (2) Gaster rubbing: an active worker bit one antenna of a target worker and rubbed it against her gaster bent forward (Figure 1b). The target either did not react or timidly tried to escape. (3) Gaster curling: resembled gaster rubbing, but the active worker did not bite and rub the antenna of the target worker. She only bent her gaster forward between her legs, exposing its dorsal region toward the target. This resembles one of the agonistic behaviors observed by Bourke (1988a) in the slave-making ant *Harpagoxenus sublaevis*, where workers compete for the production of males in the presence of the queen. (4) Antennal boxing: an active worker repetitively hit the head of a target worker with her antennae. Generally the target crouched, with mandibles opened, forelegs placed on each side of her head, and antennae either folded against her head or stretched backward. (5) Immobilization: one to six ants grasped a target worker by the legs, antennae, or mandibles (Figure 1c). When several workers succeeded each other, a target could be immobilized for several hours, or even days. (6) Leg biting: unlike immobilization, a single active worker bit a leg of a target, and this only lasted 1 or 2 s. Blocking

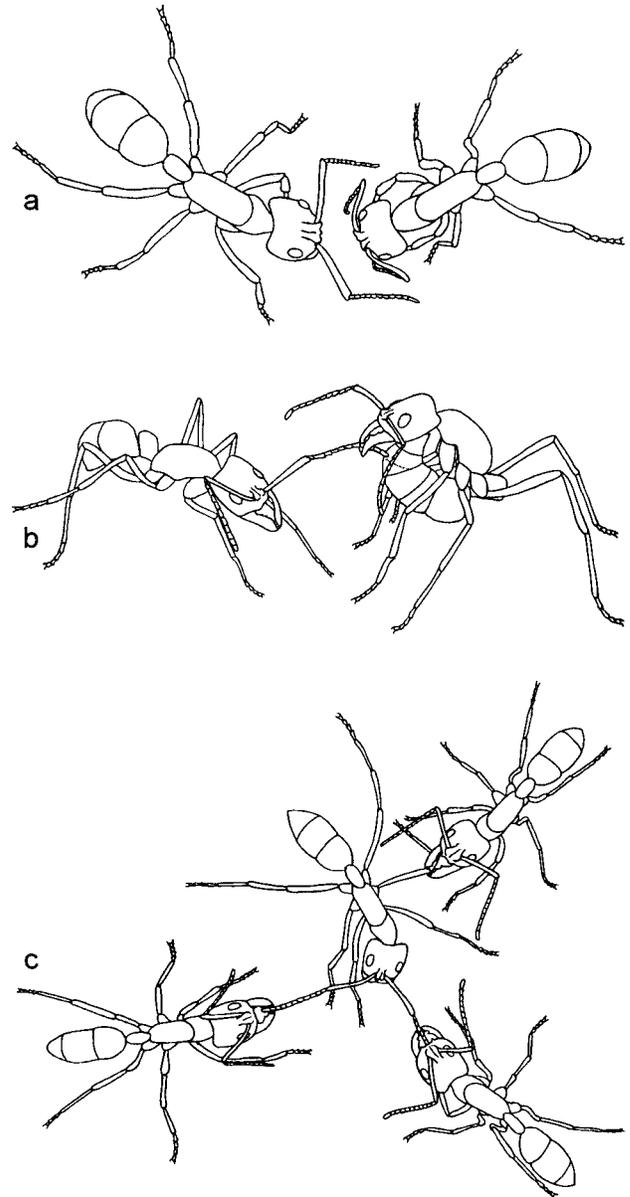


Figure 1
Drawings of various dominance interactions (based on photographs): (a) blocking, (b) gaster rubbing, and (c) immobilization.

and gaster rubbing have never been described in other species.

Data analysis

During each observation bout we recorded the identity of the workers involved in agonistic interactions and the direction of the behavior. When an interaction was repeated on the same day (involving the same active and target ants), it was not recorded because this gives no additional information about hierarchical relationships. The direction of agonistic interactions (who is active? who is the target?) rarely changed during one day and, if it did, the pattern of interactions with other nest mates enabled ranks to be determined.

Unlike various other queenless ants that exhibit one or two types of agonistic interactions, six types occur in *D. quadriceps*,

Table 1
Example of a matrix of agonistic interactions, recorded during 800 min of observations from May 1994 to December 1994

Active ant	Types of behaviors	Target ant #						Total behaviors performed
		16	96	92	12	39	22	
16 (gamergate)	Blocking	X	15	1				16
	Gaster rubbing	X	12	5				17
	Gaster curling	X	8	5				13
	Antennal boxing	X	4	1				5
	Immobilization	X						
	Leg biting	X	4	2				6
96 ^a	Blocking		X					
	Gaster rubbing		X		1	1		2
	Gaster curling		X	3		2	1	6
	Antennal boxing		X		1			1
	Immobilization		X					
	Leg biting		X		1	1		2
92 ^b	Blocking			X				
	Gaster rubbing			X				
	Gaster curling	1	2	X	1			4
	Antennal boxing		1	X	2	3	2	8
	Immobilization			X				
	Leg biting			X				
12	Blocking				X			
	Gaster rubbing				X			
	Gaster curling				X			
	Antennal boxing			3	X	5	4	12
	Immobilization			6	X			6
	Leg biting			16	X			16
39	Blocking					X		
	Gaster rubbing					X		
	Gaster curling					X		
	Antennal boxing			3		X	3	6
	Immobilization			4		X		4
	Leg biting			5		X		5
22	Blocking						X	
	Gaster rubbing						X	
	Gaster curling						X	
	Antennal boxing			1			X	1
	Immobilization			4			X	4
	Leg biting			1			X	1
Total behaviors received	Blocking		15	1				16
	Gaster rubbing		12	5	1	1		19
	Gaster curling	1	10	8	1	2	1	23
	Antennal boxing		5	8	3	8	9	33
	Immobilization			14				14
	Leg biting		4	24	1	1		30

Although 32 workers interacted, only the six highest ranked workers (involved in 59.9% of the agonistic interactions) are shown for clarity.

^a Callow, eclosed on 24 November 1994.

^b Callow, eclosed on 5 December 1994.

and this creates methodological problems. Summing interactions could lead to a loss of information, and even be misleading, because some highly significant interactions were rare. Thus, we ranked the agonistic interactions from the most to the least characteristic of dominant workers: (1) blocking, (2) gaster rubbing, (3) gaster curling, (4) antennal boxing, (5) immobilization, and (6) biting. This subjective ranking was justified by the good descriptive and predictive values of the hierarchies obtained, as shown below.

We computed 107 matrices of agonistic interactions. Each matrix corresponded to 2 weeks of observation, or less if a major event had occurred (e.g., replacement of the top-ranking worker, or eclosion of a worker in a colony with few young

workers). Each matrix allowed hierarchical relationships to be determined by inspecting every pair of interacting workers. The dominant was the worker performing the agonistic behavior defined above as more informative. For example, in Table 1, worker #92 was dominant relative to worker #12 because she performed one gaster curling, while #12 did none, even though #12 performed more antennal boxings. Simply summing up all the agonistic interactions would have resulted in #12 being more dominant than #92. When both antagonists interacted with behaviors of the same level, the dominant was the worker performing behaviors of the highest level on the greater number of days. For example, worker #96 was dominant relative to #92 because she directed gaster curling at #92

on 3 different days, while #92 did so only on 2 days (Table 1).

We qualified the hierarchy as stable when there were no major changes in the rank of high-ranking workers compared with the matrix of the previous 2 weeks, or as disturbed when major changes occurred. Oviposition was monitored, and at the end workers were dissected to check spermathecal content and ovarian development and thus determine whether reproductive activity correlates with rank.

Replacement of the top-ranking worker (alpha)

Alpha was naturally replaced on 19 occasions ($n = 11$ colonies of 39 ± 18 workers, mean \pm SD, range 19–86). These colonies were observed for 51 ± 23 days (range 25–100) after the replacements to determine who would become the new alpha. If the hierarchy is linear, beta should always be the replacement individual, but if the hierarchy is near-linear beta simply has a high likelihood of replacing alpha, with other high-ranking individuals also capable. We also observed the fate of the overthrown alpha to see whether she would become a sterile worker or would be expelled from the nest. These observations were completed by experimentally removing alpha ($n = 10$ in 7 colonies of 18 ± 7 workers, range 12–32), alpha and beta ($n = 4$ in 4 colonies of 14 ± 10 workers, range 7–26), or alpha, beta, and gamma ($n = 1$ colony of 31 workers). The individuals removed were dissected and colonies were observed for 19 ± 16 days (range 2–46) after the experimental removal.

Influence of age on dominance rank

One hundred forty-three workers eclosed in the laboratory. Workers younger than 2 weeks old are hereafter called “callows”; they were initially easily recognized because of the yellow/light brown color of the tip of their mandibles and antennae and especially the tarsi of their legs, whereas older workers are uniformly black. We observed the fate of 64 callows individually marked soon after eclosion to study the temporal dynamics of the dominance hierarchies. One possibility is that young workers initially have low rank and gain higher rank with age, eventually becoming alpha and reproducing. Alternatively, they can initially have high rank and either replace alpha and reproduce or else lose rank when other workers eclose.

To determine if rank correlates with polyethism, we recorded the occurrence of brood care (licking or carrying eggs, larvae, or cocoons), nest maintenance (digging and moving pieces of plaster), and foraging in three colonies of 40, 86, and 98 workers.

RESULTS

Agonistic interactions occurred in a consistent pattern that allowed the ranking of 5–10 workers in a dominance hierarchy. Such hierarchy was usually stable, with no major changes in the relationships among high rankers (73 matrices of agonistic interactions in 15 colonies, 675 h of observation). In contrast, the hierarchy was disturbed following the natural replacement or experimental removal of alpha or the eclosion of a worker gaining a high rank (34 matrices in 13 colonies, 209 h of observation).

Pattern of agonistic interactions in stable hierarchies

In a stable hierarchy, workers of different dominance ranks clearly exhibited distinct behavioral profiles, performing and receiving different amounts of each type of agonistic interac-

tion (Table 1 and Figure 2a). Blocking can be used as a behavioral marker of the alpha rank, and it also identifies beta. Alpha performed 94.1% of blocking and received none, while beta performed only 4.1% of blocking and received 68.6%. Beta was unable to interact with other workers while blocked by alpha. Blocking was relatively rare, representing only 3.1% of the agonistic interactions, but each bout lasted from a few minutes to more than 1 h. Alpha could spend much of her time blocking beta, as shown by continuous video recording in one colony where she blocked beta for 183 min out of 297 min (i.e., 62% of the time).

Gaster rubbing was mainly performed by alpha (55.8%) and beta (27.4%). Alpha was the target of only 0.5% of this behavior, whereas beta received 17.2%. Workers ranking just below beta received similar percentages of gaster rubbing. Callows sometimes performed gaster rubbing within minutes after eclosion. Rarely, gaster rubbing was directed at eclosing callows still partially in their cocoon.

The distribution of gaster curling was similar to that of gaster rubbing, except that alpha performed more gaster rubbing (55.8%) than gaster curling (45.2%). Rarely, two callows of similar age performed reciprocal gaster curling, each exposing her gaster toward the other.

Antennal boxing was the commonest agonistic behavior, representing 44.5% of total interactions. In contrast to the preceding agonistic interactions, it was not performed principally by alpha (2.2%), but by beta, gamma, and delta (18.8, 19.5 and 15.3%, respectively), and to a lesser extent by individuals ranking five to seven (10.7, 7.5, and 6.6%, respectively). The major targets were workers with ranks three to seven, which each received from 7.5% to 9.8% of antennal boxing. Reciprocal antennal boxing (each worker alternatively boxing the head of the other during the same interaction) was rare.

Immobilization was only exceptionally performed by alpha (0.2%) and seldom by beta (2.6%), but individuals ranking three to eight performed 53.2% (8.2–10.8% each). The major targets were individuals from ranks two to six (17.8–10.5%, totaling 70.6%). Callows were the target of 31.6% of immobilization.

The pattern of distribution of leg biting is roughly similar to that of both gaster rubbing and gaster curling. It was mainly performed by alpha (23.9%) and beta (21.9%), and to a lesser extent by gamma (7.6%) and delta (7.8%, totaling 61.2%). Workers having ranks two to six were the major targets, receiving 15.2–10.9% (totaling 63.2%).

Blocking, gaster rubbing, and gaster curling are characteristic of alpha partly because we defined them as the more informative behaviors (see Methods). However, the finding that blocking is almost exclusively performed by alpha and essentially directed toward beta is independent from this assumption. Similarly, the observations that alpha did not perform antennal boxing and, more interestingly, that immobilization was not performed by the highest ranking workers, are not influenced by our assumption.

Pattern of agonistic interactions in disturbed hierarchies

Disturbed hierarchies had a higher proportion of more aggressive dominance interactions than stable hierarchies. The frequencies of gaster rubbing and gaster curling increased from 12.8% and 13.9% in stable hierarchies to 28.2% and 22.8% in disturbed hierarchies, respectively. In contrast, the frequency of antennal boxing decreased from 44.5% in stable hierarchies to 23.0% in disturbed hierarchies. However, the pattern of agonistic interactions (Figure 2b) remains similar to the one observed in stable hierarchies (Figure 2a). Only the behavioral profiles of the new alpha and the new beta changed. The new alpha was more aggressive than an estab-

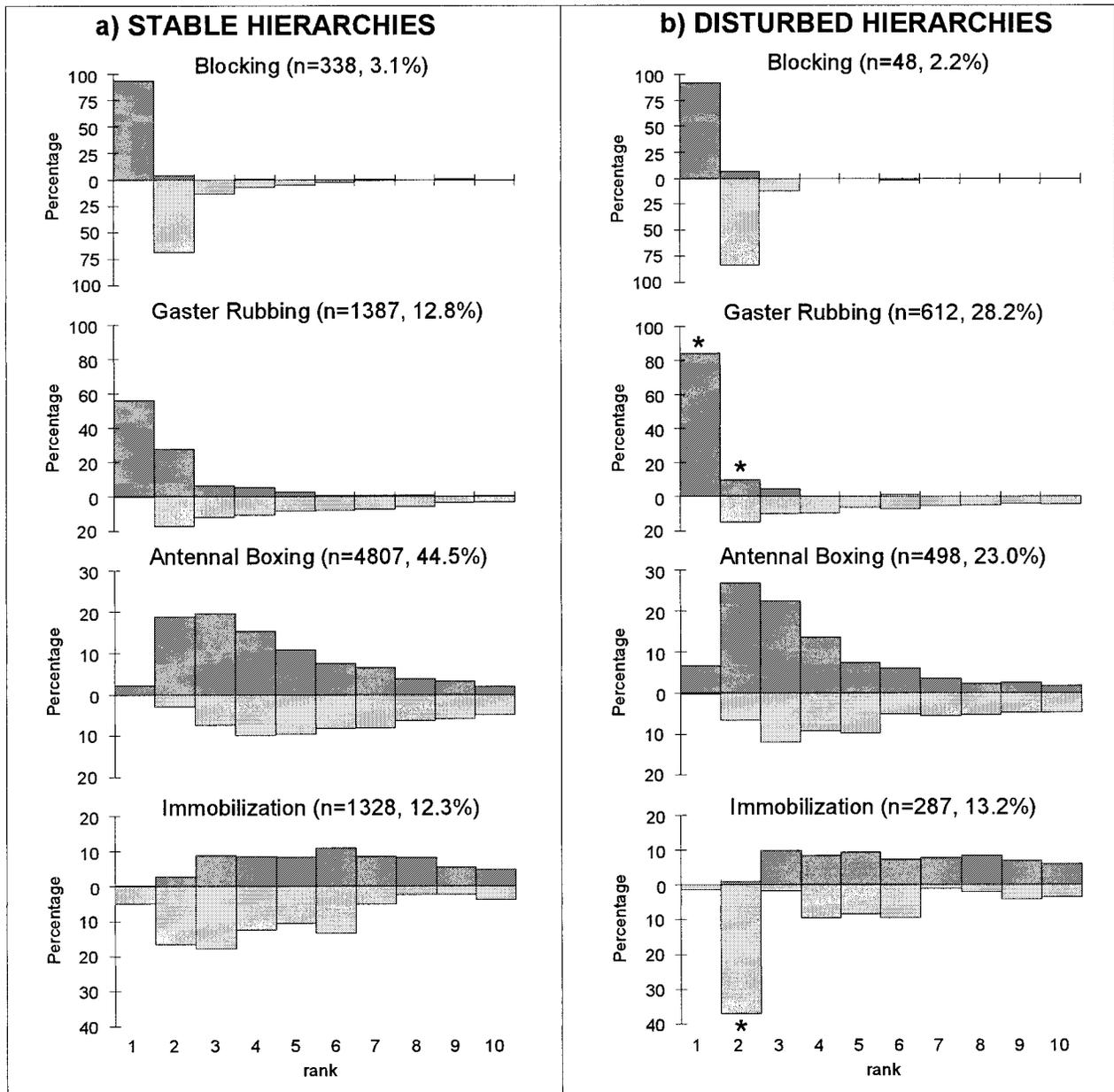


Figure 2

Relationship between the pattern of agonistic interactions and dominance ranks. Stable hierarchies (73 matrices totaling 10,797 interactions) are contrasted with disturbed hierarchies (34 matrices and 2169 interactions). The interactions initiated and received are plotted in the upper and lower parts of each graph, respectively. Only workers of ranks 1–10 are included; they performed and received 89.4% and 72% of all behaviors, respectively. Gaster curling and leg biting (not included) accounted for 13.9% and 13.3% of the total number of interactions in stable hierarchies, and 22.8% and 10.6% in disturbed hierarchies, respectively.

lished alpha, performing more gaster rubbing (84.3% versus 55.8%), gaster curling (84.8% versus 45.2%), and leg biting (64.6% versus 23.9%). Indeed, in the first days after replacement the new alpha interacted with almost all individuals passing close to her. In contrast, the new beta was less aggressive and was attacked more often than an established beta, performing less gaster rubbing (9.8% versus 27.4%), gaster curling (10.1% versus 28.9%), and leg biting (6.6% versus 21.9%), and receiving more blocking (83.3% versus 68.6%) and immobilizations (36.9% versus 16.5%). These differences are significant at $p < .001$ (chi-square analysis), except for the number of blocking received by the new beta, which is significant at $p < .05$.

Replacement of alpha

The alpha worker lost her rank naturally in 19 cases (11 colonies). She was usually replaced by beta ($n = 12$), but sometimes by gamma ($n = 5$) or by delta ($n = 2$). The overthrow of alpha is unambiguous because she is never challenged in stable hierarchies. There was no instance of alpha losing her rank and gaining it back later because she usually died soon after replacement. The number of gaster rubbings and immobilizations greatly increased during the replacement of alpha (Figure 3). In particular, all immobilizations directed at alpha occurred during the replacement period: 61 within the 8 days preceding her replacement (i.e., 4.6% of the 1328 im-

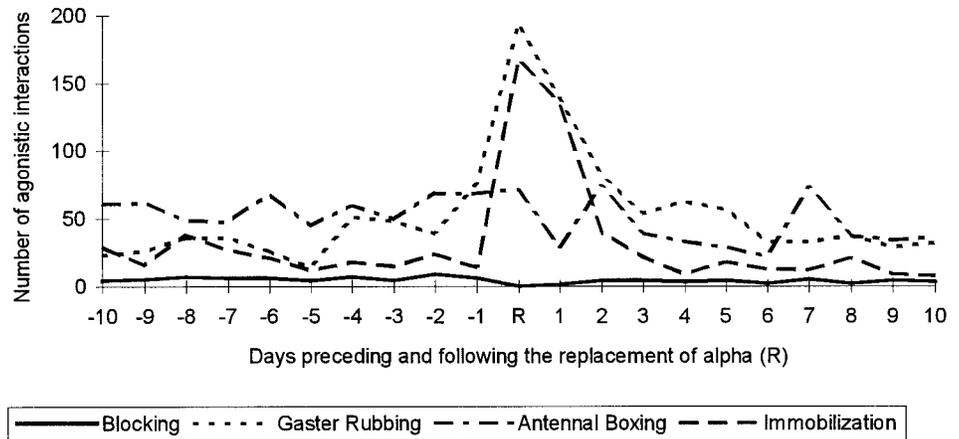


Figure 3
Number of agonistic interactions observed per daily session before and after the replacement (natural or experimental) of alpha ($n = 26$), summed for 11 colonies.

mobilizations observed in stable hierarchies), and 78 within the 7 days after her replacement (i.e., 27.2% of the 287 immobilizations observed in disturbed hierarchies). Alpha ended up being immobilized continuously. Most of the 19 overthrown alphas died soon after replacement: 5 mated alphas (gamergates) and 4 virgin alphas died within 2 days, while 5 virgin alphas survived longer (5, 6, 12, 24, and 44 days). These workers were dissected immediately after their deaths, and the remaining three gamergates and two virgin alphas were sacrificed for dissection 1 or 2 days after replacement. Seven of the overthrown alphas had totally regressed ovaries with no yolky oocytes (including the 3 alphas that survived more than 6 days), 7 had partly regressed ovaries with no mature oocytes and a few yolky oocytes, and 5 had well-developed ovaries with 1–4 mature oocytes and 8–12 smaller yolky oocytes. Thus, five alphas (three gamergates and two virgin alphas) were replaced despite being able to lay eggs and producing high proportions of 9-C₃₁, as was measured in two of them (10.3% and 9.5% of all cuticular hydrocarbons C₂₁ to C₃₇; Monnin and Peeters, unpublished data). The fact that most overthrown alphas died within a few days after replacement strongly suggests that they were killed or died as a consequence of being continuously immobilized. 34.5% of the immobilizations directed against beta occurred during the replacement of alpha (8.9% within 8 days preceding the replacement, and 25.5% against the new beta within 7 days after the replacement). In contrast, the new alpha was the target of only a few brief immobilizations.

When an experimentally isolated alpha was reintroduced, or during some episodes of alpha replacement (successful or not), alpha and beta initially chased each other within the nest and sometimes started to fight. Both workers strongly bit one another, but these fights were short and never induced death. An exceptional agonistic behavior (“sting smearing”) was then often observed, involving mainly alpha and beta and sometimes gamma. The active worker curled her gaster forward and extruded her sting, positioning it close to the target and often rubbing it on the cuticle of the target, but never stinging her. The active worker usually ran away after smearing her opponent. Sting smearing was often associated with immobilization behavior, and it seems to induce the immobilization of the targeted ant (C. Peeters, personal observation). This agonistic behavior was not used to compute the matrices of interactions because it was rare. Furthermore, it only occurred when beta attempted to overthrow alpha.

When alpha was experimentally removed, beta always became the top-ranking individual ($n = 10$). Beta reacted within 1–3 h to the removal of alpha, aggressing nearly all workers passing close to her with gaster rubbing and gaster curling.

When both alpha and beta were removed, gamma reached the alpha rank ($n = 4$), and on one occasion when alpha, beta, and gamma were all removed, delta became alpha. Therefore, alpha was replaced by the highest-ranking worker left in the colony in 79.4% of both natural overthrows ($n = 19$) and in experimental removals ($n = 15$) of alpha.

Rank of callows within the hierarchy

Callow workers tend to reach high ranks in the hierarchy: they had ranks 1–10 in 94% of cases ($n = 64$; Figure 4) and ranks 1–4 in 56% of cases. The rank they gained depended on the age of the existing high-ranking workers. Callows dominated old high rankers, except alpha who generally remained unchallenged. For example, one worker eclosed in a colony where there had been no callow for 3 months because the brood died. She behaved aggressively, performing gaster rubbing, and immediately achieved the beta rank without resistance from the former beta. Alpha started blocking her and performing gaster rubbing, whereas she had not done so with the previous beta. Callows were less likely to become dominant when high rankers were young, and it was sometimes difficult to rank callows of similar age. For example, one worker eclosed in a colony without other callows and became beta. Seven days later four workers eclosed, and three more eclosed within the next 9 days. None of these seven callows was able to replace the new beta. However, they clearly dominated all other workers and excluded older workers from ranks 3–10. In colonies without callows, beta kept her rank for several months.

Division of labor

The pattern of dominance interactions correlated well with the performance of helper activities. The 10 highest ranking workers in each colony specialized in activities inside the nest, performing 57.0% of egg care, 28.7% of larvae care, 12.6% of cocoon care, and only 4.0% of nest maintenance and 4.1% of foraging (Figure 5). The almost exclusive contribution of alpha was to care for the eggs. In contrast, workers rarely involved (1% each) in dominance interactions were less specialized and accounted for an important percentage of each task (30.4% as a minimum), although they preferentially cared for larvae and cocoons (66.9% and 77.8%, respectively). Finally, workers that were never involved in dominance interactions specialized in nest maintenance and foraging (51.1% and 65.5%, respectively).

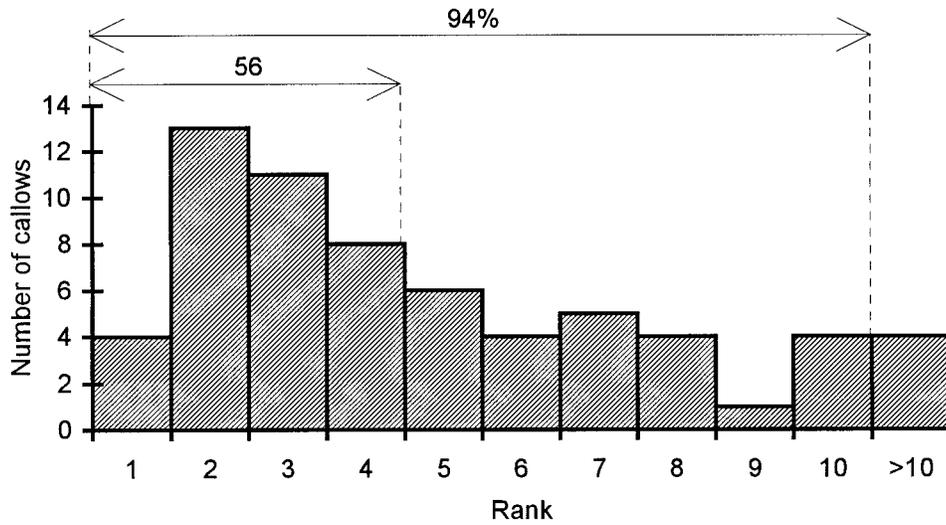


Figure 4
Hierarchical ranks gained by calls ($n = 64$ calls from 11 colonies), within 2 weeks of emergence.

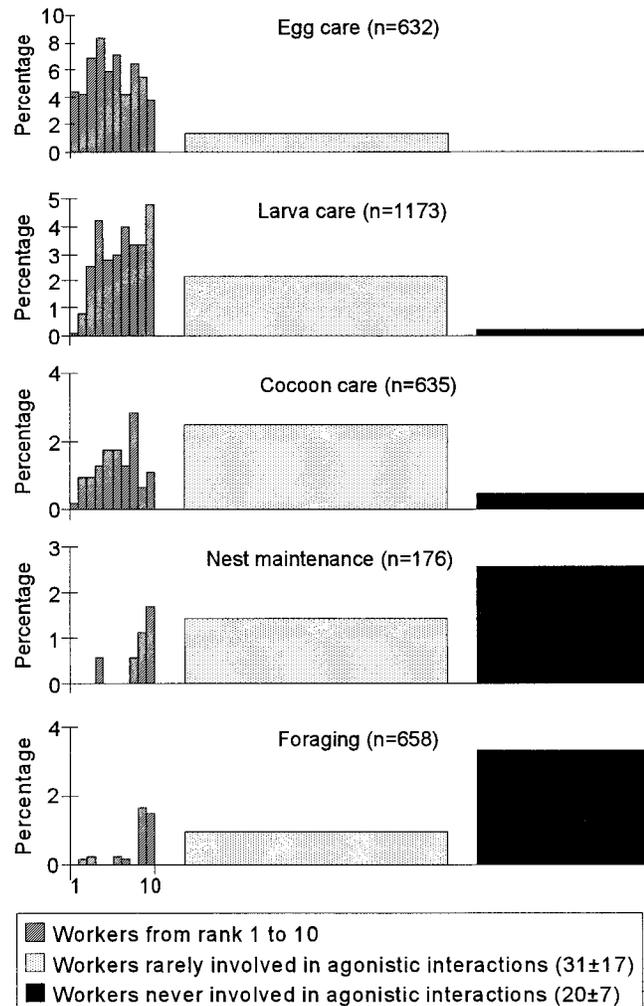


Figure 5
Relationship between rank and the performance of brood care, nest maintenance, and foraging. Polyethism was studied in three colonies of 40, 86, and 98 workers, and ranks were determined using 18 matrices of interactions: 31 ± 17 workers were rarely involved in agonistic interactions (mean ± SE, range 9–57), while 20 ± 7 were never involved in such interactions (range 10–30).

DISCUSSION

Different degrees of reproductive skew often result from dominance interactions in societies in which all individuals are morphologically similar, such as cooperative breeding birds and mammals (Stacey and Koenig, 1990; Solomon and French, 1997) and primitively eusocial insects (Breed and Gamboa, 1977; Reeve, 1991; Röseler, 1991; Röseler and Van Honk, 1990). Permanently queenless ants exhibit similar competition for reproductive status as in other cooperative breeders, and conflicts are also resolved behaviorally. Six types of agonistic interactions occurred in a consistent pattern in the ponerine ant *Dinoponera quadriceps* and allowed us to rank workers in a hierarchy. Blocking was highly characteristic of alpha and can be used as a marker of this rank. This behavior also allowed beta to be identified because she was usually the target (Figure 2). Furthermore, gaster rubbing was characteristically performed by alpha, and to a lesser extent by beta. The hierarchy had good descriptive and predictive values: (1) only alpha was able to mate (Monnin and Peeters, 1998). (2) The probability of ovipositing and the number of eggs laid were well correlated with rank: alpha, beta, gamma, and delta oviposited in 15, 6, 3, and 2 of the colonies studied ($n = 15$), and laid 111, 11, 4, and 3 eggs, respectively. Furthermore, alpha selectively destroyed the eggs laid by high-ranking subordinates (Monnin and Peeters, 1997). (3) The probability of replacing alpha was also correlated with rank: alpha was usually replaced by beta ($n = 12$), sometimes by gamma ($n = 5$), and more rarely by delta ($n = 2$). Therefore, the dominance hierarchy controls reproduction in *D. quadriceps*. Although mating and oviposition are both regulated by dominance rank, these are independent mechanisms. Virgin alphas exhibited similar ovarian development to that of gamergates (Monnin and Peeters, 1997), demonstrating that mating does not control egg laying. Conversely, ovarian activity does not regulate mating: new alphas were able to mate before they had developed ovaries, but subordinate egg-layers with slightly developed ovaries were unable to mate (Monnin and Peeters, 1998).

Statistical tests have been developed to determine the degree of linearity of hierarchies because ranking individuals can sometimes generate a linear hierarchy by chance (Appleby, 1983; review by de Vries, 1998). In *D. quadriceps* many workers are seldom involved in agonistic interactions, and this can cause the hierarchy to be nonlinear if we include all workers. However, only high rankers compete for reproduction, and the pattern of mating and oviposition as well as the pat-

tern of agonistic interactions among high rankers (Figure 2) and the probability of becoming the replacement alpha are all consistent with a near-linear hierarchy. Such an experimental demonstration is superior to statistical tests because it illuminates the biological function and the dynamics of the hierarchy, whereas statistics only describe its structure. Importantly, ranks are more distinct for workers ranking alpha to delta, who performed 99.1% of blocking, 94.7% of gaster rubbing, and 91.1% of gaster curling (Figure 2), than among lower ranking workers. Indeed, it makes an enormous difference to be beta or gamma (these have a distinct probability of ovipositing and of replacing alpha), rather than a low-ranking worker.

The function of agonistic interactions among lower-ranking workers remains unclear. They may either be a by-product of the regulation of reproduction among high-ranking workers or be linked with the division of labor. Indeed, polyethism is related to dominance hierarchy in some polistine wasps (*Parischnogaster jacobsoni*, Turillazzi, 1988; *Polistes dominulus*, Pratte, 1989, Théraulaz et al., 1992; *Ropalidia marginata*, Premnath et al., 1995) and ants (*Harpagoxenus sublaevis*, Bourke, 1988a; *Protomognathus americanus*, Franks and Scovell, 1983). In *D. quadriceps* high rankers mainly perform brood care (Figure 5). This was particularly true for alpha, whose participation consisted almost exclusively of egg care. It is noteworthy that she was able to recognize and destroy the eggs occasionally laid by high-ranking workers (Monnin and Peeters, 1997). In contrast, workers never engaging in dominance behavior essentially performed nest maintenance and foraging. A similar correlation between rank and polyethism has been shown in *Pachycondyla sublaevis* (Ito and Higashi, 1991), but because colonies are exceptionally small (9 ± 2.5 workers), high rankers are more involved in maintenance activities than in *D. quadriceps* (89 ± 50 workers). The single gamergate performs more than 60% of egg care in *P. sublaevis* versus 4.4% in *D. quadriceps*. Other factors such as age, quantity of brood, availability of resources, and colony size also regulate polyethism (reviewed for ponerine ants in Peeters, 1997).

Queenless ant colonies are potentially immortal because old gamergates can be replaced by younger nest mates. A new gamergate will differentiate when the current gamergate is too old or when a gamergate is lacking after colony fission. Natural replacements and experimental removals of alpha in the laboratory allowed us to study both situations. The number of interactions increased when alpha was replaced (Figure 3), and the types of aggression changed, with the proportion of gaster rubbing and gaster curling roughly doubling, while the proportion of antennal boxing decreased by half. Furthermore, sting smearing and overt fighting only occurred while alpha was overthrown. The new alpha performed most gaster rubbing and gaster curling and was involved in most sting smearing and fights. This high aggressiveness may allow her to secure her rank. Interestingly, the number of aggressions decreased back to normal within a few days (Figure 3), although it took several weeks for the new alpha to acquire the cuticular hydrocarbon profile characteristic of the alpha rank (Peeters et al., manuscript submitted). This means that the fertility signal is not necessary for the establishment of rank. Indeed, it does not prevent high-ranking workers from replacing alpha, as discussed below. Stable and disturbed hierarchies also differed by the direction of the immobilizations. In stable hierarchies callow workers were the main targets of immobilizations, whereas in disturbed hierarchies low-ranking workers also immobilized the overthrown alpha and the new beta. This may allow the new alpha to establish her rank more rapidly, thus minimizing colony disruption and increasing the inclusive fitness of low rankers. Similarly, low rankers interfere

with high rankers when the gamergate is experimentally removed in *Pachycondyla sublaevis* (Higashi et al., 1994).

When alpha was experimentally removed, beta reacted to the absence of alpha within 1–3 h by attacking almost any worker she encountered. The most probable interpretation is that beta's subordinate status is maintained by the reception of agonistic behaviors from alpha. However, it is also possible that this reproductive suppression results in whole or in part by close-range pheromones emitted by alpha, which are normally detected by beta while walking close to alpha. Volatile pheromones are unlikely to be involved: when beta was separated from alpha by a mesh ($n = 2$ colonies separated into two parts with evenly distributed workers and brood), she became alpha in the orphaned part despite the presence of the original alpha on the other side of the mesh (Monnin and Peeters, in preparation). This supports the hypothesis that the chemical signal of alpha is nonvolatile, which is the case of the long-chain hydrocarbon 9-C₃₁. This chemical signal may be transferred from alpha's gaster toward the antennae of her victim during gaster rubbing. However, gaster rubbing performed by a worker yielding little or no 9-C₃₁ (e.g., a newly established alpha) cannot have this function. When one of the above colonies was reunited after 11 days of separation, the newly differentiated alpha immediately behaved submissively when she encountered the original alpha, prior to any physical contact, demonstrating that alpha can be recognized at a short distance, probably by olfaction (Monnin and Peeters, in preparation). The hydrocarbon 9-C₃₁ may be responsible for this because even apparently nonvolatile substances may be projected a short distance from the individual through the formation of an aerosol of cuticular particles (Arpino et al., 1972). This would also explain the gaster curling behavior, where there is no contact between the active ant and her target.

Callows tend to become high-ranking workers and frequently accede to beta rank. A similar trend was shown in *Pachycondyla* sp. from west Java (Ito, 1993a), *P. sublaevis* (Higashi et al., 1994) and in the wasp *Polistes instabilis* (Hughes and Strassmann, 1987). Although *D. quadriceps* callows replaced older high-ranking workers (alpha excepted), they did not necessarily replace young high rankers. Therefore, the age distribution has a major influence on the dynamics of the dominance hierarchy. Alpha maintained her dominance for months in the laboratory, while other high rankers were frequently displaced down the hierarchy by callows and lost any chance to reproduce. Given this important turnover, high-ranking workers (alpha excepted) are always young individuals. This means that the worker who can replace alpha will have a long life expectancy and would probably not be replaced for the next 1 or 2 years. This is important because overly frequent replacements of alpha would decrease colony efficiency.

In established colonies of *D. quadriceps* all workers are daughters of the singly mated gamergate and thus full-sisters. How can workers of ranks beta to delta maximize their fitness? The gamergate provides them inclusive fitness (relatedness = 0.75 for full-sisters and 0.25 for brothers) equal to the direct fitness they would gain by becoming alpha and producing their own offspring (relatedness = 0.5 for both sexes). Therefore, they should be indifferent to becoming alpha. However, if the gamergate was replaced by another high-ranking worker, they would have to rear nieces and nephews (relatedness = 0.375). Their best strategy may then be to test the gamergate continually and be ready to replace her. Therefore, high-ranking workers are in conflict over who should replace alpha, and this may account for the fact that they are involved in most agonistic interactions (Figure 2). Beta is dominant over other high rankers, which may have a positive feedback

on her ovarian activity. However, beta's behavior probably accounts for the persistence of antagonism from alpha, especially blocking, which apparently decreases the opportunities for beta to interact with other subordinates. Furthermore, alpha's dominance behavior may have a negative effect on beta's rate of egg production. Because workers' eggs develop in grandsons of the gamergate, the latter gains in fitness, unless these eggs develop at the expense of her own. This may well be the case, as alpha destroys worker-laid eggs (Monnin and Peeters, 1997). It is also possible that high rankers are more likely to replace alpha if they can lay eggs than if they cannot. When alpha is replaced, or after the event of colony fission, the new alpha is initially a full-sister of the high-ranking workers. These would therefore increase their fitness by overthrowing the new alpha and producing daughters and sons instead of rearing nieces and nephews. In conclusion, high-ranking workers are in conflict over who should become the next alpha, independently of whether the current alpha is their mother or sister.

In contrast to high-ranking individuals, low-ranking subordinates can no longer become alpha. When the gamergate is their mother, they gain more inclusive fitness through her (full-sisters and sons, relatedness = 0.75 and 0.25, respectively) than through a full-sister of high rank which would replace her and mate with a foreign male (nieces and nephews, relatedness = 0.375). Therefore, low-ranking workers are in conflict with high-ranking workers and should prevent the replacement of alpha unless alpha's fecundity is declining. When alpha has already been replaced or when colony fission has occurred, low rankers and high rankers are equally related to the new alpha (full-sisters). However, preventing the replacement of alpha would minimize colony disruption and increase their fitness. Low rankers sometimes immobilized high rankers; for example, when a colony separated into two halves by a mesh was reunited after 11 days, low-ranking workers immobilized the alpha that had newly differentiated in the orphaned half, but not the original alpha. The newly differentiated alpha still had undeveloped ovaries and presumably yielded low amounts of 9-C₃₁, whereas the original alpha had fully developed ovaries (Monnin and Peeters, in preparation). This strongly suggests that 9-C₃₁ supplies honest information about ovarian activity, which is used by low-ranking workers. Although high-ranking workers may continually test alpha in an attempt to replace her, alpha's 9-C₃₁ signal may also provide them with reliable information because her ovarian activity is likely correlated with her aggressiveness. This would explain why alpha regularly displays her cuticular signal toward high rankers using gaster rubbing and gaster curling.

Polistes wasps exhibit an interesting parallel with *D. quadriciceps*. All adult females are able to reproduce, but egg laying is mostly monopolized by alpha (Reeve, 1991; Röseler, 1991), and this regulation is based on both agonistic interactions and chemical cues. When alpha was tethered in *P. fuscatus*, her reproductive status was still recognized, probably by olfaction, but she lost her rank and reproductive control because she was unable to attack her subordinates (Downing and Jeanne, 1985). This demonstrates that chemicals cannot inhibit reproduction on their own. On the other hand, ovariectomized foundresses of *P. dominulus* can become and remain alpha, but they inhibit oviposition by subordinates less than an intact alpha does (Röseler and Röseler, 1989). This is probably because they lack the cuticular hydrocarbon profile characteristic of alpha, which is related to ovarian activity (Bonavita-Cougourdan et al., 1991), thus supporting the implication of chemicals in the behavioral regulation of reproduction. Similarly, both aggressions and chemicals regulate reproduction in the polistine wasp *Ropalidia marginata*, where most attacks occur only during the supersedeure of the single reproductive

female. Thereafter the new reproductive ceases to be the most aggressive individual, and other females probably suppress their oogenesis when they detect her chemical cues (Premnath et al., 1996).

This study provides a basic understanding of the function and dynamics of the dominance hierarchy in *D. quadriciceps*. Behavioral observations followed by dissections of ovaries and spermathecae enabled us to determine which worker produces diploid progeny (alpha), which are likely to produce males (beta, more rarely gamma and delta), and which may become the next alpha (beta, more rarely gamma or delta). Young workers tend to have high ranks and displace older high-ranking individuals down the hierarchy, thereby ensuring that future reproductives have a long life expectancy. However, it remains poorly understood whether alpha is replaced when her fecundity declines (possibly detected by a decrease in 9-C₃₁) or when the vigor of her interactions with beta weakens. Our data show that the agonistic behavior of alpha is essential to establish and maintain her status. Alpha's cuticular signal provides honest information which may be used by low-ranking workers to stabilize the system, although further studies are needed to clarify the respective functions of dominance behavior and chemical signals.

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