

Reproductive monopoly enforced by sterile police workers in a queenless ant

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In societies of totipotent insects, dyadic dominance interactions generate a hierarchy that often underlies an extreme reproductive skew. Subordinates remain infertile but can maximize their indirect fitness benefits through collective power (worker policing): interference with challenging high-rankers can prevent an untimely replacement of the reproductive. However, police workers only benefit if they favor individuals with high fertility. In the monogynous queenless ant *Streblognathus peetersi*, we used behavioral, physiological, and chemical methods to show that police workers have the primary role in the selection of the reproductive, and that they probably use reliable information about fertility encoded in the cuticular hydrocarbons to make their decision. We successfully decreased an alpha's fertility by using a hormonal treatment (Pyriproxyfen, a juvenile hormone analogue), and she was always removed from the hierarchy by police workers. In the preceding days, one of the high-rankers became aggressive, although her interactions were not directed at the treated alpha. All treated alphas ($n = 10$) remained aggressive but ended up immobilized by low-ranking workers after a median time of 11.5 days. By then, the challenging high ranker exhibited dominance behaviors typical of the alpha rank. In parallel, the cuticular profile of the treated alpha exhibited predictable and opposite modifications to that of the challenger's. This is the first study that uncouples dominance and fertility in a social insect: it gives a better understanding of the crucial role of sterile helpers in the control of reproductive skew in animal societies. *Key words*: cuticular hydrocarbons, fertility signal, gamergate, juvenile hormone, *Ponerinae*, reproductive skew, worker policing. [*Behav Ecol* 15:970–975 (2004)]

Animal societies are characterized by cooperation as well as conflict. In the large number of social insects in which the worker caste can never mate, thus being restricted to laying unfertilized eggs, conflicts are mostly expressed over male progeny. One mechanism that reduces this intracolony conflict is worker policing (Ratnieks, 1988; Whitfield, 2002). Sterile workers can maximize their fitness by manipulating the colony's brood; this can be favored by either colony-level costs (Cole, 1986; West-Eberhard, 1975) or patterns of relatedness (Woyciechowski and Lomnicki, 1987). In honeybees, police workers destroy the male eggs laid by their virgin nest mates, because these eggs are less related to them compared with the queen's male eggs (Ratnieks and Visscher, 1989). This behavior, expressed at the scale of the whole colony, reduces the potential benefits of attempted worker reproduction, leading to suppression of self-reproduction by workers (Ratnieks, 1988). Also, in various wasps worker policing is confined to differential oophagy (Foster and Ratnieks, 2000, 2001), restricting worker control to a relatively minor aspect of colony reproduction, that is, the production of males.

The scope of worker policing is considerably extended in societies composed of totipotent individuals. In ants lacking a queen caste, conflicts occur among monomorphic workers having equivalent potentials for sexual reproduction (Peeters, 1993). Workers cannot leave their colony to found independently and have only two options: when young they engage in dominance interactions to try and accede to the alpha rank if it becomes vacant, or they delegate reproduction to a relative.

Monnin and Ratnieks (2001) broadened the notion of worker policing to the selection of mated reproductives (gamergates) in queenless ants: when infertile workers immobilize a dominant worker for hours or days, she loses her rank and no longer has a chance to become a gamergate. Police workers thus have collective power that is arguably as important as the dominance interactions among hopeful reproductives. Unlike dominance behaviors, which rely on the physical attributes of contenders, policing is not a demonstration of strength: a few workers recognize a target based on olfactory information about fertility, and immobilize her (Gobin et al., 1999; Liebig et al., 1999). Tsuji et al. (1999) demonstrated that ants can detect the fertility of nest mates through a contact pheromone. Moreover, recent studies indicate that cuticular hydrocarbons encode reliable information about fertility: ant and wasp reproductives have characteristic blends of these long-chained hydrocarbons, compared with that of sterile nest mates (Cuvillier-Hot et al., 2001; Dietemann et al., 2003; Hannonen et al., 2002; Heinze et al., 2002; Liebig et al., 2000; Peeters et al., 1999; Sledge et al., 2001). In *Myrmecia gulosa*, the hydrocarbon fraction of a cuticular extract from reproductives elicits high interest from nestmate workers (Dietemann et al., 2003).

The fertility of a reproductive is a crucial parameter that affects the inclusive fitness of sterile subordinates: their interests converge with the gamergate's only as long as she produces enough offspring. Because reproductives generally live longer, it is likely that the gamergate is the mother of all workers in a colony. Low-ranking helpers, who are no longer physiologically capable of direct reproduction, should prefer the current gamergate (their mother) to a high-ranking sister that needs to mate with a foreign male before she can produce daughters. However, this alliance no longer holds when the gamergate's fertility drops below a critical threshold (estimated as 25% loss of productivity; Monnin and Ratnieks, 2001).

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Until now, reproductive division of labor in species without morphological castes was mostly thought to be controlled by the alpha female, who has supposedly the physical power to enforce her rank and egg-laying monopoly. In the queenless ant *Streblognathus peetersi* (Robertson, 2002), we investigated the importance of worker policing relative to dominance behavior. In this species, the alpha is the only worker that produces eggs and mates (Cuvillier-Hot et al., 2004). We experimentally reduced the fertility of the alpha without negative effects on her dominant behavior. We compared the subsequent reactions of low-ranking and high-ranking workers. In parallel, we monitored modifications in the cuticular hydrocarbons of the treated alphas to determine if reduced fertility was reflected in a change of the cuticular profile. Such information about the altered physiological condition of a reproductive could be used by workers to maximize their own fitness, thus supporting Keller and Nonacs's (1993) requirements for an honest signal of fertility.

METHODS

Ant collection and rearing

Nine complete colonies of *S. peetersi* (73 ± 35 workers; range = 32–146) were collected in Magoebaskloof (Limpopo Province, South Africa) in February and April 2001. To increase the number of experimental groups, the larger colonies were divided in equal parts, taking care of distributing all age classes evenly (16 groups of 19–57 ants; median = 30). Ants were reared in plaster nests at 25°C, with a 12-h light/12-h dark cycle. All individuals were marked with number tags, and aggressive interactions were recorded to determine social ranks in each group.

Pyriproxyfen treatment

Juvenile hormone (JH) modulates vitellogenin synthesis by the fat bodies in adult insects, with either a stimulatory or inhibitory effect according to species (Hartfelder, 2000). It is thus an appropriate tool to manipulate fertility. We used pyriproxyfen (PPN, Sumitomo), which mimics the effects of JH but is metabolically more stable, thus eliminating the need for repeated applications. In *Apis mellifera* workers, a single application of 1.25–10 µg inhibits *in vivo* and *in vitro* synthesis of vitellogenin (Pinto et al., 2001). Because a *S. peetersi* worker has approximately the same body volume as does an *Apis* worker, one dose (1 µg, $n = 3$ alphas, or 5 µg, $n = 7$ alphas) of PPN diluted in 0.5 µl acetone was applied on the thorax. The alpha was then left alone for 10–15 min before being returned to her nest. Control alphas ($n = 5$) were manipulated in the same way, but received acetone only.

Behavioral observations

In the queenless ant *S. peetersi*, dyadic aggressions lead to a hierarchy, and only alpha mates (with one foreign male) and lays eggs. Alpha is easily recognized because she is the only one to perform the conspicuous behavior of “gaster curling” (see Figure 2D). When she gets old (or after creation of a new colony by fission), she is replaced by a high-ranking daughter (Cuvillier-Hot et al., 2004). We decreased the fertility of egg-laying alphas ($n = 10$) with a single application of PPN. The behavior and interactions of treated alphas with nest mates were then recorded until the hierarchy changed conspicuously. Five groups (created from three colonies) with a treated alpha (all treated with 5 µg PPN) were video-recorded daily for 3 h, starting 1 day before PPN application to get a control observation. Observations ended when alpha was immobilized continuously, and we then killed her for vitellogenin

measurement. We focused on the behavior of alpha and of the prospective alpha (the high ranker that will replace the treated alpha), and counted the occurrences of gaster curling, gaster rise, biting, and antennal boxing. Control colonies (alpha applied with acetone only) were regularly checked for aggressions during 16–30 days; then the alpha was killed for vitellogenin measurement.

Vitellogenin dosage

Preliminary experiments showed that dissections and visual observation of the ovaries are not sensitive enough to detect short-term changes in the ovarian development of treated alphas compared with control alphas. We thus decided to look at a more proximal marker of ovarian activity: the level of vitellogenin. This protein is produced by fat bodies, released in the hemolymph and specifically taken into the oocytes to form vitellins, the major storage proteins of the eggs (yolk). The measurement of the circulating level of vitellogenin in natural conditions previously showed that egg-layers have high levels of this protein, whereas low-ranking workers have almost none. High-ranking ants have intermediate levels, even though their ovaries are only slightly enlarged (Cuvillier-Hot et al., 2004). In the present study, we checked that the PPN treatment has the same negative effect on the hemolymphatic vitellogenin level as that already shown in honey bees (Pinto et al., 2001). Hemolymph extracts were obtained from treated and control alphas, killed after the behavioral observations. A reference solution was made up with fresh eggs to build a standard curve, and the levels of vitellogenin were measured by ELISA for eight treated alphas (the other two treated alphas yielded less than 0.5 µl of hemolymph, which did not allow a reliable measurement of the vitellogenin titer) and the five control alphas (acetone only). The vitellogenin level of treated and control alphas were compared with that of unmanipulated egg-laying alphas ($n = 10$, data from Cuvillier-Hot et al., 2004) by using nonparametric permutation tests.

Analysis of cuticular hydrocarbons

The cuticle of live ants was sampled by the nondestructive technique of solid-phase microextraction, and analyzed by gas chromatography. A polydimethylsiloxane fiber (SUPELCO) was rubbed for 2 min against the intersegmental membranes of the ants' gaster. The fiber was then inserted for 5 min into the injector of an HP6890 gas chromatograph, fitted with a 30-m-long HP-5 column (95% dimethylsiloxane, 5% biphenyl polymeric). The temperature of the injector was set at 270°C, and the oven temperature program was 60°C (5 min), 60°C–230°C (50°C/min), 230°C (1 min), 230°C–280°C (2°C/min), 280°C–300°C (6°C/min), and 300°C (10 min). Chromatograms were integrated with the HP Chemstation Software.

The cuticular hydrocarbons of treated ($n = 10$) and control ($n = 5$) alphas were measured twice: once before the treatment, and a second time when treated alphas were immobilized or 15 to 30 days after acetone application in the case of control alphas. Before each PPN treatment, the beta ant was also measured. She was measured a second time after alpha immobilization, provided she had become the new alpha (confirmed by her performance of gaster curling).

There are about 60 different hydrocarbons on the cuticle of *S. peetersi*. To reduce the data set, only the 17 peaks with the highest factorial weights in a principal components analysis were selected, and their corrected relative proportions (Reyment, 1989) were used as variables in discriminant analyses. A previous study (Cuvillier-Hot et al., 2004) investigated the patterns of the cuticular profile for five social categories encountered in natural (unmanipulated) colonies: egg-laying alphas, new alphas that have not yet begun to lay eggs (it takes

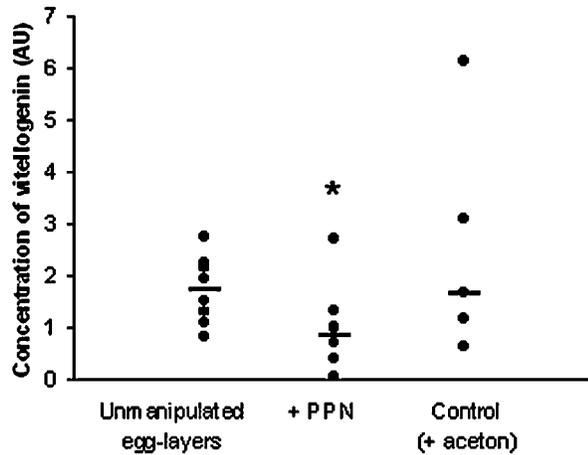


Figure 1
Levels of vitellogenin in the hemolymph of treated alphas (+PPN, $n = 8$) and control alphas (control, $n = 5$) compared with that in unmanipulated egg-laying alphas (Unmanip. egg-layers, $n = 10$). Arbitrary units refer to a standard solution made up of fresh eggs of *S. peetersi*. Bars give the median value for each group. Asterisk indicates a significant difference relative to unmanipulated alphas (nonparametric permutation test).

about 4 weeks for a new alpha's ovaries to become sufficiently developed for oviposition), high-ranking ants, foragers (old sterile ants that collect food outside the nest), and callow ants less than 4 days old. This discriminant analysis was used as a framework to detect changes in the cuticular signature owing to the PPN treatment: we used its predictive function to position the cuticular profiles of treated alphas, prospective alphas, and control alphas on the graph.

RESULTS

Physiological effect of PPN treatment

We verified that the treated alphas' ability to produce eggs had been affected (Figure 1): they exhibited reduced levels of vitellogenin in their hemolymph, relative to that of unmanipulated egg-laying alphas (permutation test: $p = .03$). Applying either 1 or 5 μg PPN made no difference to vitellogenin levels, and they were treated as one in the analysis. Control alphas, applied with acetone only, had similar levels to that of unmanipulated egg-layers studied previously (permutation test: $p = .3$).

Social effect of PPN treatment

All treated alphas were replaced by a high ranker; the prospective alpha was beta in five of 10 groups, gamma in three of 10. Replacement occurred 2–33 days after PPN application (median = 11.5 days). This time range highlights the physiological nature of the induced effect: if PPN had chemically interacted with cuticular compounds, there would have been an immediate response from the workers, which is not the case here. Detailed behavioral data on five groups reveal that the treated alpha remained aggressive until the last day (Figure 2A). In particular, she performed many gaster rises (Figure 2E; characteristic of all high-rankers) and gaster curls (Figure 2D, characteristic of alpha only), many of which were directed at the prospective alpha (data not shown). Nevertheless, in all 10 colonies, several low-ranking workers eventually immobilized the treated alpha. On that day, the prospective alpha was always observed to perform gaster curling (Figure 2B). On previous days, the prospective

alpha had attacked (antennal boxing and biting) many ants (Figure 2B) but rarely the treated alpha (Figure 2C). In contrast, one or a few days before being immobilized, the treated alpha received unusually frequent aggressions from low-ranking workers (antennal boxing and biting) (Figure 2C). Note that in the natural situation, the alpha is never the target of any aggression (Cuvillier-Hot et al., 2004).

Neither immobilization nor increased aggression occurred in the colonies with a control alpha. We checked that PPN has a similar effect to JH III by applying several doses of 2.5 μg of this hormone diluted in acetone on another three alphas. These were eventually immobilized as well.

PPN effect on the cuticular profile

The cuticular hydrocarbons of the different actors of these conflicts changed predictably: the profile of treated alphas shifted toward that typical of sterile workers (Figure 3A), whereas the profile of prospective alphas became more similar to that of fertile individuals (Figure 3B, profiles shifted along an "axis of fertility," which represents the expected change in the profile of a beta ant when she reaches the alpha rank and then begins to lay eggs). The profile of control alphas also followed the axis of fertility (except for one outlier) (Figure 3C), which is expected because their ovaries become more developed with time. The cuticular hydrocarbon profiles thus reflected the internal physiological changes of each category.

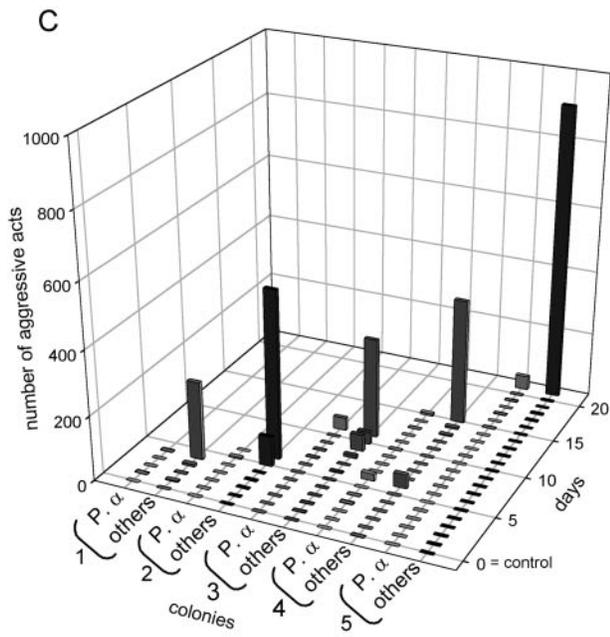
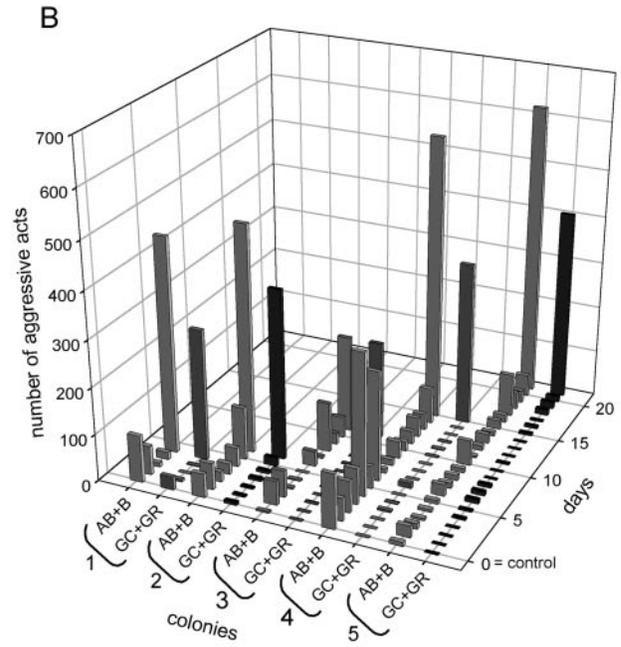
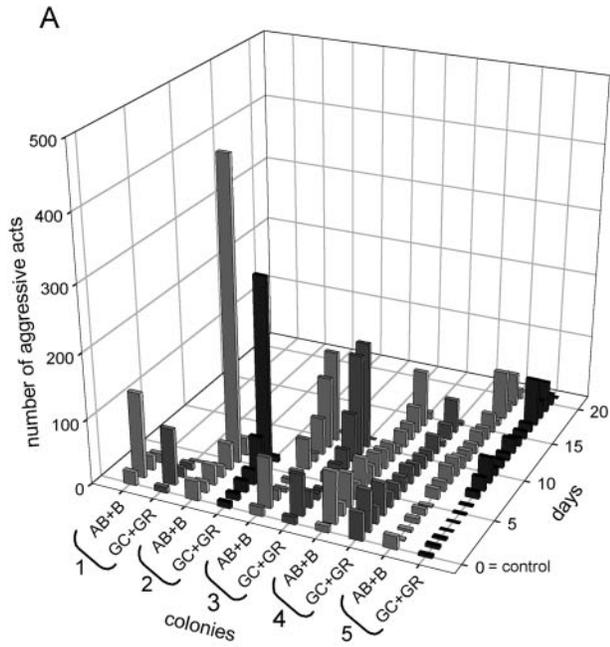
DISCUSSION

Our results show that in queenless ants, JH represses ovarian activity and does not influence dominance behavior. This is confirmed by the low JH level detected in gamergates of another queenless ant (*Diacamma pallidum*: Sommer et al., 1993). In contrast, in primitively eusocial bees and wasps, JH stimulates ovarian development and promotes behavioral dominance (see Agrahari and Gadagkar, 2003; Bloch et al., 2000). It seems that the loss of the queen caste, which is a derived event in ants, induced a change in JH function. Accordingly, similar social organizations of various social insects can be achieved by opposite roles of the same hormone.

The induced decrease of an alpha's fertility always led to her ejection from the top rank. This was preceded by an increase in antagonistic interactions in each group, the prospective alpha quickly becoming aggressive and the treated alpha later becoming the target of frequent attacks by low-rankers. This suggests that these workers detected a change in the treated alpha's odor. The prospective alpha attacked many nest mates but rarely the treated alpha, who

Figure 2

Dominance behaviors in five observation groups with a treated alpha. Different categories are presented separately: behaviors performed by treated alphas (A), behaviors performed by the prospective alphas (B), and aggressive acts received by treated alphas (C). The treated alphas considered here were replaced 4–20 days after PPN application. Day 0 corresponds to a control observation made the day before treatment; PPN application was made on day 1. AB+B indicates antennal boxing + biting; GC+GR, gaster curling (see D) + gaster rise (E); P. α , aggressive acts performed by the prospective alpha; others, aggressive acts performed by workers other than the prospective alpha. In group 5, alpha was the original gamergate (i.e., fertility above average) and received two doses of PPN. The increase in aggressions by alpha on day 1 is an artifact of our manipulation of alpha, as indicated by the colonies with a control alpha (data not shown).



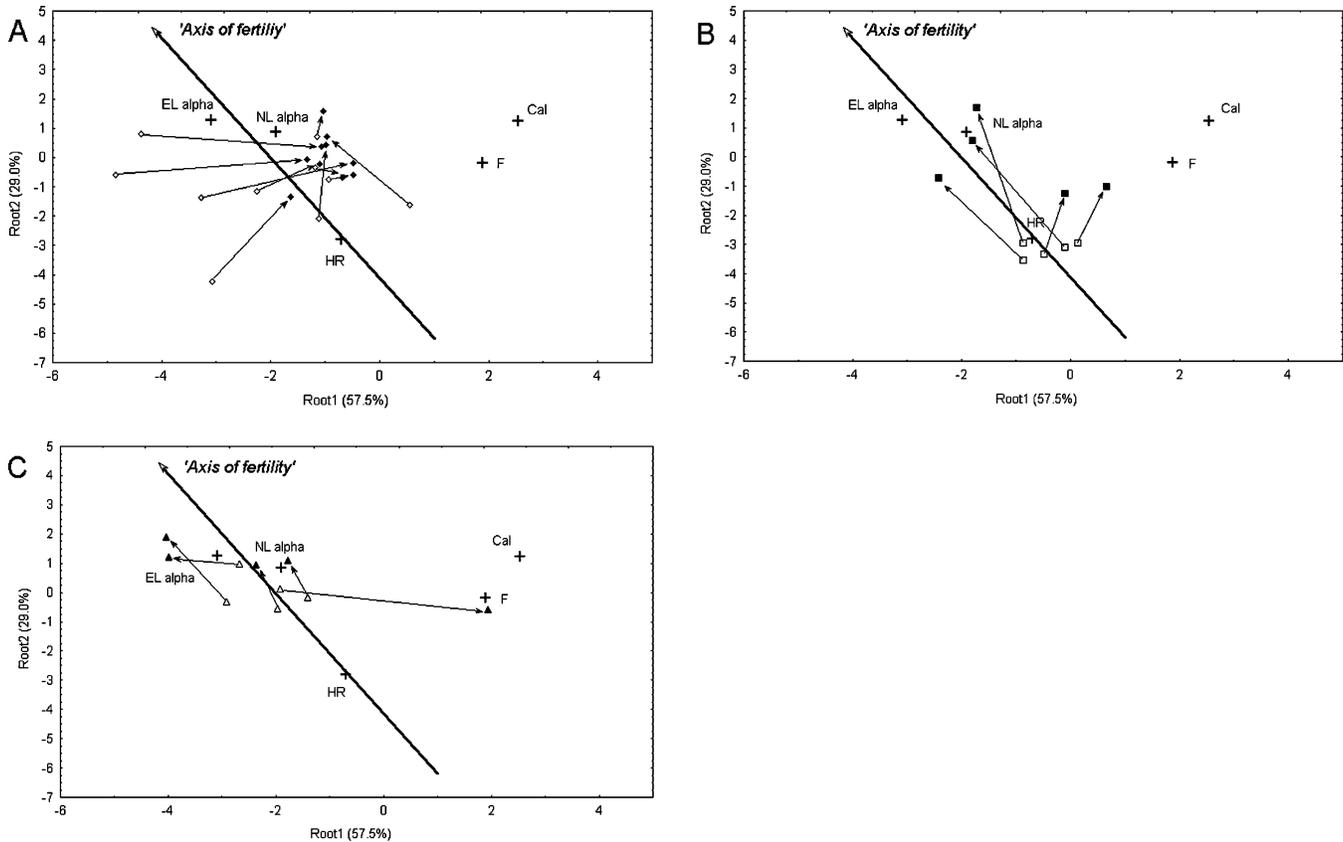


Figure 3

Chemical modifications of the cuticular hydrocarbons of alphas treated with PPN ($n = 10$) (A), prospective alphas in colonies with a treated alpha ($n = 5$) (B), control alphas ($n = 5$) (C). Open symbols refer to the measures before treatment; solid symbols refer to the measures after immobilization of the treated alpha (A, B), or 15 to 30 days after acetone application on control alphas (C). Arrows connect the two consecutive measures for each individual ants. Data about treated alphas, prospective alphas, and control alphas are positioned on the graphs by using the predictive function of a discriminant analysis done with the following five social categories, indicated by their centroids (crosses): egg-laying alphas (EL alpha; $n = 26$), new alphas that have not yet begun to lay eggs (NL alpha; $n = 17$), high-ranking ants (HR; $n = 27$), foragers (F; $n = 32$), and callow ants less than 4 days old (Cal; $n = 28$). The "axis of fertility" represents the expected shift of the profile of a beta ant when she reaches the alpha rank and then begins to lay eggs.

remained aggressive toward her. Our cuticular hydrocarbons data reveal that oogenesis had begun in the prospective alpha, because her profile had become similar to that of established alphas by the time each immobilization occurred (Figure 3B). The large intergroup variability in the time taken for the treated alphas to become immobilized is probably owing to different levels of fertility at the time of PPN application.

Our manipulation of alpha's fertility triggered two different forms of aggression in nontreated individuals: dominance behaviors by high-ranking workers who competed to reach the vacant first rank, and worker policing by low-ranking workers who immobilized the deficient alpha. This dual effect highlights the crucial role of low-rankers in settling reproductive conflicts in this species. They are not passive witnesses of the hierarchy struggles among high-rankers but have the power to favor whichever contender will be more productive. For this, the fertility of nest mates needs to be monitored.

To be evolutionarily stable, worker policing has to be based on a reliable assessment of fertility. Our chemical results show that the link between fertility and cuticular hydrocarbons, documented in several ants and in a *Polistes* wasp (see Introduction), goes beyond a simple correlation. The profile of the alphas with experimentally reduced fertility shifted and became more similar to that of infertile workers (Figure 3A); this change is likely to be detectable by nest mates. Low-ranking workers can rely on this information about fertility to

cooperate with a related egg-layer and prevent her overthrow as long as she is sufficiently productive. High-ranking workers can also use this fertility signal to detect the first signs of an aging gamergate's decline. The same signal is thus involved in two natural contexts: untimely challenges for the alpha position, and senescence of a gamergate. The cuticular hydrocarbons correspond to an honest signal as postulated by Keller and Nonacs (1993): they provide information that recipients can use to their own advantage, and they do not have an inhibitory function because physical actions are needed to enforce the sterility of high-rankers.

Our results also show that dominance behavior is ineffective relative to a collective behavior such as worker policing. Treated alphas continued to be aggressive, but this did not prevent their immobilization and replacement. This emphasizes that physical dominance is not the major regulatory force even in small insect societies, such as those of *S. peetersi*. For most of the time, it is policing by infertile workers that enforces reproductive stability, whereas the role of dominance interactions is restricted to the very short period of social instability that accompanies a replacement (also colony fission, when a new reproductive is needed). Indeed, the selection of the future gamergate does rely on aggressive encounters among high-ranking individuals (Cuvillier-Hot et al., 2004). This suggests that reproductive regulation based on worker policing only may not be stable: physical dominance is

needed as an additional check on the quality of a future reproductive. Dominance interactions thus function as physical tests, whereas worker policing (and its underlying communication) is more focused on fertility checks with eventual punishment. Both mechanisms are needed at different stages in the regulation of reproduction, although it is worker policing that ensures stability over long periods of time.

The kind of worker policing reported in *S. peetersi* is clearly different, in its proximate and ultimate aspects, from the immobilization triggered by alpha's marking behavior in another queenless ant. In *Dinoponera quadricaps*, when the alpha rubs her sting against the cuticle of a target worker, the chemicals deposited release aggression from the police workers (Monnin et al., 2002). Similarly in *Apis*, police workers destroy some eggs because they are not labeled by the queen (Martin et al., 2002b). In both of these cases, policing is organized by the current reproductive and serves primarily her interests. In *S. peetersi*, the target of policing is not chemically labeled by a competitor, but is detected by low-rankers because of some intrinsic characters linked to fertility. Policing thus serves the interests of low-rankers, irrespective of the reproductive's interests.

Worker control has been documented in the contexts of sex ratio conflicts (see Hastings et al., 1998; Sundstrom, 1996) or the timing of the production of sexuals (Henshaw et al., 2002). Descriptions of direct worker interference in the selection of the reproductive(s) are scarce. Strassmann et al. (2002) demonstrated the predominant role of the infertile workers in aggressively suppressing the production of new queens in queenright colonies of an epiponine wasp. Our results on *S. peetersi* are a decisive step forward because we succeeded in dissociating dominance and fertility, and showed that low-ranking workers eliminate a reproductive deficient in the latter of these attributes. The crucial role of sterile helpers in the regulation of reproduction may prove to be widespread in insect societies.

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