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Workers agonistic interactions in queenright and queenless nests of a polydomous ant society

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In eusocial Hymenoptera, the ability of workers to reproduce is a cause of conflict, both between the queen and workers and among workers. Reproductive decisions by workers depend on parameters such as colony size (which reduces the cost of selfish reproduction for the colony) or queen fertility. Indeed, queen signals inhibit reproduction from workers either by coercion or because of self-restraint. Multinest societies called polydomous facilitate the collection of scattered food and colony movement in an unstable environment. In these societies, queen signal dispersion could be hindered by the physical absence of queen in some nests. Here we investigate how polydomy influences worker behaviour in a monogynous polydomous ant, the ponerinae *Pachycondyla goeldii*. A recent field study has shown that *P. goeldii* workers display a significantly higher ovarian development in secondary (queenless) than in primary (queenright) nests. In the present study we show that *P. goeldii* workers change they behaviour when they colonize new nests, displaying significantly more agonistic behaviours in secondary than in primary nests. Behavioural changes and ovary development of workers from secondary nests are probably because of a decrease of queen signal intensity in their nests. The rise of agonistic behaviour in these nests stems from mechanisms regulating the reproduction of workers such as policing or hierarchy set-up. Our results lead us to favour the hypothesis of hierarchy establishment between workers in the secondary nests, which characterize large colonies. *Pachycondyla goeldii* colonies therefore maximize their reproductive output by triggering worker reproduction when colony size increases.

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In eusocial Hymenoptera, relatedness asymmetries within colonies lead to potential conflict over reproduction between colony members (Hamilton 1964a, b; Wilson 1971; Hölldobler & Wilson 1990). In most species, workers cannot mate but retain functional ovaries and are able to lay unfertilized eggs which develop into males. This ability of workers creates a conflict among them over male production because individuals (be it a queen or a worker) are always more related to their own sons than to their brothers or nephews (Trivers & Hare 1976; Ratnieks 1988). When workers lay eggs, a reproductive hierarchy with dominants and subordinates workers typically sets up with only the former actively reproducing (Heinze et al. 1997, 2002; Heinze & Oberstadt 1999; Trunzer et al. 1999). However, when the queen is alive this conflict is usually resolved by social regulation mechanisms such as policing, where workers prevent each other from reproducing by coercive actions, and self-restraint from most individuals, leading to a queen monopoly on male production (Fletcher & Ross 1985; Bourke 1988; Ratnieks 1988; Keller & Nonacs 1993; Ratnieks et al. 2006; Wenseleers & Ratnieks 2006).

Two factors account for the evolution of such mechanisms. The first is relatedness. For example in polyandrous species, workers as a group are on average more related to the queen's sons than to the workers' sons and therefore benefit from preventing each other from reproducing (Ratnieks 1988). The second is the cost of selfish reproduction by workers to colony productivity, since laying

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workers are usually less involved in productive tasks (Wenseleers et al. 2004). These two parameters combine as selfish reproduction by workers is at a cost to the production of related individuals (both male and female), so often the cost to inclusive fitness will outweigh the direct fitness benefits of reproduction (Wenseleers et al. 2004; Helantera & Sundstrom 2005; Ratnieks et al. 2006).

Reproductive decisions by colony members will depend on these parameters and on the amount of information available to them to assess colony state (Bourke & Ratnieks 1999; Beekman & Ratnieks 2003). Several colony characteristics can influence the costs of reproduction. The first is colony size as diminishing returns to scale or reproductivity effect (Michener 1964; Oster & Wilson 1978; Bourke & Ratnieks 1999) reduces the cost of selfish reproduction as colony size increases. The second is queen fecundity. If the queen fecundity decreases, or is less than the amount of brood that the colony workforce could successfully rear, then both queen and workers can increase their inclusive fitness with worker reproduction (e.g. Wenseleers et al. 2004; Liebig et al. 2005). Thus, fertility signals from the queen are thought to play an important role in the assessment of colony productivity. Queen pheromonal signals inhibit reproduction from workers by coercion (Fletcher & Ross 1985; Bourke 1988; Wenseleers & Ratnieks 2006) or self-restraint (Ratnieks 1988; Keller & Nonacs 1993).

How queen pheromones spread through the colony heavily depends on nest structure, colony size and social organization. In small societies, each individual is potentially in direct contact with the queen, whereas in larger ones the queen signal has to spread via interindividual contacts (Seeley 1979; Naumann et al. 1991, 1992, 1993; Passera et al. 1995; D'Ettorre et al. 2004; Dietemann et al. 2005; Endler et al. 2004, 2006). Multinest societies (called polydomous) facilitate the collection of scattered food and colony movement in unstable environment (Foitzik & Heinze 1998; Holway & Case 2000; Debout et al. 2003, 2007; Denis et al. 2006a). Polydomous species are adequate models to investigate the mechanism of queen signal dispersion because the colony is separated in distinct physical units. An additional division occurs in species that are both polydomous and monogynous since colonies contain two distinct types of nests: one with the queen and workers (primary nest, hereafter), and one or several nests with only workers (secondary nests, hereafter). Direct interactions between the queen and workers in secondary nests of these colonies are rare or absent, which is not the case for primary nest workers. Workers in primary and secondary nests of monogynous polydomous societies could therefore physiologically and behaviourally differ. Nonsterile workers in secondary nests could indeed develop their ovary similarly to nonsterile workers from orphaned colonies (Brian 1980; Bourke 1988), such physiological change being often linked to the development of agonistic behaviours. Denis et al. (2007) have recently shown on *Pachycondyla goeldii* that worker ovarian activity is linked to the queen presence in the nest, but none to our knowledge has reported direct evidence of behavioural changes for workers in secondary nests.

Pachycondyla goeldii is a neotropical arboricolous species, well-known for its capacity to initiate ant–plant structure called ant-garden (Orivel et al. 1998; Orivel 2000). Mature colonies of *P. goeldii* usually occupy several ant-gardens dispersed over several square meters, a structure adaptively advantageous in coping with the microclimatic instability of pioneer areas by providing colonies with easily accessible nests (Denis et al. 2006a).

We studied how polydomy influences worker behaviour in *P. goeldii*. We designed experiments to investigate the behavioural changes in workers colonizing a new queenless nest. Following this we focused on queen pheromone transmission, asking whether queen signal needs direct queen–worker contacts to be effective in *P. goeldii*. Additional data on worker behaviour and physiology after queen loss (i.e. orphaned colonies) provide a point of comparison for all these experiments.

METHODS

Colonies

Fourteen *P. goeldii* colonies were collected in September 2002 near the dam of Petit Saut, French Guiana (grouping nests by colony). We found a queen in 12 polydomous colonies; two colonies were considered as orphaned (i.e. no associated queenright nest could be found during collection). Colonies were installed in plaster nests (19 × 26 × 3.5 cm), reared at 28 ± 2°C with 80 ± 2% relative humidity and fed twice weekly with diluted honey, drosophilae and crickets.

Dissections

We used an ovarian index for each ant as the total length of all chorionated basal oocytes (i.e. reproductive eggs, see Dietemann & Peeters 2000), to provide us with a continuous variable for correlation with agonistic behaviours.

Behavioural Analysis

We monitored the occurrences of three easily discernable agonistic behaviours through all experiments: (1) biting, (2) asymmetrical antennal boxing and (3) symmetrical antennal boxing.

Antennal boxing is a well-documented ritualized behaviour representative of dominance relationships (e.g. Ito & Higashi 1991; Heinze et al. 1996; Cuvillier Hot et al. 2004), during which a worker intensely pummels the head of a target individual. The assailed worker can become submissive or not. In the first case, it adopts a submissive posture by lying down with its antennae pressed flat over its head. This behaviour was scored as asymmetrical antennal boxing (AA; one dominates, one is dominated). In the second case, the recipient worker does not display any submissive posture but rather performs, in turn, a similar antennal boxing behaviour. This was scored as a symmetrical antennal boxing (SA; no obvious dominant).

Symmetrical antennal boxing (SA) thus has no clear dominance outcome, and could correspond to the lack of stabilized dominance relationships between the two protagonists; we assume that they could also have other social significance, such as being involved in the regulation of division of labour.

We designed an index of agonistic interactions within groups of workers based on antennal boxing behaviours. This Ritualized Agonistic Index (RAI) was defined as the ratio of the number of asymmetrical antennal boxing to the total number of antennal boxing behaviours performed by the n workers of a given group:

$$\mathrm{RAI} = \sum_{i=1}^{n} N_i\mathrm{AA} / \sum_{i=1}^{n} (N_i\mathrm{AA} + N_i\mathrm{SA})$$

where N_iAA is the number of asymmetrical antennal boxings behaviours given and received by worker i; and N_iSA is the number of symmetrical antennal boxings performed by worker i.

The rationale here is to normalize the number of AA for quantitatively and qualitatively distinct groups. Both the group size and the characteristics of individuals (e.g. impeded or not, see experiment 3) could indeed impact the total number of antennal boxing behaviours in a complex, nonlinear way. Ritualized Agonistic Index (RAI) represents the proportion of accepted dominance interactions within ritualized antennal interactions between workers. It ranges from 0 to 1, 0 meaning that no submissive response could be observed and 1 that all ritualized antennation resulted in submission.

Statistics

Comparisons between groups were performed using the exact Mann–Whitney test with strata. We applied Bonferonni correction to determine P values in post hoc tests when necessary. Comparisons within groups were performed using the exact Chi-squared test. Last we calculated the Pearson's r exact test using Monte Carlo procedure for correlations. All statistical tests were calculated using StatXact-3 version 3.1 (Cytel Software Corporation, Cambridge, MA, U.S.A.).

Experiments

Behavioural responses to queen loss

First, we checked that agonistic interactions take place between workers after queen loss. Six colonies (containing 213–446 workers and 263–551 brood items) were divided each in two equal parts except for the presence of the queen in one of the nests. Each colony was observed for 1 h just before division (day 0, control) and on days 1, 2, 3, 4, 5, 6, 9 and 12 following division. Twenty workers were randomly collected in each group (orphaned and queenright) 1 month after colony orphaning for two of those colonies to assess ovarian development.

Second, we investigated the link between AA and ovarian development in stabilized orphaned colonies. For this purpose, the two orphaned colonies collected in the field were divided 6 months later into six experimental groups of forty workers each. Workers were individually labelled using enamel paint. We observed each group 1 h daily for 1 month, afterwards all workers were frozen pending dissection.

Worker behaviour in secondary nests of queenright colonies

We designed an experiment in two phases to investigate how agonistic interactions could take place in newly colonized secondary nests when polydomous colonies develop. In a first step (colonization phase) ants were offered the opportunity to colonize a new nest, and observation took place without further manipulation. In a second step (impeding phase) part of those workers were impeded either in the primary or secondary nest of colonies.

Colonization phase

Six large queenright colonies (containing 226–419 workers and 267–459 brood items) were installed into six plaster nests connected to a foraging container. Three days later an extra empty nest was connected to each foraging container (internests distance of 1.5 m). Rapidly, ants moved into these new nests, occasionally bringing brood. The empty nests were considered as colonized when they contained more than 50 workers and 10 brood items. Both nests were observed 1 h daily from this moment on days 1, 2, 3, 4, 5, 6, 9 and 12. We hereafter refer to newly colonized nests as secondary nests, and to former nests as primary ones. Queens never left the primary nests.

To estimate the rate of workers commuting between nests, we individually labelled 32 workers in each nest on day 1 for two colonies. Marked workers location was then monitored twice daily for 5 successive days.

Impeding experiment

On day 13, a total of 50% of the workers were randomly selected and individually marked in each of the six colonies. We glued a piece of plastic string (0.5 mm in diameter, 10 mm long) on the alitrunk of half of those (impeded workers, hereafter). All workers and brood were then equally distributed between the primary and secondary nests, so that each nest contained 50% of the colony workers (25% nonmarked, 12.5% marked and 12.5% marked and impeded) and brood. Impeded workers could not move from their original nest of installation (see Tsuji et al. 1999).

We therefore obtained three distinct groups within marked workers: those stuck in primary and secondary nests, and those free to commute. We recorded their agonistic behaviours 1 h daily for 5 days. Free workers that had always been observed in the same nest were discarded during analysis since we could not assume that they functionally differed from impeded workers.

At last 1 month after the end of the behavioural observation (day 50), 20 marked workers were randomly picked up from each nest in two colonies and frozen for dissection.

RESULTS

Behavioural Responses to Queen Loss

Pachycondyla goeldii workers displayed agonistic behaviours in response to queen loss. Queenright and orphaned groups differed for all three behaviours from the first or second day on (Fig. 1). Agonistic behaviours were rare in queenright colonies, except for a base level of SA. Bites never occurred in this group. On the contrary, we noted a drastic increase of agonistic behaviours in orphaned nests: we observed biting behaviours during the 4 days following orphaning and AA showed a persistent fourfold increase compared with queenright levels. As a consequence, SA nearly disappeared after queen loss. Ritualized Agonistic Index (RAI) significantly differed between queenless and queenright nests from the second day after separation (Fig. 2).

We found more workers with developed ovaries in queenless groups (65–70%) than in queenright groups (respectively 10–15%; $P < 0.001$ for both colonies) 1 month after queen loss. More finely, we found that 40% and 50% of the orphaned workers were potentially fertile (i.e. their ovaries contained at least one oocyte more than 1.13 mm long, which corresponds to the minimum size of an egg) while none was in queenright groups ($P < 0.01$ for both colonies).

As mentioned earlier all antennations start off with the potential of ending either symmetrically or asymmetrically. We found a negative correlation between the two (Pearson's r exact test using Monte Carlo procedure, 24 nests considered as statistical units: $r = -0.79$, $P < 0.001$). Therefore, using the RAI as a normalized measure to compare agonistic levels between groups is justified. As expected, the RAI observed in colonies at least 6 months after queen loss was low and similar to RAI in queenright colonies. We observed developed ovaries in 57 ± 10% of the workers (mean ± standard error), 25 ± 13% of them being potentially fertile. As observed in Denis et al.'s (2006b) study, the percentage of given AA (ratio of given AA over the sum) during the observation period was significantly correlated with ovarian development (Fig. 3).

Worker Behaviour and Physiology in Secondary Nests of Queenright Colonies

Colonization experiment

No biting behaviour could be observed in this experiment, neither in secondary nor primary nests. Nevertheless, the significant increase of the AA (counterbalanced by a decrease of the SA) reflects the rise in agonistic interactions in secondary nests (Fig. 4a, b). The proportion of asymmetrical relationships is significantly higher in secondary than in primary nests, both remaining relatively stable for the whole observation period (Fig. 4c).

We could verify that our experimental (primary and secondary) nests were functionally connected since 73% of the marked workers ($N = 128$) were seen at least once in each nest. Nevertheless, a majority of workers (60.84%) showed a preference for one nest or the other, where they were observed in more than 80% of the observations.

Impeding experiment

Workers from the three functional groups (impeded in secondary or primary nests, and commuting workers) did not perform the same number of AA. Using RAI, we found that workers impeded in secondary nests showed a much higher rate of dominance interactions than both workers impeded in primary nests and commuting workers (Fig. 5). *Pachycondyla goeldii* workers physically separated from their queen therefore develop agonistic interactions even when there are workers and brood exchanges between their nest and the primary nest. We have indeed observed, 99 of the 203 marked free workers in both nests during the time of this experiment. Those commuting workers showed an RAI similar to the one of primary nest workers, indicating that they did not engage in dominance relationships.

We could not detect any significant difference in the physiological status of workers in primary and secondary nests of the two studied colonies (respectively, 10% versus 10% and 10% versus 15% of workers displayed developed ovaries). No potentially fertile worker could be found, neither in primary nor in secondary nests.

DISCUSSION

Our experimental data clearly indicate behavioural differences between workers of primary and secondary nests in *P. goeldii* polydomous societies. *Pachycondyla goeldii* workers modify their behaviour when they colonize new nests, displaying significantly more agonistic behaviours in secondary than in primary nests. The emergence of agonistic behaviours between *P. goeldii* workers in experimental queenless nests indicates that some mechanisms regulating worker reproduction are developing. Two types of social regulation mechanisms between workers are conceivable: (1) hierarchy between reproductive workers which limit the number of reproductives (Heinze et al. 1994, 1997, 2002; Heinze & Oberstadt 1999; Gobin & Ito 2003; Gobin et al. 2003) or (2) policing behaviours which prevent worker reproduction when the main reproductive is alive because of relatedness asymmetries with male brood of varying origin and/or of the possible costs involved (reviewed in Ratnieks et al. 2006).

A recent field study (Denis et al. 2007) showed that worker ovarian activity is linked to queen presence in the nest in polydomous *P. goeldii* societies: highest in orphaned nests, intermediate in secondary nests, and lowest in primary nests. Our experimental data differ since ovarian development was similar for primary and secondary nest workers of our experimental colonies. The reason why secondary nests workers did not show ovarian development in the lab is probably the laboratory set-up itself as it only partly recreates natural situation.

However, the intensity of agonistic behaviours closely mirrored the ovarian activation levels observed in the wild. The high levels of aggression in orphaned colonies, including frequent biting and asymmetrical antennal boxing, correspond to the highest ovarian activity. In primary nests where worker ovarian activation was negligible, no significant aggression was observed. In secondary

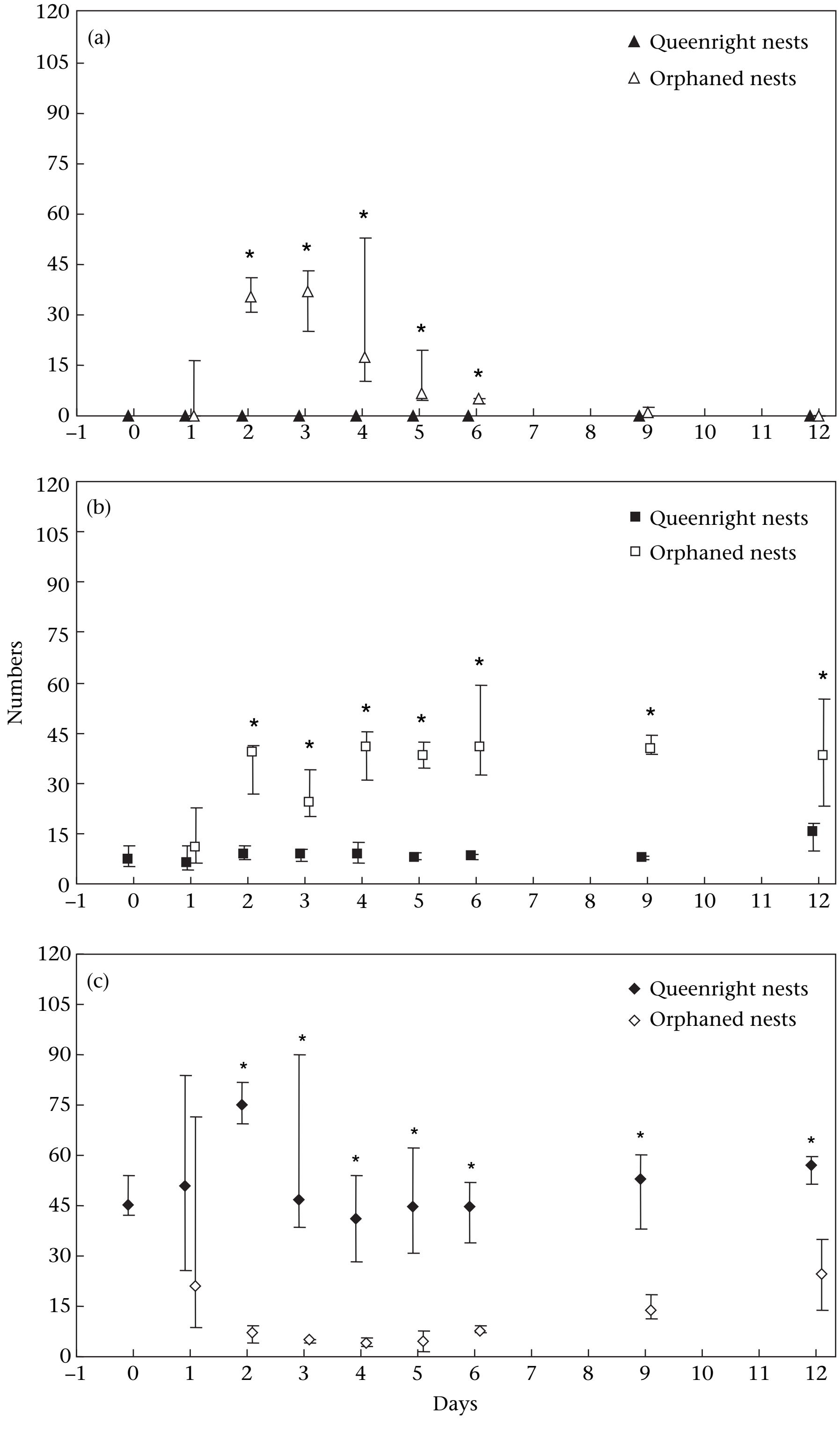

Figure 1. Temporal change in the occurrence of agonistic behaviours in queenright and orphaned nests (respectively closed and open symbols; $N = 6$ for both groups). Separation occurs between days 0 and 1. (a) Bites. (b) Asymmetrical antennal boxing. (c) Symmetrical antennal boxing. Symbols represent medians, whiskers represent the first and third quartiles. Exact Wilcoxon signed rank between queenright and orphaned nests; $*P < 0.05$.

nests, only asymmetrical antennal boxing was recorded. A possible explanation for the lesser agonistic interactions in laboratory secondary nests could be the lack of workers' ovarian development under our experimental conditions. Nevertheless biting was observed in orphaned colonies only 2 days after colony set-up, much too quickly for physiological changes to take place. Behavioural alterations can indeed occur very quickly despite physiological

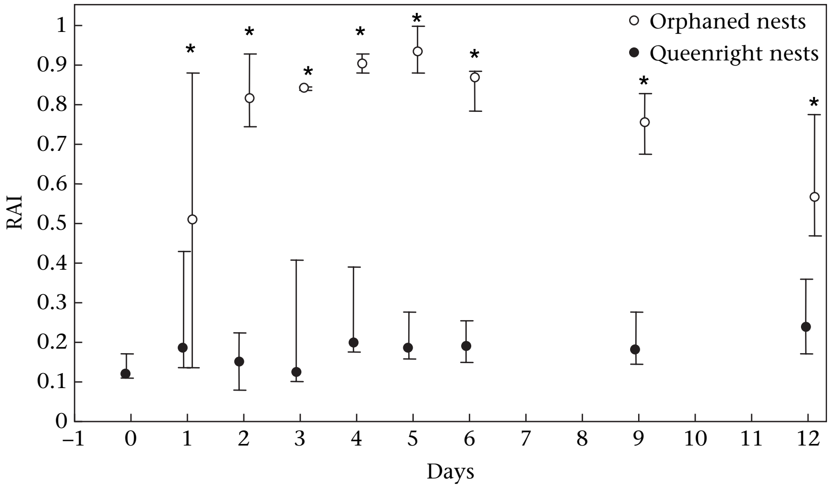

Figure 2. Temporal change in the Ritualized Agonistic Index (RAI) in queenright and orphaned nests (respectively closed and open symbols; $N = 6$ for both groups). Separation occurs between days 0 and 1. Six hundred and thirty-nine recorded behaviours in the queenright nests on day 0 {411, 487, 443, 387, 270, 282, 314, 338} and {404, 200, 147, 208, 209, 274, 301, 326} recorded behaviours in, respectively, queenright and queenless nests on days {1, 2, 3, 4, 5, 6, 9, 12}. Symbols represent medians; whiskers represent the first and third quartiles. Exact Wilcoxon signed rank between queenright and orphaned nests; $^*P < 0.05$.

changes being slower (Peeters & Tsuji 1993; Tsuji et al. 1998; Monnin & Peeters 1999; Gobin et al. 2003). Behavioural differences between experimental secondary nests and queenless colonies could therefore mainly reside in the intermediate situation of the former. That would be consistent with the natural situation where intermediate ovarian activity exists. In *P. goeldii*, the signal of queen presence available in the secondary nests could be insufficient to prevent worker reproduction. The efficiency of queen signal transmission from primary to secondary nests probably depends on the level of exchanges between nests. The decrease in queen signal intensity would therefore be much lower in laboratory secondary nests than in field ones, resulting in the absence of marked ovarian development in our experimental conditions. We did not investigate the nature of this signal. However, *P. goeldii* reproductive workers show characteristic variations in their cuticular hydrocarbon profiles (Denis et al. 2006b) and fertile workers and queen signature have been shown to differ similarly from sterile workers in a close ponerine species *Pachycondyla inversa*, (Heinze et al. 2002). Therefore, it is likely that hydrocarbons could be used by workers to assess the presence of a fertile queen. The transmission of this signal could occur in various ways. For example, in honey bees, the queen signal is indirectly spread through the colony by workers (Seeley 1979; Naumann et al. 1991, 1992, 1993; Passera et al. 1995). In ants, direct contact with the reproductive can be necessary (Tsuji et al. 1999). Alternately, queen-laid eggs can also inform workers of the presence of a fertile reproductive (D'Ettorre et al. 2004; Endler et al. 2004, 2006).

Asymmetrical Antennal Boxing behaviours in queenless nests could have a policing function. In *Diacamma* sp., worker reproduction is prevented by agonistic behaviours from other workers (preovipositional policing; Kawabata & Tsuji 2005) or by the destruction of worker progeny (postovipositional policing; Kikuta & Tsuji 1999). In our study, asymmetrical antennal boxings could correspond to mild behaviours of preovipositional policing. In that case, fertile workers staying away from the queen could suffer aggression from workers still commuting and therefore exposed to regular contacts with the queen. This prediction conflicts with the results of our third experiment, where some workers of the secondary nests were prevented from commuting to the primary nest, and therefore completely lacked direct contact with the queen. In that context, impeded workers and not commuting ones initiated aggression in secondary nests.

Thus, the increase of RAI could rather be the consequence of a hierarchy set-up between workers in queenless nests. Worker behaviour in secondary nests is indeed similar to those in orphaned nests where policing is by nature absent (Wenseleers & Ratnieks 2006) and where a strong correlation between ovarian development and asymmetrical antennal boxing suggests a reproductive hierarchy, with potentially fertile workers behaving aggressively (Heinze et al. 1997, 2002; Heinze & Oberstadt 1999; Trunzer et al. 1999).

Hierarchy set-up between workers implies the expression of conflict over reproduction between workers. Localized worker reproduction could not be explained by relatedness-based conflicts since relatedness between workers is the same in primary and secondary nests. In contrast, the costs involved in the conflict over male production shed light on our findings. The cost of selfish reproduction on colony productivity might decrease when colony size increases (Michener 1964; Oster & Wilson 1978; Bourke & Ratnieks 1999). In Ponerinae species like *P. goeldii* in which queen fecundity is low (Peeters 1997), worker reproduction could be adaptive when the worker force exceeds what is needed to successfully rear all the queen-laid brood.

Denis et al. (2006a) have shown in a field study that populous colonies of *P. goeldii* are polydomous (two to

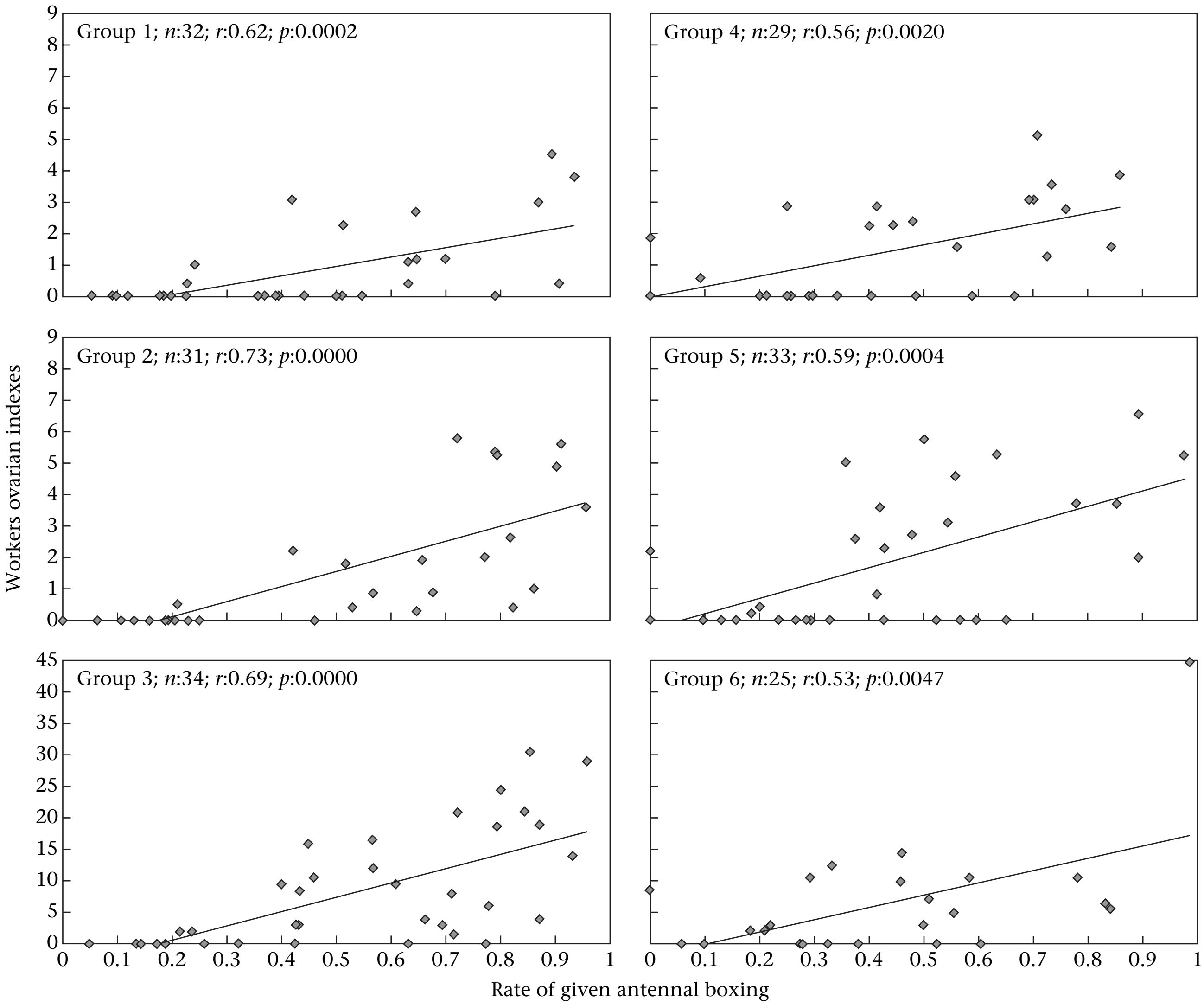

Figure 3. Correlation between the given/received asymmetrical antennal boxing rate and ovarian development of each ant for six orphaned groups of workers. Pearson's *r* exact test using Monte Carlo procedure.

four nests and 401 ± 126 workers (mean ± SD) per colony). Moreover, they have found only small monodomous queenright colonies (respectively, 204 ± 178 and 73 ± 34 workers, mean ± SD). This suggests that polydomy only develops as colony size increases, leading to the colonization of additional queenless nests. In the field, workers activate their ovaries only in those queenless nests (Denis et al. 2007). Worker reproduction therefore occurs only when colonies reach larger sizes and become polydomous.

The potential cost of reproduction is nevertheless greater in queenright than in orphaned colonies, as the decrease in productivity affects both female and male production in the former (Wenseleers et al. 2004). It is also of primary importance for a worker to start reproducing immediately after queen loss, whatever the cost, mainly because of the short life expectancy of orphaned colonies (Brian 1980; see also prediction for annual species; Tsuji & Tsuji 2005). In the third experiment, the rise in agonistic interactions obtained for impeded workers in secondary nests is intermediate between (1) the minimum observed for both impeded workers in primary nests and commuting workers and (2) the maximum observed for orphaned workers. Our results thus prove that isolated workers do perceive some cues revealing the presence of the queen (they do not behave as orphaned workers) but that these cues are lower than in the primary nests (they do not behave as workers in contact with the queen). Exposed to a weak (but not null) queen signal, workers display a ritualized agonistic behaviour, avoiding potentially harmful bites and thus setting up a hierarchy at a low cost. This could also explain the escalation of conflict from ritualized to overt in orphaned colonies. The development of additional nests is a good indicator of colony size and worker force and could therefore be a proximate factor used by workers to trigger reproduction.

A major conclusion of our study is that *P. goeldii* workers in a polydomous colony can adopt a different behaviour depending on their nest location. The rise in agonistic behaviour in secondary nests is presumably linked to an

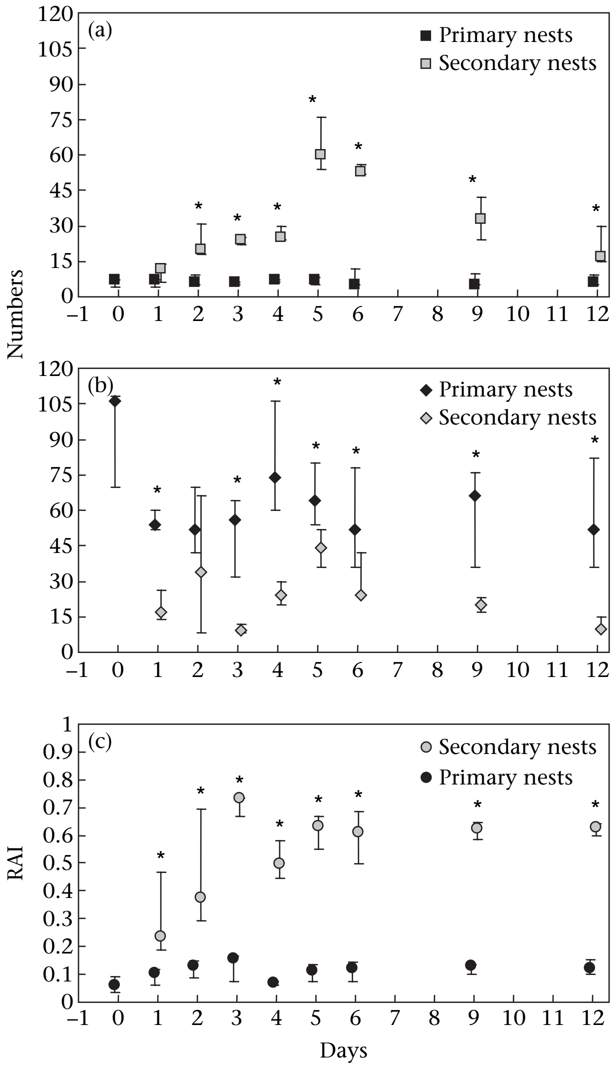

Figure 4. Temporal change of the occurrence of agonistic behaviours in primary and secondary nests (respectively, closed and shaded symbols; $N = 6$ for both groups). Empty nest is considered as colonized from day 1. (a) Asymmetrical antennal boxing. (b) Symmetrical antennal boxing. (c) Ritualized Agonistic Index (RAI). Five and hundred and fifty-two recorded behaviours in primary nests on day 0, {374, 392, 314, 480, 400, 426, 360, 350} and {173, 337, 196, 284, 519, 410, 277, 169} recorded behaviours in respectively primary and secondary nests on days {1, 2, 3, 4, 5, 6, 9, 12}. Symbols represent medians, whiskers represent the first and third quartiles. Exact Wilcoxon signed rank between queenright and orphaned nests; *$P < 0.05$.

increased ovarian activity of workers as a result of partial queen isolation (Denis et al. 2007). Worker reproduction becomes adaptive when colony rearing capacities outweigh the queen productivity (Monnin 2006). This can occur either when queen fertility decreases or when colony productivity increases. Liebig et al. (2005) have shown an effect of a decreased queen fecundity on worker reproduction. Our study constitutes the first experimental demonstration to our knowledge of an effect of increased colony productivity. *Pachycondyla goeldii* colonies therefore maximize their reproductive output by triggering worker reproduction when colony size increases.

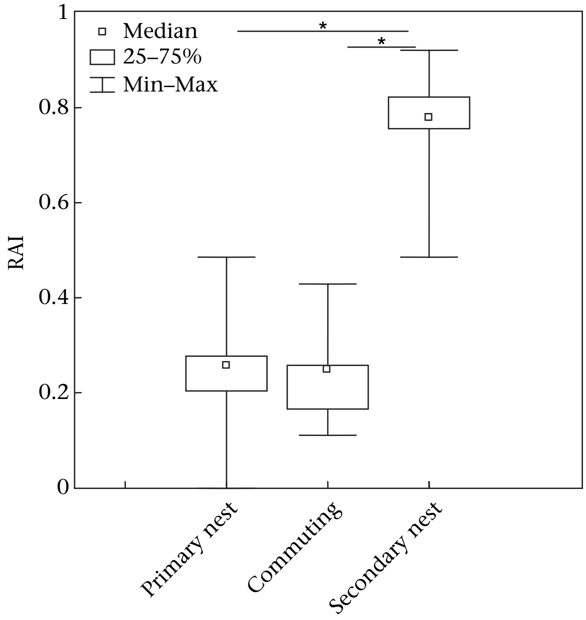

Figure 5. Ritualized Agonistic Index (RAI) for impeded workers in primary and secondary nests, and for commuting workers (respectively, 111, 142 and 1331 recorded behaviours). Kruskall–Wallis test and post hoc exact Wilcoxon signed rank with Bonferroni correction; *$P < 0.05$.

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