Research article

Reproductive division of labour without dominance interactions in the queenless ponerine ant *Pachycondyla* (= *Ophthalmopone*) *berthoudi*

M. F. Sledge[1], C. Peeters[2] and R. M. Crewe[1,3]

[1] *Communication Biology Research Group, Department of Zoology, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa;*
 Present address: Dipartimento di Biologia Animale e Genetica, Università di Firenze, Via Romana 17, I-50125, Firenze, Italy,
 e-mail: msledge@dbag.unifi.it

[2] *CNRS UMR 7625, Laboratoire d'Ecologie, Université Pierre-et-Marie Curie, 7 Quai Saint Bernard, F-75252 Paris cedex 05, France,*
 e-mail: cpeeters@snv.jussieu.fr

[3] *Present address: Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002, South Africa, e-mail: rmcrewe@scientia.up.ac.za*

Summary

In the queenless ponerine ant *Pachycondyla* (= *Ophthalmopone*) *berthoudi*, a variable proportion of the workers in each colony are mated and lay diploid eggs ("gamergates"). Virgin workers do not reproduce. The mechanism of reproductive regulation was investigated by splitting colonies to create groups of workers containing one, several or no gamergates. In the monogynous groups, no aggressive interactions were observed and virgin workers showed no signs of ovarian development. In polygynous groups, aggressive interactions were also not observed and the gamergates rarely interacted with each other; again, virgin workers showed no signs of ovary development. The groups without gamergates consisted of a large proportion of callows, with a variable number having developed ovaries in each. No aggressive interactions were observed, and there was no correlation between inter-individual behaviours and reproductive status. Although the eggs produced in these virgin groups were embryonated, none hatched. When sterile workers were separated from gamergates by a double mesh screen, several developed their ovaries and laid eggs. However, when separated by a single mesh antennate screen and allowed to gamergates, virgin workers remained infertile. Our results suggest that chemical signals produced by reproductive individuals are important in reproductive regulation in this species. The absence of any form of behavioural interactions in the regulation of reproduction is unusual for a queenless ant.

Key words: Pachycondyla berthoudi, dominance, gamergates, reproduction, ants, pheromones, hydrocarbons.

Introduction

Reproductive division of labour is an essential feature of all insect societies. In the ants it is generally associated with the existence of morphologically distinct queen and worker castes (Hölldobler and Wilson, 1990). In several species belonging to the subfamily Ponerinae, queens are never produced and reproduction is performed by mated workers (= gamergates) (Peeters, 1993). In these queenless ants, all workers have the potential to mate and hence to be reproductives. Competition for reproduction generates intense conflicts over which individuals become gamergates (Peeters, 1993; Monnin and Ratnieks, 1999). Dominance interactions among nestmates generally regulate their ovarian activity. This contrasts with the majority of ants in which antagonism is absent within colonies, and pheromonal information emitted by the queen(s) is the only basis on which workers suppress their oogenesis (Fletcher and Ross, 1985).

Many queenless ants are monogynous, but others have multiple gamergates. Such species exhibit diverse patterns of association between mating and ovarian activity. In *Gnamptogenys menadensis*, only the behaviourally dominant workers are able to mate, and these can already lay eggs while virgin (Gobin et al., in press). In other species, a large number of workers can mate in each colony, indicating that behavioural regulation is absent at this stage. In *Harpegnathos saltator*, many young workers mate, but only a few of these oviposit (Peeters and Hölldobler, 1995). Similarly, in *Pachycondyla* (= *Bothroponera*) sp. from Indonesia, most colonies contain multiple mated workers, but ritualised dominant-subordinate behaviour results in only one of these individuals laying eggs (Ito, 1993a). In *Pachycondyla* (= *Ophthalmopone*) *berthoudi*, up to 63% of workers were inseminated in nests collected immediately after the period of male activity,

but they all laid eggs (Peeters and Crewe, 1985). It appears that age of workers is the only determinant of sexual activity; choice tests indicated that callows less than 20 days are most attractive to foreign males (Sledge, 1997). In *P. berthoudi*, egg production is not behaviourally regulated since all inseminated workers have active ovaries, and variation in the number of active egglayers in a nest is large (Peeters and Crewe, 1985; Sledge et al., 1996). Although field colonies never yielded virgin workers that were able to oviposit (Peeters and Crewe, 1985), Villet (1992) reported that eggs were laid in one experimental group of young virgin workers that were isolated without gamergates. This discrepancy between field and laboratory observations indicated that a more detailed study of virgin oviposition was required. The lack of dominance interactions among mated workers was further investigated in this study.

Material and methods

Experimental animals

Thirteen nests (from 12 colonies) of *P. berthoudi* were collected from Dunstable farm (30°50′E; 25°25′S) in South Africa between November 1994 and August 1996. Care was taken to collect all the ants and as much of the brood as possible. Colonies are polydomous and thus the terms "colony" and "nest" are used here with distinctly different meanings (Peeters and Crewe, 1987). The ants were maintained in modified Lubbock nests placed in large wooden arenas (80 × 160 × 10 cm). Environmental conditions in the laboratory were 12:12 light / dark photoperiod, temperature 25–30°C, and humidity 50%–70%. Termites (*Hodotermes mossambicus*) were provided as food. All workers were individually colour-coded using Tippex® correcting fluid.

Manipulation of gamergate number

Groups of between 15 and 29 workers (in the lower range of nest size in natural conditions) were isolated from 11 of the 13 original nests, and all brood (including eggs) was removed. Since gamergates of this species are not readily identifiable by morphological (Peeters and Crewe, 1985) or behavioural means (Sledge et al., 1999), the controlled manipulation of gamergate number is a difficult procedure. Thus, the actual number of gamergates could only be confirmed at the end of the observations, once workers were dissected to check for development of ovaries and for presence of sperm in the spermatheca. Dissections showed that four monogynous, three polygynous and five virgin isolates had been created. Behavioural observations were initiated 24 h after isolation and continued during 10–30 days, each group being monitored for 30 min to one hour per day. Interactions between workers were observed with a dissecting microscope, and all cases of egg-laying were noted. In order to get a detailed assessment of the behaviour of gamergates in natural conditions, two additional unmanipulated nests (nests X and Y) (Table 1) were studied. Analysis of the movements and behaviour of gamergates and virgin workers within the nest was performed by focal sampling of individuals with a Panasonic® CP-210 video camera and AG-6010 video recorder. Each nest was recorded for two 3-hour periods on alternating days. Workers were observed for 20 min at the beginning and end of the 3-hour period.

To investigate the nature of olfactory signals produced by gamergates, groups of 15–20 virgin workers were separated by wire mesh from groups consisting of gamergates and virgins. Virgin workers were selected by disturbing ants near the nest entrance with forceps. Gamergates never leave the nest and are extremely shy during such nest disturbances. We thus selected only those individuals leaving the nest and responding in a typical aggressive fashion (heads raised with mandibles open). Two types of separation were used. In the first case (n = 3), a double layer of mesh (1.5 cm apart) prevented any physical contact between groups (although movement of air was possible). Secondly (n = 3), a single layer of mesh allowed antennal contact between workers. These isolates were left until eggs were produced by the virgin workers, or for three months.

Egg production by virgin workers and the role of insemination

As all workers in the nests were individually marked, newly eclosed workers (callows) were easily identified. These callows were then isolated (two days after emergence) from 6 of the natal nests and maintained in separate groups. Twenty isolates of varying worker compositions were established, sometimes including foragers with different numbers of callows, or with callows only. Isolates were maintained until eggs were laid. Detailed behavioural observations of these isolates were not made.

To assess whether eggs laid by virgin workers were embryonated, eggs produced by both virgin and mated workers were fixed in three parts methanol to one part DMSO (dimethylsulfoxide) overnight and then re-hydrated in consecutive washes of 70%, 50%, and 30% methanol for one hour each. They were then left overnight in phosphate buffer solution (PBS), and stained the following day with 50 µg/ml propidium iodide (PI) (a DNA fluorescent stain) for 30 min. Stained eggs were then viewed under a Zeiss confocal microscope for the presence of DNA. Morphometric comparisons (lengths and widths) between virgin-laid and gamergate-laid eggs were also made.

Analysis of worker reproductive status

On completion of the experiments, the ovaries of all workers were dissected to determine reproductive status. The number of mature oocytes and number of yolk-containing immature oocytes were counted. The presence and colour of yellow bodies (corpora lutea, i.e. remains of nurse cells after eggs are laid) at the base of individual ovarioles was also recorded. Spermathecae were checked for the presence of sperm.

Results

Nest composition

Field-collected nests consisted of 91 (s.d. 60, range: 20 to 207) workers (Table 1). There was much fewer brood in those nests collected during the dry winter months (May to August), suggesting that either larval mortality increases at these times, or egg production by gamergates is reduced. Gamergates were present in all nests and varied in number from one (3% of Nest I) to 23 (17% of Nest L).

Monogynous isolates

In four groups with a single gamergate, no aggressive behaviours were observed between any workers. The only interaction observed among workers was reciprocal antennation which was never followed by a submissive response from one of the participants. Antennation never lasted more than three seconds, and both individuals always ceased contact simultaneously. Importantly, this behaviour was also observed in the non-manipulated nests (X and Y), and foragers were involved in the majority of these interactions: gamergates antennated significantly more with foragers than with other virgin workers (mean hourly frequencies: gamergate-virgin =

Table 1. Demographics of 13 nests of *Pachycondyla* (= *Ophthalmopone*) *berthoudi* at the time of collection. The number of gamergates was determined after dissections of all the workers

Nest code	Date of collection	No. of workers	Brood			No. of gamergates
			Pupae	Larvae	Eggs	
C	23/11/94	127	69	32	18	2
E	24/11/94	79	21	16	9	6
F	24/11/94	29	8	3	0	3
G	02/03/95	86	42	28	15	2
H	03/03/95	112	71	22	7	2
I	03/03/95	33	16	2	5	1
L	22/04/95	135	107	52	29	23
S	14/05/96	207	31	21	16	14
T	14/05/96	183	16	4	12	23
U	15/05/96	84	26	17	18	13
V	15/05/96	67	18	12	11	6
X	23/08/96	26	2	6	9	5
Y	23/08/96	20	3	11	7	8

Table 2. Estimates of fertility of gamergates in monogynous and polygynous isolates (between 15 and 29 workers) of *P. berthoudi* after 30 days of observation

Isolate type	Isolate code	No. eggs laid in 30 days	No. mature oocytes	No. immature oocytes	Maximum ovariole length (mm)	Yellow bodies
Monogynous	E2	17	1	4	4.0	yes
	F2	20	1	4	3.9	yes
	G1	18	3	3	4.5	yes
	H1	21	4	3	3.9	yes
Polygynous	E1 – 5 gamergates	32	0.8 (0.7)	1.6 (0.8)	3.6 (0.24)	4 yes, 1 no
	F1 – 2 gamergates	26	1 (0)	2.5 (0.5)	3.7 (0.14)	yes
	L1 – 13 gamergates	39	0.15 (0.36)	1.2 (0.42)	2.56 (0.17)	3 yes, 10 no
Differences between isolate types (Mann-Whitney tests)			U = 8.0, p = 0.01	U = 2.0, p = 0.001	U = 1.0, p = 0.002	

In isolates with multiple gamergates, mean values and standard deviation are given.

0.51, s. d. = 0.62, gamergate-forager = 3.32, s. d. = 1.17, U = 117.5, p = 0.001). The same was found for antennations between foragers and virgin workers in the nest (mean hourly frequencies: virgin-virgin = 4.64, s. d. = 4.45, virgin-forager = 9.74, s. d. = 7.89, U = 1688.0, p = 0.001). It is unlikely, therefore, that this behaviour is involved in regulation of reproduction.

Mated workers in these monogynous isolates possessed well developed ovaries with dark, well defined yellow bodies (Table 2). Virgin workers showed no signs of ovarian development, and lacked yellow bodies.

Polygynous isolates

Three isolates contained several gamergates: two in isolate F1, five in E1 and 13 in L1. As in the monogynous isolates, no aggressive behaviours were observed among workers. In general, gamergates rarely interacted with each other (hourly mean = 0.48, s. d. = 0.64), and virgin workers inside the nest were usually ignored. In contrast, foragers were involved significantly more in reciprocal antennation, as described for monogynous isolates (F = 74.294, p = 0.001; mean hourly frequencies: gamergate-forager = 4.79, s. d. = 2.38; gamergate-virgin = 1.64, s. d. = 1.28; virgin-virgin = 1.04, s. d. =

1.19, virgin-forager = 4.08, s. d. = 2.40; pairwise comparisons with Bonferroni correction all p = 0.001).

Gamergates possessed fewer oocytes in their ovarioles compared to those in the monogynous isolates, while some lacked yellow bodies (Table 2). Based on ovariole lengths and the number of immature oocytes present in the ovaries, there was no indication of reproductive skew within these isolates. The number of mature oocytes present, on the other hand, ranged from one to three, but sampling gamergates of *P. berthoudi* before/after an egg-laying event can greatly affect this variation (Peeters and Crewe, 1985). Additional variation in ovarian development within polygynous isolates may arise from differences in age of gamergates, with younger gamergates assumed to have lower ovarian development. Unfortunately, we had no information regarding the age of the gamergates.

Virgin isolates

Behavioural observations were made of five isolates containing only virgin workers (Table 3). There were no aggressive behaviours, and hierarchical relationships could not be detected. As in the monogynous and polygynous isolates, antennation occurred between workers, but it differed from

Table 3. Ovarian and behavioural status of workers in virgin isolates of *P. berthoudi*. All isolates were a mixture of 2–10 day old callows and foragers, and were observed for 10 (isolate I2) or 23 h (remaining isolates). No aggressive behaviour was observed in any of the isolates. See text for description and relevance of recruitment behaviour. Values for recruitment behaviour refer to both "recruitment" and "being recruited". Numbers in parentheses indicate the mean number and standard deviation of recruitment interactions for workers without developed ovaries (per hour) in the isolates

Isolate code	No. of workers	Days to first egg	No. of eggs laid	No. of workers with active ovaries	No. mature oocytes	No. immature oocytes	Max. ovariole length (mm)	Participation in recruitment behaviour per hour
H2	30	23	12	3	0	1	1.9	0.60
					0	2	2.4	0.82 (0.56, s.d. 0.38)
					1	1	2.5	0.34
I2	17	–	0	1	1	1	3.1	7.9 (3.04, s.d. 1.62)
L2	27	24	3	1	1	1	2.6	0.82 (0.87, s.d. 0.42)
virg 4	25	20	1	1	1	0	1.8	0.30 (0.48, s.d. 0.39)
virg 5	25	–	0	1	0	1	1.9	0.30 (0.38, s.d. 0.29)

that in the gamergate isolates, resembling instead the interactions preceding social transport between nests observed in natural conditions in *P. berthoudi* (Peeters and Crewe, 1987). One worker vigorously antennates another, whereafter the solicited worker either allows itself to be carried or refuses. This behaviour is clearly distinguishable from aggressive behaviours such as antennal boxing. Antennal boxing is usually highly directed and accompanied by a typically submissive lowering of the body. We observed successful recruitment on several occasions. All adults in virgin isolates (including foragers) participated in these bouts of antennation. The majority of all interactions (>60% in all isolates) took place outside the laboratory nests, and included many callow workers that were frequently outside. Quantification of this behaviour confirmed that it was not correlated with ovarian development; we found no differences between the mean hourly frequencies of participation in recruitment bouts between workers with developed ovaries and between those without across isolates (Mann-Whitney U = 386, p = 0.799).

Four virgin isolates were observed for 23 h each over a period of 30 days. A variable number of workers were found to have developed ovaries and they participated infrequently in recruitment interactions (Table 3). Indeed, the three workers with developed ovaries in isolate H2 were never observed to interact with each other. Twelve eggs were laid by these individuals, the first egg being laid 24 days after isolation of the workers. Similarly, in the remaining isolates, a single worker per isolate had developed ovaries, but interacted infrequently with other workers. In a further isolate observed (I2, 17 workers), a single worker possessed active ovaries, containing one mature and one immature oocyte. This worker participated frequently in recruitment events, both in attempting to recruit other workers and being recruited (Table 3). No yellow bodies were observed in this individual, and no eggs were laid in the observation period. All other workers in this isolate had undeveloped ovaries and lacked yellow bodies.

Ovarian activity of virgin workers

Nineteen virgin worker isolates were established with newly eclosed workers and foragers, and an additional one consisted only of foragers (see dissection results in Table 4). Only one isolate failed to produce eggs. The length of time taken for eggs to be laid varied considerably, ranging from 20 days to 115 days (mean = 54.4, s.d. 23.3) (Tables 3 and 4). In all virgin isolates (except the one with foragers only) egg-layers were callows. There was no relationship between the number of callows in the isolate and the time taken for eggs to be laid (r = 0.05, p = 0.864). Similarly, the number of egg-layers did not depend on the number of callows originally isolated (r = 0.32, p = 0.10). The pattern of ovarian activity varied in these isolates, but in no case did every callow worker have developed ovaries (Table 4). Behaviour of workers in these isolates was similar to that described above for virgin worker isolates. Interactions between workers were identical to those associated with inter-nest recruitment.

Eggs laid by virgin workers appeared similar to those laid by gamergates; they did not differ in size (mean length (mm): gamergates = 1.74, s. d. = 0.1, virgin-laid = 1.69, s. d. = 0.08, t = 0.27, p = 0.79; mean diameter (mm): gamergates = 0.55, s. d. = 0.04, virgin-laid = 0.56, s. d. = 0.01, t = 0.44, p = 0.65; n = 22). Nonetheless, eggs produced by virgin workers never hatched in the presence of the workers. This was not due to developmental anomalies as the eggs were found to be embryonated (presence of DNA confirmed by microscopy). Furthermore, when several (n = 8) virgin-laid eggs were removed from the workers and placed alone in a sterile petri dish, they always hatched. Embryos with distinct segmentation became visible after approximately 20 days, and hatched into larvae at 30 to 35 days. These larvae developed normally when re-introduced in worker isolates.

Mesh isolation and olfactory communication

Focal sampling of individuals within non-manipulated nests revealed that gamergates spend the majority of their time resting. They stayed together and moved very infrequently within the nests. On the other hand, virgin workers walked about the nests, often encountering and investigating gamergates. When separated from gamergates by a double mesh (n = 3) (see methods), virgin workers were found to develop their ovaries and lay eggs; two ovipositing workers occurred

Table 4. Ovarian status of workers in 20 virgin isolates of *P. berthoudi*. Isolates were mixtures of two day old callows (C) and several foragers (F), or callows only. In one case (isolate 6) only foragers were isolated. In all cases where callows and foragers were mixed, only callows were found to have active ovaries. The number of eggs produced in each isolate could not be fully assessed because workers often discarded them (see text)

Isolate code	Composition of isolate	Days to appearance of first egg	No. of workers with active ovaries	No. mature oocytes	No. immature oocytes	Presence of yellow bodies?
6	16F	68	4	1/1/1/2	1/0/1/0	all yes
7	1C+7F	33	2	1/1	1/0	all no
11	1C+7F	74	1	0	2	no
8	2C+7F	83	1	0	1	no
12	2C+7F	46	2	1/1	3/2	no
1	2C+8F	49	1	1	1	yes
9	3C+7F	26	3	2/0/1	2/2/2	yes/no/yes
17	4C+6F	61	1	0	2	no
18	7C+7F	52	2	2/0	1/2	no
3	7C+8F	46	1	1	1	no
2	8C+6F	45	5	2/2/2/0/0	1/1/0/1/1	all yes
4	9C+3F	45	4	1/2/0/0	0/1/1/2	no
5	9C+4F	Eggs never produced				
10	9C+7F	47	2	0/1	2/1	no/yes
13	7C+0F	115	1	1	1	no
19	11C+0F	69	4	1/0/0/1	1/2/4/2	all no
15	14C+0F	35	4	0/1/0/1	3/4/2/1	all no
20	15C+0F	95	1	0	2	no
14	16C+0F	52	2	1/1	2/0	all no
16	23C+0F	54	2	0/0	2/2	all no

in each isolate. In contrast, when separated only by a single mesh (n = 3), no eggs were produced by virgin workers, and subsequent dissections revealed that none possessed developed ovaries. When gamergates were perceived across the single mesh by infertile workers, the latter were attracted to the gamergates and often attempted to remove the mesh. Workers also antennated through the mesh for extended periods and this was only initiated by virgin workers in the separated groups. Workers and gamergates in gamergate groups rarely antennated through the mesh.

Discussion

Egg production by virgin workers and the role of insemination

Young virgin workers laid eggs in all but one isolates from which gamergates had been excluded. The number of egg-laying virgins varied among the isolates, and was not related to group size. Age partly determined ovarian activity in these experimental groups as only the young workers oviposited. However, some remained sterile, suggesting that additional factors regulate oogenesis. This is supported by the fact that four workers also produced eggs in an isolate that contained only foragers. The earliest appearance of eggs was 20 days (mean = 54 days), which is longer than an earlier study (14 days; Villet, 1992), despite the age of callows being similar in both studies. Virgin-laid eggs were clearly viable because embryos developed normally. Nonetheless, these eggs did not hatch in the laboratory nests, which was also reported by Villet (1992). This is unlikely to be an artefact of the laboratory since eggs laid by gamergates develop

normally (pers. obs.). A possible explanation is that virgin-laid eggs were incompletely matured and their chorion was too thin. Thus, egg-handling by workers resulted in their accidental destruction. Insemination may also play an important role. In another ponerine ant with gamergates, *Gnamptogenys menadensis*, virgin-laid reproductive eggs are also indistinguishable from those produced by gamergates, but they very rarely develop into larvae (Gobin et al., 1998). However, when these workers become mated, 50% of their eggs develop into workers. Since colonies of *P. berthoudi* are polydomous, and workers are carried between nests (as well as the fact that a large number of inseminated workers are found), the natural occurrence of nests without gamergates is unlikely.

Pachycondyla berthoudi is exceptional among ponerine ants because so many mated workers are able to lay eggs. Field-collected colonies of *P. berthoudi* never yielded virgin workers with developed ovaries if gamergates were present (1373 workers dissected in Peeters and Crewe (1985); 619 workers dissected in this study). Only mated workers have mature oocytes and oviposit, which led Peeters and Crewe (1984, 1985) to propose that insemination controls reproductive division of labour in *P. berthoudi*. Despite the fact that young virgin workers can lay eggs when isolated experimentally (Villet, 1992; this study), insemination has a clear influence on oogenesis. As the length of time taken for virgin workers to lay eggs was usually very long, it is possible that insemination shortens the time taken for oocytes to develop. Worker age will also be an additional influence in this regard (young callows were used in our isolates).

It is also worth considering whether workers intentionally destroy the haploid eggs produced by other virgin workers, thus preventing the production of male offspring. There is

strong evidence from several social insect species that infertile workers prevent each other from reproducing, a phenomenon known as worker policing (Ratnieks, 1988; Ratnieks and Visscher 1989). Workers may either physically impede other individuals from developing their ovaries (e.g. Visscher and Dukas, 1995; Gobin et al., 1999; Liebig et al., 1999; Monnin and Peeters, 1999), or they may selectively destroy unfertilised eggs (Ratnieks and Visscher, 1989; Kikuta and Tsuji, 1999). This always occurs in colonies that contain queens or gamergates, though, and the reason for such policing in orphaned groups in *P. berthoudi* is unclear. It is likely that reproduction by virgin workers in addition to that of gamergates will be energetically costly, especially when nests contain large proportions of gamergates. Furthermore, males are active for only a short period during the year (January to March) (Peeters and Crewe, 1986), and haploid egg production during other months may be wasted. Oophagy has never been observed in *P. berthoudi* (Peeters and Crewe, 1985; pers. obs.), though, and it remains to be experimentally tested whether virgin workers can actually differentiate eggs of varying origins.

Lack of dominance relations and regulation of reproduction

Our results confirm the absence of antagonism between gamergates and other workers in *P. berthoudi* (Peeters and Crewe, 1985). Physical interactions are restricted to reciprocal antennations, and foragers take part in the majority of these. However, in the absence of gamergates, a proportion of virgin workers proceeded to oviposit, indicating that they were previously suppressed. Similarly, in *Rhytidoponera* sp. 12, no aggression occurs among gamergates and workers, yet virgin workers produce eggs when gamergates are removed (Tay and Crozier, 2000). In *Gnamptogenys striatula*, workers can mate (in the absence of the queen), but there is also no aggressive behaviour between these gamergates and sterile workers (Blatrix and Jaisson, 2000). The lack of dominance interactions among workers provides circumstantial evidence that ovarian activity is entirely regulated by olfaction in *P. berthoudi*. We have also shown that variations in individual fecundity occur despite the lack of physical interactions among gamergates, and this suggests some form of mutual pheromonal inhibition (Sledge et al., 1996). This has been proposed by Ito (1993b) for *Amblyopone* sp., and may also occur in *Rhytidoponera aurata* (gamergate fecundity decreases in relation to gamergate number in this ant) (Komene et al., 2000). Additional factors, such as nest size, may also affect gamergate fecundity, with energy intake by the ants controlling the extent to which gamergates reproduce. Further support for olfactory-based reproductive regulation in *P. berthoudi* is provided by the lack of aggression in virgin worker groups. Recruitment behaviour among workers was the only interaction observed. There was no general pattern to this behaviour and it is not correlated to ovarian activity. The likely significance of this behaviour is that it facilitates recruitment of gamergates from other nests (within a colony) in the unlikely event that gamergates become absent in any one nest.

Physical separation of gamergates from virgin workers by a double mesh allowed the latter to lay eggs, indicating that the signals produced by gamergates are not air-borne, and are probably perceived by workers during physical contact. Direct physical contact between individuals is required to regulate egg-laying in another ponerine ant, *Diacamma* sp. from Japan (Tsuji et al., 1999). In our experiments this was confirmed by the lack of egg production by virgin workers when they could antennate with gamergates through a single mesh. It is therefore likely that low-volatility signals crucial in regulating reproduction are present on the cuticle. This is supported by recent research on several other social insects, where it has been shown that proportions of epicuticular hydrocarbons are correlated with ovarian activity (Monnin et al., 1998; Peeters et al., 1999; Liebig et al., 2000; Cuvillier-Hot et al., 2001). It is also known in several species that workers are able to recognise the development of nestmates ovaries by olfactory means (Visscher and Dukas, 1995; Liebig et al., 1999; Gobin et al., 1999). It is likely that this is mediated by changes in cuticular hydrocarbons associated with ovarian activity. Similarly in *P. berthoudi,* oogenesis in gamergates may result in the production of characteristic chemical signals, and these could be involved in the regulation of oogenesis in both virgin workers and gamergates. Bioassays are required to confirm this, though.

In the monogynous ponerine *Dinoponera quadriceps*, dominance interactions occur during episodes of gamergate replacement (Monnin and Peeters, 1999). It remains unclear why aggressive interactions are absent among virgin workers in *P. berthoudi* when they are isolated from gamergates. Queenless ants are a polyphyletic assemblage, and thus a variety of mechanisms can be expected to regulate reproductive division of labour across species. Mating is regulated in many species, and this determines the number of gamergates in each colony (Peeters, 1993). In *P. berthoudi*, all young workers are able to mate when foreign males enter their nests. As the proportion of gamergates varies between nests and there is a low skew in fecundity (Sledge et al., 1996; this study), olfactory information emitted by gamergates seems important in regulating individual fecundity – suppressing oogenesis in virgin workers may be another consequence of this regulation. The effects of polydomy on regulation of reproduction in *P. berthoudi* require further attention.

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