

Research article

Potential and realized reproduction by different worker castes in queen-less and queen-right colonies of *Pogonomyrmex badius*

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Abstract. Workers of the Florida harvester ant (*Pogonomyrmex badius*), the only North American *Pogonomyrmex* with a polymorphic worker caste, produce males when colonies are orphaned. In this study, we assessed the reproductive potential of workers of each caste group, minors and majors, in the presence and absence of the queen, and tested whether males produced in natural queen-right colonies are derived from workers. Worker size was positively correlated with ovariole number such that major workers had approximately double the number of ovarioles as minor workers. The number of vitellogenic oocytes, a measure of reproductive potential, was greater in major compared to minor workers and increased in both worker castes when queens were removed. Major workers have greater reproductive potential than minors although they represent a minority within the colony (~5% of workers are majors). Worker produced eggs were visible in colonies 28–35 days after queen removal. This time lag, from queen removal to egg production, is similar to other ants and bees. Though workers are capable of producing viable eggs, we found no evidence that they do so in queen-right colonies, suggesting that worker reproduction is controlled via some social mechanism (self restraint, policing, or inhibition). This result supports predictions of kin selection theory – that due to multiple mating by the queen workers are more related to queen-produced males than most worker-produced males and should thus favor reproduction by the queen and inhibit reproduction by other workers.

Keywords. Polymorphism, worker reproduction, worker sons, male parentage, queen removal.

Introduction

Truly sterile workers, those with no potential to reproduce, have evolved multiple times in the social insects (Oster and Wilson, 1978). Body size is often correlated with increased reproductive development within species (Heinze et al., 1995; Tsuji, 1995; Gobin and Ito, 2003; Dijkstra et al., 2005), such that large workers capable of reproducing may interact antagonistically with the queen over access to reproduction (Oster and Wilson, 1978; Bourke, 1988). Therefore, Oster and Wilson (1978) postulated that the evolution of physical worker castes may be facilitated by worker sterility because size and reproductive potential are decoupled. Morphologically distinct worker castes, however, have evolved repeatedly in diverse ant genera without worker sterility as a precursor step (Fielde, 1905; Heinze et al., 1995; Gobin and Ito, 2000, 2003; Dijkstra et al., 2005; Dijkstra and Boomsma, 2006).

Ant species lacking the ability to share resources via trophalaxis (regurgitative food sharing) can distribute resources within the nest via trophic eggs (Gobin and Ito, 2000, 2003). Trophic egg-laying requires a developed and functional reproductive system in workers. Thus, in species where trophic egg-laying is necessary, an increase in worker reproductive potential will be a byproduct of the evolution of physical worker castes. In such taxa, large

workers may be selected for as trophic egg-laying specialists (Heinze et al., 1995). In these cases, however, a mechanism to prevent worker reproduction is probably necessary to ensure colony efficiency (Cole, 1986).

In many ant species, workers are capable of male production, but lack a spermatheca and the ability to produce diploid female offspring (Hölldobler and Wilson, 1990). Although exhaustive data on worker reproduction is not available, a recent review (Hammond and Keller, 2004) found that male production by workers occurs in 58 % (14 of 24) of surveyed ant species, although queens still produced the vast majority of males in most species.

Kin-selection theory makes specific predictions of worker reproduction in queen-right nests: as the relatedness among workers within the nest decreases, via polygyny or polyandry, worker reproduction should decrease (Bourke and Franks, 1995). The decrease in worker reproduction is often proximately enforced by nest-mate policing, the selective killing of worker laid eggs by queens or workers, which has been demonstrated in bees, wasps and ants (Ratnieks, 1988; Liebig et al., 1999; Hartmann et al., 2003; Wenseleers et al., 2004). Furthermore, general patterns of worker egg-laying and policing appear consistent with the predictions of kin selection (Wenseleers and Ratnieks, 2006a). However, colony-level selection may also favor non-reproducing workers, independent of the kin structure of the nest, because workers that are reproducing are not working and whole colony reproductive efficiency decreases (Cole, 1986; Hammond and Keller, 2004). The predictions of both kin selection and colony-level (efficiency) selection are not mutually exclusive.

Pogonomyrmex harvester ants are a new world seed-harvesting genus composed of approximately 60 species (Taber, 1998). *Pogonomyrmex* workers lack the ability to share resources via trophalaxis, yet are efficient at distributing nutrients stored as fat reserves (Smith, 2007), likely via trophic egg production. Two species of *Pogonomyrmex* have evolved physical worker castes (polymorphism) independently, *P. badius* in the southeastern USA (Cole, 1968) and *P. coarctatus* in Argentina (Kusnezov, 1951). *P. badius* is the only ant in the genus where workers are documented to produce males in queen-less situations (Smith and Tschinkel, 2006) despite the long history of scientific study on this genus (Taber, 1998). Colonies of *P. badius* have a single queen and an average effective mate frequency of 10 (Rheindt et al., 2004), and thus low worker-worker relatedness.

The aim of the present study was to examine the effect of queen presence/absence on potential and realized worker reproduction. We predicted that major workers would have greater reproductive potential than minor workers, and that all workers would increase in reproductive potential in the absence of the queen. Given the relatedness structure of the colony, due to high levels of polyandry, we predicted that workers would not reproduce in the presence of the queen. We discuss our results

in the context of the evolution of worker castes and conflict over reproduction.

Methods

Ovary dissection

We dissected live workers or individuals stored at -80°C . Dissections were done in water under a stereoscope at variable magnifications. We extracted the entire reproductive system by gently pulling the sting, and clearing away the fat body to expose the ovaries. We defined ovarian activity as the number of white (likely vitellogenic) oocytes, summing across ovarioles and both ovaries. In some individuals the ovaries were highly reduced, likely due to age. In these individuals the number of ovarioles and oocytes was counted as zero. Similarly, in some individuals a single ovariole was either lacking or reduced leading to ovariole counts that are odd numbered.

We assigned workers to sub-caste, minor or major, *a priori* by visual inspection. If the vertex of the head was concave they were called majors; individuals lacking this concavity were defined as minors. This system of classification yielded assignment of individuals with complete agreement with the designation of minors and majors by Tschinkel (1998), where he defined minors and majors using a normality plot of head width, where the minor-major designation was at the point of inflection in the curve. To confirm sub-caste assignments, head widths (maximum diameter including the eyes) of all dissected individuals were measured to the nearest 0.001 mm using a stereoscope with a stage micrometer (Micro-DRO, Semprex Corp).

Queen removal

To examine the effects of queen removal on worker reproductive characteristics and egg production, we maintained workers of both castes in the absence of queens. A total of seven nests were used to perform the experiments. We collected two large (4,000–5,000 workers), queen-right colonies (“Shlurpy” and “Croc”) in late March, 2005 from the Apalachicola National Forest (ANF) in Leon Co., ~15 km from Tallahassee in northern Florida [N30° 22'21.0", W84° 23'41.6"]. These colonies were placed in laboratory nests consisting of interconnected Petri dishes half filled with plaster of Paris. On 2-Nov., 2005 queens were removed from both colonies; at this time the colonies had been reduced to ~500–1000 workers each. At the time of queen removal 10 minor and 10 major workers were sampled from each nest to ascertain worker ovary activity in queen-right nests. These colonies were then maintained in a queen-less state for three and one-half months (until 16-Feb., 2006) when 10 minors and 6–7 majors were sampled from each colony to assess ovary activity of workers in queen-less nests (Table 1). Queen-less nests were maintained in the lab at -27°C and fed *ad libitum* seeds. Insect prey, large mealworms (*Tenebrio* sp.), was provided once per week. The other five nests consisted of workers freshly collected from field colonies. In late March, 2006 workers were collected from the top 30 cm of nests from the same population in the ANF. At least 20 majors and exactly 210 minors were collected from each of five colonies, hereafter referred to as EX 1–5. Between 6 and 11 individuals of each sub-caste were stored in a -80°C freezer within two days of being collected (“queen-right” samples) (Table 1). The remaining 200 minors and 10 majors from each colony were placed in lab colonies as described above; the proportions of minors and majors used was typical of naturally occurring colonies (5 % of workers were majors; Tschinkel, 1999). When transferred to lab nests we confirmed that no eggs were present with the ants, which was expected because colonies in this population do not typically begin brood production until April, and colonies are dormant from late December through February (Smith and Tschinkel, 2006). The presence of a queen in the field source colonies was directly confirmed in two of the five nests, where the queen was in the top 30 cm. Newly emerged workers without fully darkened

cuticle (callows) were present in all nests, suggesting that all colonies possessed a reproductive queen prior to going dormant in winter. The colony fragments were maintained in the lab at $\sim 27^{\circ}$ C and fed *ad libitum* seeds; insects were provided as food once per week.

Table 1. Sample sizes of workers sampled in queen removal studies.

Colony	Queen-right		Queen-less	
	Majors	Minors	Majors	Minors
EX-1	7	11	7	11
EX-2	4	11	4	10
EX-3	8	10	8	10
EX-4	6	10	9	10
EX-5	8	10	9	10
Croc	10	10	6	10
Shlurpy	10	10	7	10

Colony fragments were observed for the presence of eggs or larvae twice per week. On 16-May, 2006 the experiment was terminated and 4–10 of each minor and major workers were sampled and stored at -80° C until they were dissected (Table 1). Oocyte number was counted in workers from all seven colonies while ovariole number was only counted in the colonies collected in 2006.

Ovary activity statistical analysis

We used repeated-measures ANOVA to assess the effect of queen removal on ovary activity of both worker sub-castes. The response variable was the average number of vitellogenic oocytes per sub-caste (minor or major) per colony state (queen-right or queen-less) (sample sizes in Table 1). The analysis contained two repeated measures, each with two levels; worker sub-caste composed of minors and majors, and colony state composed of queen-right and queen-less. We used independent contrasts to test the effect of queen removal on each worker sub-caste. We combined the data from the two lab colonies collected in 2005 and the five colonies collected in 2006. Because assignment of individuals was done *a priori* it was necessary to rule out the possibility that worker size was different between queen-right and queen-less samples. To confirm that individual size did not bias our results we again used a repeated measure ANOVA with individual head width in place of oocyte number.

The relationship between worker size (head width) and ovariole number was assessed with linear regression using data from both queen-right and queen-less treatments in the second queen removal experiment. Individuals with highly reduced ovaries, recorded as zeros, were eliminated from this analysis because the ovaries in those individuals were likely degenerate due to old age.

Worker reproduction in queen-right nests

We genotyped males in 6 field colonies to infer if they were produced by workers or queens using two microsatellite loci, Pr2 (Gadau et al., 2003) and Pb5 (Volny and Gordon, 2002). Both loci had been used previously in *P. badius* (Rheindt et al. 2004, 2005). The 6 colonies sampled, all from the same population described previously, were part of a field starvation-supplementation experiment (Smith, 2007). To decrease the potential bias associated with the experimental treatments, starvation or supplementation, we sampled from three colonies in each treatment group. We genotyped 20–30 males, the queen, and at least 65 workers from each field colony (Table 2). The queen was

collected in all but one colony; for this colony the queen's genotype was inferred by the worker genotypes using Matesoft (Moilanen et al., 2004). Due to a possible patriline bias between worker castes in *P. badius* (Rheindt et al., 2005), we genotyped equal numbers of minor and major workers (Table 2).

Table 2. Sample sizes, per colony, for each caste successfully genotyped at both microsatellite loci.

Colony	# Minors	# Majors	# Males	# Queens
E2	43	53	19	1
E5	52	52	32	1
E8	55	42	32	1
E9	56	39	22	0
E10	33	32	23	1
E11	52	32	32	1
Total	291	250	160	5

After collection, we stored ants in 95% ethanol and extracted DNA as follows: 1) the ant was taken out of alcohol, briefly dried, and its gaster was removed; 2) the ant was pulverized with a pestle in liquid nitrogen; 3) the crushed ant was then dissolved in ~ 100 μ l 10% Chelex 100 and 5 μ l Proteinase K; 4) the mixture was incubated at 57° C for 1 hour, and subsequently heated for 5 min at 95° C; 5) lastly, the mixture was centrifuged at 14,000 rpm for 15 min and the supernatant containing the DNA was extracted.

PCR reactions for each locus followed the protocol described by Gadau et al. (2003) for Pr2 and Volny and Gordon (2002) for Pb5 using 2 μ l of the extracted DNA in solution. Allele length was detected by running denatured PCR product (1:1 with formamide/EDTA) on a 5% polyacrylamide gel in a LiCor 4300 DNA Analysis System, and using SAGA^{GT} software for microsatellite analysis.

Power to detect worker reproduction

Our ability to detect worker reproduction was dependent on both our ability to detect a worker produced male and the proportion of males in the colony that are worker produced. We defined our power as the probability of detecting a single worker produced male, and calculated this for varying levels of worker reproduction in colonies. For any given level of worker reproduction and for any given male, the power of ascertaining it being worker produced is: $1 - (\text{the proportion of alleles not shared between workers and the queen})$. Due to direct descent from a single queen in the colony, all workers share a minimum of 50% of alleles with the queen. Furthermore, if the queen's mates have the same allele, the proportion of shared alleles increases. Thus, per locus and per number of males (n), the equation for power was: $[1 - (0.5^n (\text{proportion of maternal alleles not the same as paternal alleles}))^{(n)}]$. If both loci are unlinked the probability to detect worker produced males using two independent loci is multiplicative. To test for independent assortment of the two loci we tested for linkage disequilibrium in both haploid sons and diploid worker daughters in colonies where queens were heterozygous at both loci (a total of three colonies), and in the case of diploid workers, where alleles from the queen were unambiguous. A total of 73 males and 205 workers met these criteria. The observed frequencies of allele combinations of both loci were tested for independence using a chi-square test; the null hypothesis was that alleles assorted independently. The test was performed separately for each colony and for males and workers.

Results

Ovary activity and queen removal

We found a significant effect of queen removal on vitellogenic oocyte number (repeated-measures ANOVA: $F_{1,6} = 22.3, P < 0.005$), where individuals in the queen-less state had more vitellogenic oocytes than those that were in the presence of the queen. Major workers also had more vitellogenic oocytes than minors ($F_{1,6} = 65.2, P < 0.005$). The interaction between sub-caste and queen presence/absence was not statistically significant ($F_{1,6} = 3.4, P = 0.11$). The independent contrasts show that both minor and major workers increased ovary activity when the queen was removed (minor: $F_{1,6} = 7.9, P < 0.05$; major: $F_{1,6} = 12.8, P < 0.05$). On average, majors increased from 6 to 12 vitellogenic oocytes, while minors from three to five.

Head widths of individuals did not differ between queen-less and queen-right colony fragments ($F_{1,6} = 1.2, P > 0.3$), and there was no interaction between worker sub-caste and queen presence/absence ($F_{1,6} = 2.4, P > 0.15$). These results confirm that our sampling of workers was not biased by size. Head widths did, of course, differ significantly between minor and major workers ($F_{1,6} = 1017, P < 0.0001$).

The number of ovarioles increased positively with worker head width ($F_{1,162} = 488.7, P < 0.0001, r^2 = 0.75$, Fig. 2). The regression equation for this relationship is, #ovarioles = $-0.47 + 2.8(\text{head width})$. The average major had 9.1 ovarioles while the average minor about half that, 4.5.

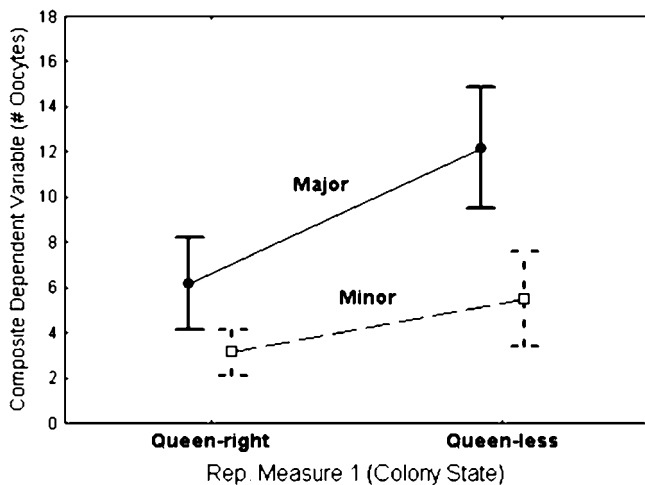


Figure 1. Interaction plot from a repeated-measures ANOVA. The dependent variable is the number of vitellogenic oocytes, represented as two repeated measures, each from 7 colonies. Repeated measure 1 is colony state, queen-right or queen-less. Repeated measure 2 is worker sub-caste, major or minor. Colony state and worker subcaste significantly affected individual oocyte number, but the interaction term is not significant ($P = 0.11$).

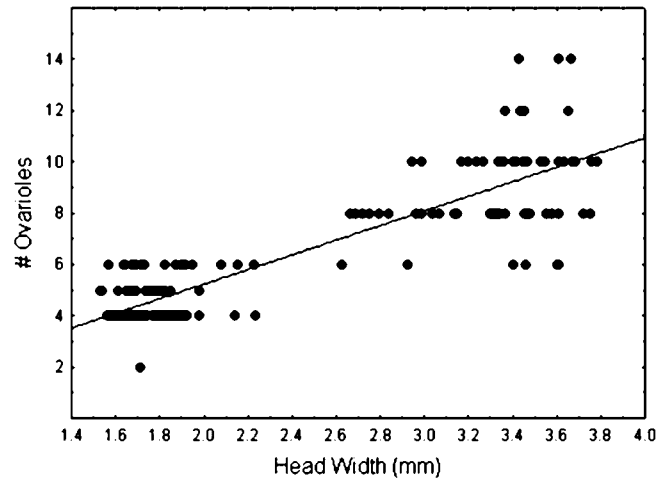


Figure 2. Regression plot of individual head width (a proxy of body size) and ovariole number. Head width is a significant predictor of ovariole number ($P < 0.0001$), and predicts 75% of the variation in ovariole number. Individuals with a head width below 2.5 mm were considered minors, and those with heads greater than 2.5 mm were majors.

In the two colonies collected in 2005, queen-less nests produced male pupae by the time the experiment was terminated (106 days). These pupae were allowed to finish development after minor and major workers were sampled for ovary activity. The males that emerged appeared normal and functional; dissections of two individuals revealed typical testes and accessory glands. Workers continued to produce males in these colonies for an additional 7 months.

In the five colony fragments collected in 2006, no pupae were produced in the 54 days of the experiment, though eggs and larvae were present in all colonies. The observation of eggs was difficult due to some tunneling by the ants under the plaster of their nests, and thus our observations should be considered conservative. We first observed eggs in colony EX-1 on 20-April, 2006. Eggs were observed latest in colony EX-4 on 27-April, 2006. Thus, a conservative estimate of the time required for workers to begin egg laying in the absence of the queen is 28 days, and had occurred in all five colonies by 35 days.

Worker reproduction and detection power in queen-right nests

From each of 6 field colonies a total of 160 males, 20–30 per colony, were successfully genotyped for two micro-satellite loci, as were queens from five colonies and at least 65 workers from all 6 colonies (Table 2). All males had a haplotype shared by the queen and therefore none could unmistakably be described as worker produced.

Neither males nor workers from any colony showed any evidence of dependence between the loci, indicating that they assort independently (for all tests: $\chi^2 < 3.47, P >$

0.17). Therefore, estimates of the power to detect worker reproduction were combined across loci.

At locus Pr2 20% of workers had both queen alleles, and only 11% at Pb5. Given these levels of allele sharing between workers and the queen, our power to detect a single worker produced male is greater than 0.95 for levels of worker reproduction greater than 5% for both loci independently. When the loci are considered together power is 0.95 for worker reproduction greater than 2%, but drops precipitously at successively lower levels of worker reproduction (Fig. 3). Since we did not detect any exclusively worker alleles in any males we can conclude with high confidence that males are not worker produced at high frequencies (>2%) in queen-right colonies.

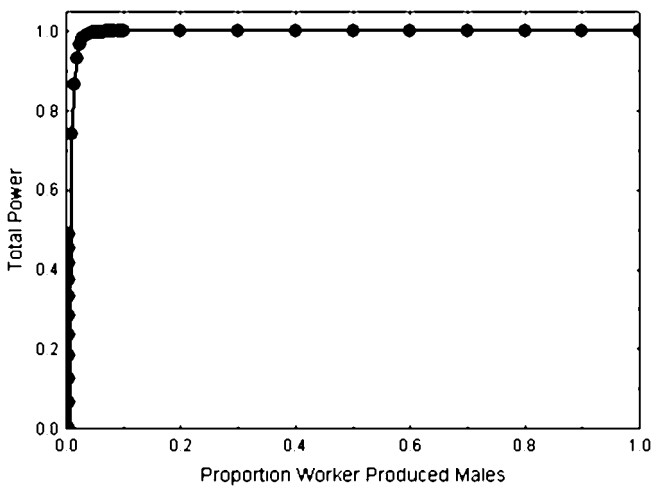


Figure 3. Total power to detect a single worker produced male for varying levels of worker reproduction within nests. Given our sampling effort, the power to detect worker reproduction is very high, >0.95 except when worker reproduction is very rare, i.e., fewer than 2% of males are worker produced.

Discussion

Worker reproductive potential

Our results demonstrate that *P. badius* workers are not functionally sterile, but capable of producing haploid, male, offspring. Furthermore, the degree of reproductive development of each worker depends on its size, with larger individuals having more ovarioles (Fig. 2). Other characteristics typical of reproductive females are also present in some major workers, including pigmented dots where ocelli occur in gynes (visible only in callow individuals) and forewing buds (pers. obs.). The correlation between reproductive potential (e.g. increase in number of ovarioles) and individual size supports a developmental link between size and reproductive development.

Reproduction and the evolution of worker castes

Oster and Wilson (1978) postulated that physical worker castes (= sub-castes) are more likely to evolve when workers are sterile. Their prediction is not supported by *P. badius*. Although Oster and Wilson (1978) do not explicitly state which species they use in their analysis of worker polymorphism and sterility, they do mention three genera that have at least one polymorphic species with sterile workers: *Eciton*, *Solenopsis* and *Pheidole*. Genera that contain polymorphic workers with functional ovaries are *Acromyrmex* (Dijkstra et al., 2005), *Atta* (Dijkstra and Boomsma, 2006), *Crematogaster* (Heinze et al., 1995), and *Camponotus* (Endler et al., 2004, 2006; Fielde, 1905). Thus it would appear that sterile polymorphic workers evolved at least 3 independent times whereas polymorphic workers with functional ovaries evolved 4 times (Brady et al., 2006; Moreau et al., 2006), suggesting that sterility is by no means the predominant factor favoring the evolution of worker polymorphism. In polymorphic species in which workers have retained reproductive potential, selection for polymorphism may have evolved along with selection on mechanisms to maintain queen control over reproduction (e.g., policing, chemical inhibition or worker self-restraint). Worker polymorphisms may be more likely to evolve in species where selection on trophic egg production and functional worker ovaries is strong, such that increasing worker size is coupled with increased reproductive potential.

Trophalaxis is not known to occur in any *Pogonomyrmex* species. Nutrient redistribution is thought to occur via trophic egg production (Gobin and Ito, 2000), which has been observed in *P. badius* (E.O. Wilson, pers. comm.), *P. barbatus*, *P. californicus* and *P. rugosus* (pers. obs.). To efficiently distribute nutrients through the colony it is likely that most individuals produce some trophic eggs. If only some individuals laid trophic eggs then nutrient stores, such as fat, would be imbalanced within the worker population in times of resource flux. This, however, is not seen; when colonies of *P. badius* were starved for two months the worker population adjusted their fat reserves equally, regardless of age or size (Smith, 2007).

Although all *Pogonomyrmex* workers are expected to have some degree of ovary development for trophic egg laying, only *P. badius* workers are known to actually produce males in the absence of the queen. The lack of positive evidence in other *Pogonomyrmex* may be due to a lack of study, though this seems unlikely due to the degree of attention this genus has received by the scientific community (Taber, 1998). In fact, workers of *P. barbatus* are reared specifically for life in a queen-less state ("ant farms"), and can be shipped nationwide (within the U.S.A.) specifically because they are functionally sterile. *P. badius* is the only N. American *Pogonomyrmex* with a major worker caste, raising the possibility that males are only produced by the major

workers, and that majors may serve a trophic egg-laying role in queen-right colonies. Majors of *P. badius* have been speculated to be seed-milling specialists, although there is little firm evidence to support this claim. In other ants, the major class of worker typically serves a defensive or foraging role (Oster and Wilson, 1978; Hölldobler and Wilson, 1990). There is no evidence that *P. badius* majors specialize in defense (pers. obs.) or foraging (Traniello and Beshers, 1991). If major workers are responsible for the majority of male production in orphaned colonies, a sibling rivalry between minor and major workers may arise because they may not share fathers (Rheindt et al., 2005). In this case, an allele that increases the probability of becoming a major may reap an increased fitness benefit through male production. This type of selfish behavior may, however, be kept under control by frequency-dependent selection where the proportion of majors is maintained at a low level to prevent anarchy ensuing from selfishness. The proportion of majors in *P. badius* nests is typically 5%, but can vary between ~0% to 25% (Tschinkel, 1999).

Reproductive females, gynes, were not included in our study, but Tschinkel (1987) reported that a single *P. badius* queen had ~35 ovarioles, about three times as many as the largest worker in our study. Furthermore, data from Tschinkel (1999) show that reproductively active queens have ~50–500 vitellogenic oocytes depending on colony size. In this study, minor and major workers had only 6 and 12 vitellogenic oocytes, respectively, even when the queen was absent. These data elucidate the stark difference between queens and workers in *P. badius*. Although major workers are intermediate between minors and queens in many respects, their reproductive capacity is much closer to a minor worker than a queen.

Worker reproduction and reproductive release

Though capable of reproducing, *P. badius* workers do not do so when a queen is present in the colony (Fig. 3). When the queen is removed the ovaries of the workers increase in activity (Fig. 1). Major workers may experience greater reproductive release than minor workers, although this difference was not statistically significant in this study (Fig. 1). Behavioral dominance is often associated with reproductive dominance in ants (Hölldobler and Wilson, 1990), and many studies have found that the chemical profile of reproductive workers is similar to that of queens (Heinze et al., 2002; D’Ettorre et al., 2004; Hartmann et al., 2005).

Due to multiple mating by the queen the workers in a *P. badius* nest are a mixture of full and half-sisters (Rheindt et al., 2004). We predicted that workers would not produce males in queenright nests as expected from kin selection theory. The prediction was supported, at least for levels of worker reproduction higher than 2%. We are unable to detect very low levels of worker reproduction, which have been de-

tected in honey bees (Ratnieks, 1993). Due to the relatedness structure of the nest, the average worker produced male will be more related to the queen than a random worker. Therefore, the queen benefits the most from worker reproduction within the colony even when she is dead.

The underlying cause of the increased reproductive potential, “reproductive release,” of workers is unknown, but may be due to one or a combination of the following factors: a lack of queen repression (Fletcher and Ross, 1985), the release of worker self restraint (Cole, 1986; Dijkstra et al., 2005), or a decrease in worker policing (Miller and Ratnieks, 2001). Under queen repression the queen emits some cue, behavioral or chemical, that actively inhibits ovarian development in the workers (Jay, 1968, 1970; Fletcher and Ross, 1985). When the queen is removed so is the inhibitory cue and workers initiate reproduction. On the other hand, under worker self restraint the queen advertises her presence and the workers do not reproduce in the interest of colony-level efficiency, but are not necessarily directly inhibited (Cole, 1986; Endler et al., 2004, 2006); worker self restraint may evolve when worker policing is very efficient and worker laid eggs have very little chance of survival (Wenseleers et al., 2004; Wenseleers and Ratnieks, 2006b). When the queen is gone, however, there is no loss in colony efficiency by a worker reproducing, and she can give up self-restraint. An increase in worker reproductive activity in the absence of the queen can cause a decrease in policing efficiency (Miller and Ratnieks, 2001) leading to high levels of worker reproduction.

Workers in our study began producing males within 28 days of queen removal. Studies on honey bees show a similarly rapid initiation of reproduction after queen removal, from an average of 24 days (Page and Erickson, 1988) to an average of 29 days (Miller and Ratnieks, 2001). In a ponerine ant, *Pachycondyla inversa*, workers begin egg-laying in as little as three weeks (D’Ettorre et al., 2004). On the other hand, *Camponotus floridanus* carpenter ants require up to 60 days to initiate worker reproduction (Endler et al. 2004), and *Atta* leafcutter ants, though physically capable of reproducing, may or may not initiate reproduction even after living in a queen-less state for 6 months (Dijkstra et al., 2006). A rapid release of reproduction in workers may enable them to gain fitness for the otherwise doomed colony, especially if queen death is more likely to precede seasonal reproduction and mating. In *P. badius*, mating is a highly synchronized event in early summer (Smith and Tschinkel, 2006), but is preceded by the most dangerous season for queens. In spring, the queen is often at the top of the nest, and thus vulnerable to predation or environmental factors; in all other seasons the queen tends to be in the deepest of chambers (Tschinkel, 1999). Furthermore, many *P. badius* colonies move nests during the spring (Carlson and Gentry, 1973; Harrison and Gentry, 1981), the only occasion when the

queen is outside of the nest other than during her own nuptial flight.

In the event that a colony is orphaned in spring, but diploid eggs from the queen are still present within the nest, workers may salvage indirect fitness by rearing the diploid brood as gynes while simultaneously producing males themselves. Interestingly, this has not been observed in three colonies whose queens died while diploid eggs and larvae were still present in the nest (pers. obs.). In one colony the queen was killed during colony excavation in early spring and only two larvae were present in the nest. These larvae pupated as gynes in the laboratory, but never eclosed and were presumably killed by the workers. Upon queen loss in two laboratory colonies that had many stages of queen produced diploid brood present, no individuals eclosed and were presumably killed by the workers. These observations, along with the results presented in this study, suggest that orphaned workers forgo indirect fitness in favor of direct reproduction. Whether this is an optimal strategy for the workers depends on the fitness returns of individual males and gynes.

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