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David C. Queller · John M. Peters · Carlos R. Solís
Joan E. Strassmann

Control of reproduction in social insect colonies: individual and collective relatedness preferences in the paper wasp, *Polistes annularis*

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Abstract Social insect colonies often have one or a few queens. How these queens maintain their reproductive monopoly, when other colony members could gain by sharing in the reproduction, is not generally known. DNA microsatellite genotyping is used to determine reproductive interests of various classes of colony members in the paper wasp, *Polistes annularis*. The relatedness estimates show that the best outcome for most individuals is to be the reproductive egg-layer. For workers, this depends on the sex of offspring: they should prefer to lay their own male eggs, but are indifferent if the queen lays the female eggs. The next-best choice is usually to support the current queen. As a rule, subordinates and workers should prefer the current queen to reproduce over other candidates (though subordinates have no strong preference for the queen over other subordinates, and workers may prefer other workers as a source of male eggs). This result supports the theory that reproductive monopoly stems from the collective preferences of non-reproductives, who suppress each other in favor of the queen. However, we reject the general hypothesis of collective worker control in this species because its predictions about who should succeed after the death of the present queen are not upheld. The first successor is a subordinate foundress even though workers should generally prefer a worker successor. If all foundresses have died, an older worker succeeds as queen, in spite of a collective worker preference for a young worker. The results support the previous suggestion that age serves as a conventional cue serving to reduce conflict over queen succession.

Key words *Polistes* · Policing · Social control · Relatedness · Kin selection

Introduction

Coloniality and relatedness

Social insects live in colonies, with most individuals serving as non-reproductive helpers. Kin selection makes it relatively easy to explain why individuals might choose to be non-reproductive helpers in colonies rather than nesting alone. There are a number of plausible explanations which posit both relatedness of helpers to reproductives and some ecologically based efficiency of group living (e.g., Hamilton 1964a,b, 1972; West Eberhard 1975; Andersson 1984; Alexander et al. 1991; Strassmann and Queller 1989; Queller 1989, 1994a, 1996). However, this does not in itself solve the problem of insect sociality, because another problem remains. Given that colonial living is advantageous, how is it decided who gets to reproduce in the colony? At its most basic, the question can be represented in terms of a single egg. It is time for the colony's next egg to be laid, and only one individual will lay it. Each colony member might well ask "Why not me?" The relative ecological efficiencies of the choices cannot play the same role here as they do in the question of grouping or nesting alone. This seems to be a zero sum game; there is one unit of fitness at stake and the only question is who gets it. It is clear that the answer, whatever it is, has to account for one fundamental fact: reproduction is highly biased. In many species only one queen per colony reproduces. Even in polygynous species the number of reproductives is generally small relative to the number of helpers. How this degree of reproductive bias is maintained in the face of the temptation of individual reproduction is one of the central problems of insect sociobiology.

One possibility, at least in haplodiploids, is that relatedness provides the crucial difference. If the queen is

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D.C. Queller (✉) · J.M. Peters[1] · C.R. Solís · J.E. Strassmann
Department of Ecology and Evolutionary Biology,
Rice University, 6100 Main St.,
Houston, TX 77005-1892, USA
Fax: 713-285-5232; e-mail: queller@rice.edu

Current address:
[1] Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

the mother of the workers, and she has only mated once, she can produce females 3/4 related to the workers, better than the 1/2 that each worker could get by producing her own offspring. Though it may work in special circumstances, the same problems arise as in the attempt of Hamilton (1964a,b 1972) to explain grouping via the 3/4 relatedness, without any help from ecological efficiencies. If the queen also produces 50% males, then the average relatedness of workers to siblings is only 1/2, and advantages due to biased sex ratios are transitory (Crozier and Pamilo 1996). To make matters worse, relatedness is often lower than 1/2 because of multiple mating, multiple queens, and queen replacements (reviewed in Crozier and Pamilo 1996). Colony foundresses in particular are usually of the same generation, so they cannot gain the 3/4 advantage (Hamilton 1972), yet reproduction may still be dominated by one individual.

Another possible explanation is physical dominance (Pardi 1948; West Eberhard 1981; Reeve 1991). If there is no agreement on who should lay the egg, perhaps reproductive rights will go to the individual most able to win an escalated fight. Considerable theory has been devoted to how reproduction would be allocated in this case (Vehrencamp 1983a,b; Reeve 1991; Reeve and Ratnieks 1993). Reproduction may not be entirely monopolized by a dominant individual because conceding some eggs to the subordinates might pay if this would prevent them from contesting the dominant's status or from leaving to nest on their own. This explanation is most appealing for very small colonies, but as the number of individuals increases, dominance becomes harder to maintain. It seems unlikely that a queen could hold hundreds or thousands of other individuals in check against their interests, either through physical force or pheromonal manipulation (Keller and Nonacs 1993).

A third category of explanation hinges on the costs of conflict. Costly conflicts can select for peaceful settlements, even if the outcome is not the ideal one for one or both parties. Selection can lead to settlement based on conventional cues, such as age, even if they have no predictive value for the outcome of a fight (Maynard Smith 1974; Maynard Smith and Parker 1976). Of course, for most organisms such conventional settlements are only possible if the conventional loser reproduces more than a fighter who flouts the convention, either by getting a smaller slice of a larger pie, or by living to play the game again. Losers in social insects often forego all reproduction, so this condition cannot apply. However, they can still be compensated in the form of indirect inclusive fitness benefits. Pollock (1996) has recently shown that indirect benefits can be enough to select for conventional settlements (see also Ratnieks and Reeve 1992). The conventional loser does not need to have greater inclusive fitness than the conventional winner in its colony; it needs greater inclusive fitness than individuals who refuse to settle conventionally and suffer costs due to fighting or due to wasted effort.

A final candidate explanation for who reproduces is collective control. Workers are the most numerous class and might therefore be in a position to exercise collective control. There is now considerable evidence that workers can control sex ratios against queen interests (Trivers and Hare 1976; Nonacs 1986; Boomsma and Grafen 1990; Mueller 1991; Queller et al. 1993a; Sundström 1994), so it seems plausible that they could control who reproduces, provided they have a collective interest. Even if each worker's first choice is to reproduce, workers may have a collective interest in favor of the queen when they are more related to the queen's progeny than to each other's progeny (Starr 1984; Woyciechowski and Lomnicki 1987, Ratnieks 1988). Selection may then lead workers to suppress each other, a strategy called worker policing. Most work on policing has focused on the unfertilized eggs destined to be haploid males. For example, honeybee workers are more related to sons of the queen than to sons of other workers, and they eat the latter (Ratnieks and Visscher 1989). However, worker control of reproduction could also be involved in other contexts, including queen elimination (Forsyth 1980; Bourke 1994), queen addition (Nonacs 1988; Pamilo 1991b), and queen succession (Hughes et al. 1987).

Distinguishing among these hypotheses is often difficult. In this study we focus on a paper wasp, *Polistes annularis*, for which extensive previous studies (Strassmann 1979, 1981, 1983, 1989a, b, 1991; Sullivan and Strassmann 1984; Hughes et al. 1987; Queller and Strassmann 1988; Peters et al. 1995) lay some groundwork for distinguishing among these hypotheses. Colonies are begun in the spring by groups of females called foundresses (mean = 4.5 at our study site near Austin, Texas). The foundresses on a given nest usually emerged from the same nest the previous autumn (Strassmann 1979) and as a consequence they are close relatives ($r = 0.62$; Peters et al. 1995). Many foundresses may lay eggs, but aggressive interactions among them gradually lead to the emergence of a dominant egg-layer, or queen (Strassmann 1981; Peters et al. 1995). The first several broods of offspring consist mostly of females called workers, but also may include a small number of males. The workers normally act as non-reproductive helpers, but they are not a morphologically distinct caste. If all the foundresses die before the end of the season, as often happens, a worker will mate and become the dominant egg layer (Queller and Strassmann 1988; Strassmann 1989b). The brood reared in the late summer or fall become males or the next year's foundresses.

The queen may die at any point before the end of the season. A major part of this paper focuses on the issue of queen succession. The succession rules are already known. When a queen dies, her successor is a subordinate foundress or, if all foundresses have died, an older worker becomes the new queen (O'Rourke 1982; Hughes et al. 1987; J. E. Strassmann unpublished work). Similar patterns hold for other Texas *Polistes* (Strassmann and Meyer 1983; Hughes and Strassmann 1988). The fact that older workers succeed in preference to younger workers is particularly interesting. The first workers to emerge in *Polistes* are commonly the smallest workers (Reeve 1991), and might therefore be expected to have poorer fighting ability (large size is often an important determinant of who becomes dominant among foundresses; reviewed by Reeve 1991). The fact that older workers succeed to queenship before younger ones may often mean that smaller workers succeed instead of larger competitors. This has not been directly demonstrated in *P. annularis*, but has been shown for *P. instabilis* (Hughes and Strassmann 1988). This suggests that age is a conventional cue in some species of *Polistes*, used to peacefully settle high-cost conflicts (Hughes and Strassmann 1988). However, another possibility has yet to be ruled out: collective worker control. It is possible that succession rules reflect average worker interests determined by average worker relatednesses to the progeny of potential successors.

In this study, we used microsatellite markers (Queller et al. 1993b; Schlötterer and Pemberton 1994) to estimate relatednesses relevant to the reproductive interests of various parties. Microsatellites are tandem repeats of short DNA motifs (1–6 bases). Loci are often highly variable, a desirable trait for estimating relatedness. Alleles usually differ in the number of tandem repeats and are easily resolved on polyacrylamide gels.

A special advantage is that microsatellites are normally amplified by the polymerase chain reaction (PCR; Saiki et al. 1988) so that very small initial samples of DNA are sufficient for genotyping. This means the sperm stored in the spermatheca of a mated female can be genotyped (Queller 1993; Evans 1993). We have previously used this method to show that most *P. annularis* foundresses are singly mated (2 out of 40 foundresses mated twice) and to perform reliable maternity assignments of progeny (Peters et al. 1995). Here we exploit the ability to genotype stored sperm in a different way. The genotypes of a female and her stored sperm together determine the alleles present in their daughters. This allows us to estimate relatedness to these daughters, even if they have not yet been produced.

We will test for three kinds of relatedness preferences in *P. annularis*. The first question concerns whether individuals would prefer to lay their own eggs, other things being equal. A total of eight preferences are relevant here. For the queen, the alternatives to direct reproduction are egg laying by a random subordinate or by a random worker. For subordinates, the alternatives are the queen, a random non-self subordinate, and a random worker. For workers, the choices are the queen, a random subordinate, and a random non-self worker.

When many individuals might prefer to be queen, then collective preferences may come into play to make choices among them. The second class of preferences we will test is collective support for the reigning queen. Four distinct choices are tested. Workers may prefer the queen over either subordinates or other workers. Likewise subordinates may prefer the queen over workers or other subordinates.

Finally, we will test for collective preferences concerning queen succession should the queen die. There are three relevant preferences here. First, is there a collective worker preference between a subordinate or worker successor? Second, is there a collective subordinate preference for the same issue? Third, if all subordinates have died, is there a collective worker preference for either a young or an old worker successor?

Models of worker preference for queen succession

The main goal of this paper is to empirically document expected preferences for queenship by measuring the appropriate relatednesses. However it is also worthwhile attempting to understand how such preferences are generated. The factors affecting some of the choices to be studied are fairly obvious. For example, an individual should prefer its own offspring unless it can substitute full sisters, the only category of kin with higher relatedness. Similarly, if the current queen is the most fecund of the foundresses, then workers should prefer her to the subordinates, because she is the most likely to supply them with full sisters (Forsyth 1980). But some of the expected preferences are less obvious, particularly the two concerning worker preferences for potential queen successors. They are not obvious a priori because the relatednesses depend, sometimes in complex ways, on the number of foundresses, how they have divided reproduction in the past, and the number of times they have mated. The goal of this section is to illustrate some of these dependencies for a simple two-foundress model system.

For the reader who wishes to proceed to the empirical findings, we will state the major conclusions of these models at the outset. Worker preference for subordinate succession is generally enhanced by high relatedness among foundresses and by multiple mating of foundresses. It is also enhanced if the subordinate is the mother of some of the workers, particularly if she produces female offspring, because daughter production by a mother will also transmit the father's genes. The preference among age classes of workers depends on how foundress contributions to the worker pool change. If the most reproductively dominant foundress becomes more dominant with time, then workers will be more related to younger workers. If the reproductive dominance decreases, workers will tend to favor older workers.

Consider a nest founded by two foundresses related by r_f which can take values from zero to 3/4. The dominant foundress lays a fraction $1-p$ of the eggs, leaving a fraction $p < 0.5$ for the subordinate foundress. These are their relative contributions to the worker pool. The daughters of each foundress may be full sisters or half sisters depending on whether they have the same father. Let x be the fraction of maternal sisters that are full sisters; $x = 1$ therefore corresponds to single mating and $x = 0$ to extreme multiple mating.

Table 1 Relatednesses of a focal worker to various progeny or potential queen successors. These are life-for-life relatednesses (Hamilton 1972) which already include a 2-fold reproductive value correction for the sexes. The appropriate reproductive value correction can differ from two if one sex goes through more generations than the other, such as when workers lay only male eggs (Taylor 1981). Here it might change slightly if worker queens are common and primarily lay male eggs, but this effect is ignored in the model

Successor	Sex of progeny	Abbreviation	Worker relatedness to progeny
Foundress (mother of focal worker)	Female	r_1	$(2x+1)/4$
	Male	r_2	$1/4$
Foundress (not mother of focal worker)	Both	r_3	$r_f/4$
Worker (from focal-worker matriline)	Both	r_4	$(2x+1)/8$
Worker (not from focal-worker matriline)	Both	r_5	$r_f/8$

Table 1 shows the relatednesses needed for the model. Consider first the choice between a subordinate foundress and a worker, assuming that the successor will produce only female progeny. Average worker relatedness to the progeny of another worker is:

$$r_{\text{worker progeny}} = \left[p^2 + (1-p)^2\right]\left(\frac{2x+1}{8}\right) + 2p(1-p)\frac{r_f}{8} \tag{1}$$

The two terms are for workers from the same matriline and workers from different matrilines, and each includes the frequency of that relationship times the relatedness. For the average relatedness of workers to the progeny of the subordinate foundress, we need to add a parameter, s, for the fraction of the subordinate's sexual progeny that would be female:

$$r_{\text{subordinate progeny}} = p\left(\frac{2sx+1}{4}\right) + (1-p)\left(\frac{r_f}{4}\right) \tag{2}$$

(The first parenthetical term represents a worker's average relatedness to brothers (r = 1/4), half sisters (r = 1/4), and full sisters (r = 3/4)).

Workers should therefore prefer succession by a subordinate rather than a worker when $r_{\text{worker progeny}} - r_{\text{subordinate progeny}} > 0$ or:

$$x(4p^2 - 4p - 4ps + 2) + r_f(4p - 2p^2 - 2) + 2p^2 - 4p + 1 > 0 \ . \tag{3}$$

Partial derivatives of the function on the left hand side of Eq. 3 (call it F) give some sense of how the various factors influence this choice:

$$\frac{\partial F}{\partial s} = -4px$$

$$\frac{\partial F}{\partial r_f} = -2(p-1)^2$$

$$\frac{\partial F}{\partial p} = 4(2xp + p + r_f - 1 - x - pr_f - sx)$$

$$\frac{\partial F}{\partial x} = 2(2p^2 - 2p - 2ps + 1)$$

Thus, the effect of increasing the fraction of females in the progeny (s) is always to decrease the function, and increase the payoff to having a subordinate queen. This is because of the high payoff of rearing full sisters (which is absent only if $p = 0$ or $x = 0$). Increasing the relatedness of foundresses, r_f, has the same effect (since p, the contribution of the subordinate, must be less than 0.5). The derivative with respect to p, the subordinate's share of reproduction, is more complex but it too is always negative in the ranges that the parameters can actually assume ($0 \le p \le 0.5$, $0 \le r_f \le 0.75$, $0 \le x \le 1$, $0 \le s \le 1$). The multiple mating parameter, x, has a more complex effect. Its derivative can be either positive or negative, depending on the values of p and s.

Figure 1 plots some sample values where the payoff for choosing a subordinate and a worker are exactly equal, obtained by setting Eq. 3 equal to zero and solving for x. For each line, which represents a fixed value of r_f and one sex of progeny, the region above and to the left is where worker succession is favored, and the region below and to the right is where subordinate succession is favored. It is clear that there are reasonable values of the parameters for which either outcome is

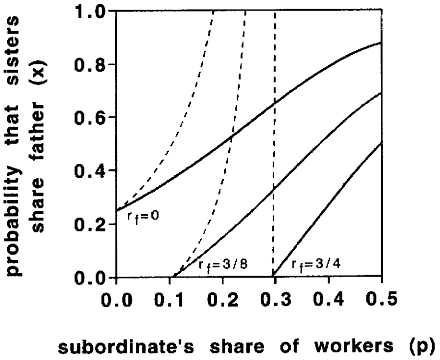

Fig. 1 Theoretical worker preferences in a colony with two foundresses related by three values of r_f. When the dominant foundress dies, workers can choose either the subordinate foundress or a random worker as successor. The preference depends on average worker relatedness to the successor's offspring which in turn depends on the fraction of workers who are daughters of the subordinate foundress and the probability that sisters who share a mother also share the same father. The *lines* show where workers have no preference for either category of successor. *Dashed lines* are for when the successor produces only females, *solid lines* for when she produces only males. Worker succession is preferred *above* and to the *left* of the line and subordinate succession preferred *below* and to the *right*

possible, so empirical work is necessary to determine worker interests.

A similar model can be constructed for the choice between an older and a younger worker. Assume again there are two foundresses related by r_f. Here there is no need to distinguish the sex of the successor's progeny because relatednesses are the same for males and females (last two lines of Table 1). Let p continue to designate the subordinate foundress's share of reproduction, but we must now distinguish time periods. For maximum simplicity, imagine there are two time periods with equal numbers of workers produced in each. The subordinate lays p_1 of the eggs in time period 1. These eggs will become the older category of workers. She lays p_2 of the eggs in time period 2. These will become the younger workers.

Table 2 shows the frequencies of the various combinations of worker-to-worker relatedness, distinguished by time period and mother. Entries marked with an asterisk are within-matriline categories, for which relatedness is $(2x + 1)/8$. The others are between-matriline categories, with relatedness $r_f/8$ (see Table 1 for relatednesses). Average relatedness of all workers to older workers is the sum of the entries (multiplied by the appropriate relatedness) in the left half of Table 2, while relatedness of all workers to younger workers is summed using the right half. The difference,

$r_{\text{age 1 progeny}} - r_{\text{age 2 progeny}}$,

can be shown to be:

$$r_{\text{age 1 progeny}} - r_{\text{age 2 progeny}} = \left(\frac{2x+1}{8} - \frac{r_f}{8}\right) \times [p_2(1-p_2) - p_1(1-p_1)]$$

The relatedness term (first parentheses) is always positive since $r_f < 1$, so which age is favored by workers for succession depends on the sign of the remaining part (second parentheses). Since $p(1-p)$ is maximized when $p = 1-p = 1/2$, old workers are favored (expression positive) when they are less evenly apportioned between the two foundresses than young workers, and young workers are favored in the reverse condition. So old workers are favored when the foundress who is initially reproductively dominant gains a smaller share of workers in the second period. Young workers are favored when the initially dominant foundress gains a larger share in the second period. Such differences might come about either through changes in dominance or through death of one of the two foundresses.

Methods

Colonies of *P. annularis* were collected in May 1992 from a cliff-face overlooking Lake Travis near Austin, Texas, a site used extensively in previous studies of this species (Strassmann 1979, 1981, 1983, 1989a, b, 1991; Sullivan and Strassmann 1984; Hughes et al. 1987; Queller and Strassmann 1988; Peters et al. 1995). At this time colonies were in the late pre-emergence stage: there were eggs, larvae, and pupae in the nests, but no adults had yet eclosed. The adults collected were therefore inseminated foundresses, the surviving subset of the original foundresses. Nine colonies were selected for detailed genetic analysis. Selection was haphazard, except that colonies with only one foundress were excluded because there would be no subordinate foundress candidate for succession (single-foundress colonies are a small minority in this population; Strassmann 1989a).

We scored genotypes at seven microsatellite loci for immature brood destined to be workers, for foundresses, and for the sperm stored in each foundress's spermatheca (Fig. 2). The seven trinucleotide repeat loci scored, their allele frequencies (the loci had 4–13 alleles), and PCR methods are given elsewhere (Peters et al. 1995; Strassmann et al. 1996). Detailed protocols are available on request. We have previously reported the use of these data to determine maternity of the foundresses (Peters et al. 1995). Here we use the same genotypes to assess reproductive interests of various parties. The pupae, larvae, and eggs (embryos) would have become workers once they emerged as adults, so we can use their genotypes to estimate worker relatedness relevant to queen succession preferences. For this purpose, the three brood stages are treated as three age cohorts of

Table 2 Frequencies of kinds of worker pairs, distinguished by two ages and two mothers. These frequencies are required for obtaining average worker relatedness to old (*time 1*) workers and to young (*time 2*) workers. The two worker ages are assumed to be equally common, so the average worker relatedness is the average for old and young workers; hence the 1/2 prefix for each entry. Entries marked with *asterisks* are for pairs from the same matriline

		Time 1 workers of:		Time 2 workers of:	
		Foundress A (p_1)	Foundress B (1-p_1)	Foundress A (p_2)	Foundress B (1-p_2)
Time 1	Foundress A (p_1)	$\frac{1}{2}p_1^{2*}$	$\frac{1}{2}p_1(1-p_1)$	$\frac{1}{2}p_1p_2{}^*$	$\frac{1}{2}p_1(1-p_2)$
	Foundress B (1-p_1)	$\frac{1}{2}p_1(1-p_1)$	$\frac{1}{2}(1-p_1)^{2*}$	$\frac{1}{2}(1-p_1)p_2$	$\frac{1}{2}(1-p_1)(1-p_2)^*$
Time 2	Foundress A (p_2)	$\frac{1}{2}p_1p_2^*$	$\frac{1}{2}(1-p_1)p_2$	$\frac{1}{2}p_2^{2*}$	$\frac{1}{2}p_2(1-p_2)$
	Foundress B (1-p_2)	$\frac{1}{2}p_1(1-p_2)$	$\frac{1}{2}(1-p_1)(1-p_2)^*$	$\frac{1}{2}p_2(1-p_2)$	$\frac{1}{2}(1-p_2)^{2*}$

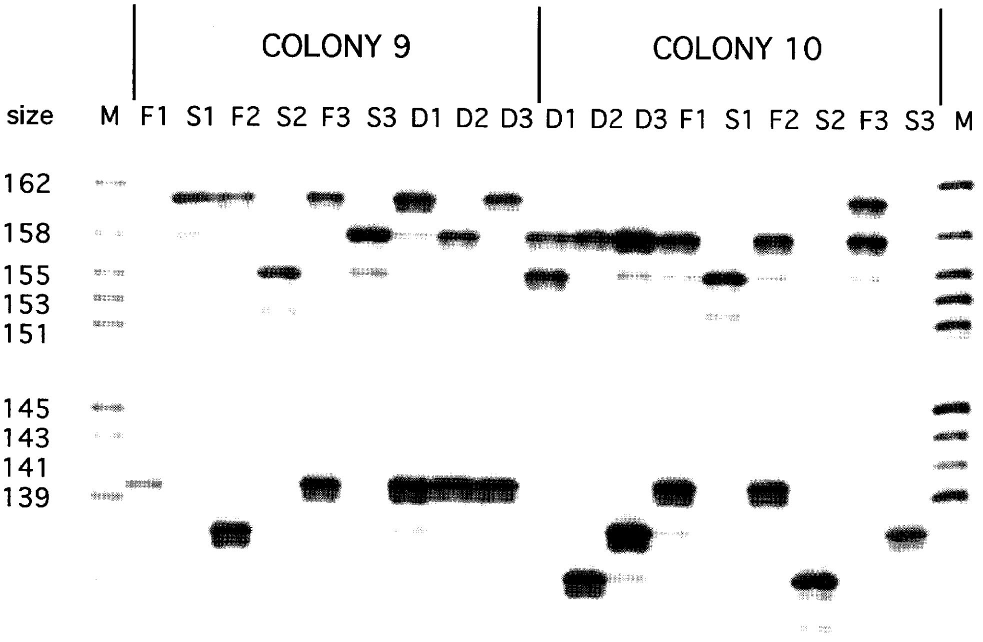

Fig. 2 Microsatellite loci. Microsatellites are tandem repeats of simple DNA sequence motifs (for our loci, trinucleotide motifs). Alleles vary in the number of repeat units. Shown are samples from two colonies for locus PBE492AAT. The outside two lanes (*M*) are size markers, the *C* lane of an M13 sequencing reaction. Foundress genotypes (*F*) are shown adjacent to the genotypes of their stored sperm (*S*). Sperm samples for these six foundresses, and for all but 2 of 40 in the study, showed a single allele at every locus, indicating single mating (Peters et al. 1995). Each daughter (*D*) shown is consistent with only one of the foundress-sperm combinations. The seven loci scored, their allele frequencies, and PCR methods are given elsewhere (Peters et al. 1995). For detailed protocols, see Strassmann et al. (1996)

workers. Pupae, larvae, and eggs represent older, middle-aged, and younger workers, respectively.

Although the worker preference model above depends on maternity fractions, and maternity has been assigned for these offspring (Peters et al. 1995), we do not use those assignments directly in this study. This is because a substantial fraction of the brood were not the daughters of any living foundress on the nest, so that not all of the required links in the pedigree can be determined. This problem is circumvented by calculating statistical estimates of relatedness (Queller and Goodnight 1989; Pamilo 1990), for which it is not necessary to know links in the pedigree. For example, we can estimate relatedness of younger workers to older workers, without knowing all of their mothers.

Relatednesses were estimated from the microsatellite data using the Macintosh computer program Relatedness 4.2b by K. F. Goodnight and D. C. Queller (available on the World-Wide-Web at http://www-bioc.Rice.edu/~kfg/GSoft.html). Colonies were weighted equally. Standard errors were estimated by jackknifing over colonies. To obtain average worker relatedness to any individual or group it is necessary to combine the separate relatednesses of the three worker age categories (pupae, larvae, eggs). We did this by weighting within colonies by the total number of individuals collected (not just those genotyped) in each age category of that colony. For example, average worker relatedness to subordinates is $(n_{\mathrm{egg}}\, r_{\mathrm{egg\text{-}subor}} + n_{\mathrm{larva}}\, r_{\mathrm{larva\text{-}subor}} + n_{\mathrm{pupa}}\, r_{\mathrm{pupa\text{-}subor}})/(n_{\mathrm{egg}} + n_{\mathrm{larva}} + n_{\mathrm{pupa}})$. This procedure somewhat overestimates the effect of older workers since many of them will have died by the time the youngest become adults, so we sometimes apply alternative weightings to explore the effects of different mortality levels.

Reproductive preferences depend on a difference between two relatednesses. For example, to assess worker preferences with respect to queen succession, we need the difference between worker relatedness to the progeny of subordinate foundresses and worker relatedness to the progeny of other workers. Since workers make their choice between colony-mates, our interest is in the relatedness difference within colonies. This is best determined, not by estimating the two relatednesses with separate jackknifed standard errors, but by first calculating the relatedness difference within each colony and obtaining a single jackknifed standard error for the mean difference across colonies (Queller 1994b). This is conceptually similar to switching from an unpaired *t*-test to a paired *t*-test. The advantage is that statistical power is often increased by eliminating the between-colony variance in relatedness, which is irrelevant to questions of preference between colony-mates. Unless otherwise noted, all statistical tests are two-tailed, even though there is sometimes a unidirectional hypothesis (e.g., individuals should prefer self, then the current queen, then the known successors). Using two-tailed tests seems unnecessarily conservative, but we do so in order to be able to detect any significant preferences in the opposite direction.

For determining who is the preferred reproductive, the relatednesses of interest are the relatednesses to the progeny of potential reproductives. In general, the required progeny do not exist (though some foundresses have some progeny in the sample). This poses no problem for male progeny. Males are just a random half of their mother's genotype, so relatedness to a male would be half of relatedness to its mother [we use life-for-life coefficients in this paper, which for female-to-male relationships are half the regression value; see Hamilton (1972)]. The same rule often works for an individual's female progeny, since they consist of half their mother's genotype mixed with the genotype of a random male (this species has no detectable inbreeding; Peters et al. 1995). The only problem arises when the candidate reproductive could be the mother of the individuals concerned. This occurs for worker relatedness to the female progeny of a foundress. In this instance, the foundress might present the worker with full sisters, transmitting the worker's paternal genes in addition to the maternal genes. Here is where the genotype of the foundress's stored sperm is essential. Worker relatedness to a foundress's future daughters is calculated as the average of their relatedness to the foundress and to her stored sperm.

Two methods were used to distinguish the dominant foundress on each nest from her subordinates (Peters et al. 1995). First, dissections of all foundresses identified the female with the most mature eggs in her ovaries on seven of the nine colonies. In the other two colonies, no foundress had mature eggs, suggesting that the dominant queen might have died just prior to collection. We also used the microsatellite-based maternity assignments of the youngest brood category (eggs). The seven foundresses identified as dominant by dissection were confirmed in this way; each had the largest share of the eggs in her nest. In the two other nests, most of the eggs had been produced by a female not collected, confirming the suspected recent death or disappearance of the dominant female. For this reason, we treated all foundresses collected on these two nests as subordinates.

The brood were all treated as diploid females. Two individuals appeared homozygous at all loci scored and therefore could have been hemizygous males, but as these represent less than 1% of the genotyped brood, they have little impact on the overall results. We report relatednesses from nine colonies. Some foundresses were genotyped from four additional colonies and these data were included to improve the estimates of population allele frequencies that are required to estimate relatedness.

Results

Relatedness

Table 3 shows the average relatednesses to potential queen successors, and also to the mates of the subordinate foundresses. Foundresses in the same colony are generally highly related, but are unrelated to their mates. The brood (future workers) are highly related to the queen (often their mother) and to the queen's mate (often their father), and are less related to the subordinates and their mates. Among the brood, relatedness tends to increase with time; that is, younger brood (eggs) were more highly related than older brood (larvae and pupae). This pattern arises because of increasing reproductive dominance by one foundress (Strassmann 1981; Peters et al. 1995).

Should individuals prefer to be reproductives?

Figure 3 shows the relatedness gains each kind of individual would accrue by producing its own offspring ($r = 1/2$) in place of some other individual's offspring (at a 1:1 sex ratio). As expected, most individuals would clearly do better by reproducing directly. The closest thing to an exception is the worker choice of reproducing instead of the queen, but even here the relatedness

Table 3 Relatednesses (± SE) of *Polistes annularis* colony-mates. Relatednesses are shown for column classes to row classes. Column classes are groups that may decide who will be queen. The relatednesses differ for different categories of brood primarily because more foundresses contributed to old brood than to young brood (Strassmann 1981; Peters et al. 1995). Sample sizes listed are for individuals genotyped and for total individuals collected. Other relatednesses of interest are: foundresses, including queens ($r = 0.62$, SE = 0.08, $n = 40$); foundresses to their mates ($r = -0.02$, SE = 0.04).

	Pupae (old workers) $n = 86, 128$	Larvae (middle workers) $n = 87, 171$	Eggs (young workers) $n = 55, 98$	Subordinate foundresses $n = 33, 33$
Pupae (old workers)	0.42 ± 0.05	0.40 ± 0.08	0.41 ± 0.09	0.30 ± 0.09
Larvae (middle workers)	0.40 ± 0.08	0.55 ± 0.08	0.57 ± 0.07	0.37 ± 0.06
Eggs (young workers)	0.41 ± 0.09	0.57 ± 0.07	0.68 ± 0.04	0.32 ± 0.07
Subordinate foundresses	0.30 ± 0.09	0.37 ± 0.06	0.32 ± 0.07	0.53 ± 0.11
Mates of subordinates	0.11 ± 0.07	0.14 ± 0.08	0.05 ± 0.08	−0.01 ± 0.05
Queens $n = 7,7$	0.39 ± 0.09	0.48 ± 0.06	0.46 ± 0.05	0.69 ± 0.07
Mates of queens $n = 7,7$	0.33 ± 0.24	0.66 ± 0.15	0.90 ± 0.04	0.04 ± 0.22

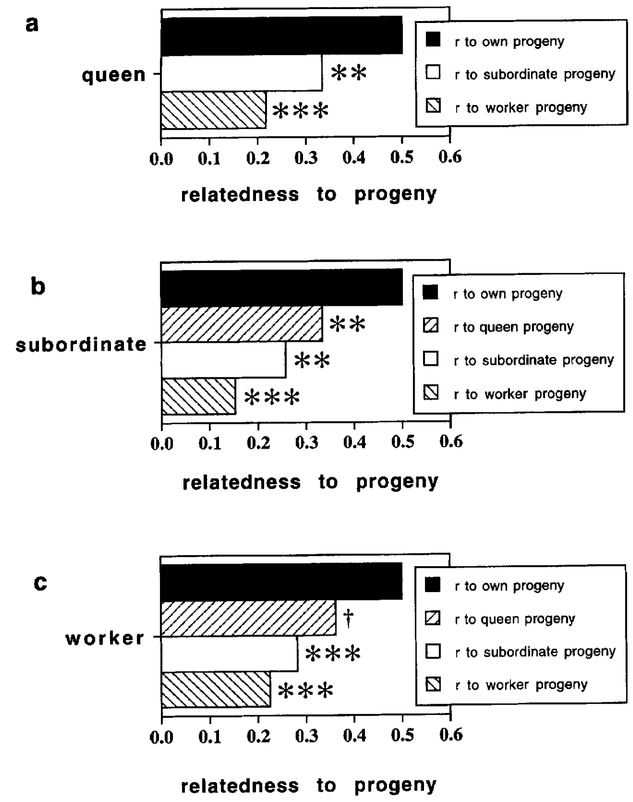

Fig. 3 Relatedness gains of direct reproduction for **a** queens, **b** subordinate foundresses, and **c** workers. Each is considered to have the choice of reproducing directly or allowing some other class to reproduce. In each case, the *solid bar* is for relatedness to one's own progeny ($r = 0.5$). The other bars show the relatedness to the progeny of other classes of potential reproductive (always excluding one's own progeny; e.g., relatedness of subordinates to subordinate progeny means relatedness to the progeny of other subordinates). A 1:1 progeny sex ratio is assumed, so relatednesses reflect an average of male and female progeny. *Asterisks* denote significant differences from $r = 0.5$: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, † indicates a nearly significant difference, $P = 0.07$. Precise relatedness estimates in top-to-bottom order are **a** 0.5, 0.33, 0.21; **b** 0.5, 0.33, 0.26, 0.15; **c** 0.5, 0.36, 0.28, 0.22

difference is 0.14 in favor of direct reproduction and it is marginally significant (two-tailed $P = 0.07$, which is significant in a one-tailed test). The advantage here is smaller because the dominant queen would produce full sisters for many workers, which are better than daughters. If we analyze the two sexes of progeny separately, workers have a very strong preference for their own male progeny over the queen's (r difference = 0.283, $P < 0.001$), but workers are indifferent with respect to the female progeny (r difference = −0.009, not significant).

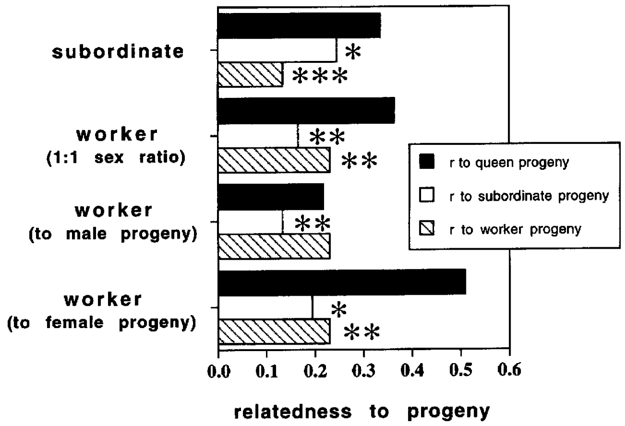

Fig. 4 Relatedness advantage of supporting the current queen. Subordinate foundresses and workers are each considered to have the choice of raising the progeny of the current queen or the progeny of an alternative queen (either another subordinate or another worker). Since worker relatednesses depend on the sex ratio of the progeny that the reproductive will produce, separate results are shown for a 1:1 sex ratio, all males, and all females. *Asterisks* denote significant differences from relatedness to the queen's progeny (two-tailed test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Precise relatedness estimates for the 12 bars, *top to bottom*: 0.33, 0.24, 0.13; 0.36, 0.16, 0.23; 0.22, 0.13, 0.23; 0.51, 0.19, 0.23

Is there collective support for the current queen?

Since most individuals would prefer to reproduce directly, but do not, it is worth seeing if collective preferences act in support of the dominant queen. In other words, is the queen the next-best choice after personal reproduction? If so, then either subordinates or workers might act to suppress alternative reproducers, including other members of their own class.

Figure 4 shows the relatedness advantages of supporting the current queen versus alternative egg layers. Subordinates gain a substantial advantage by suppressing workers in favor of the queen and, somewhat surprisingly, also seem to gain by suppressing each other.

The worker choice depends on the sex ratio produced by the reproductive (Fig. 4). For a 1:1 sex ratio, worker can gain by suppressing both subordinates and other workers. The advantage comes primarily through female progeny, although even the queen's male progeny are significantly better than those of the subordinates. Whether workers would gain by suppressing each other's male production is less obvious. When worker ages are weighted by their numbers when collected (that is, by the numbers in the three brood stages), then there is no significant preference one way or the other (though relatedness to other worker sons is slightly higher). However, this changes with the age composition of the worker force, and we do not know what the exact age composition would have been. By the time all these brood would have eclosed as workers, many of the older ones (collected as pupae) would have already died, as well as some of the middle-aged ones (collected as larvae). To assess the effect of various degrees of mortality, we recalculated the worker relatednesses for various values of m, the worker mortality per age period. That is, we assume survival fractions of $1-m$ and $(1-m)^2$, respectively, for the middle-aged workers (larvae) and older workers (pupae), and scale down the relative weighting of these two brood categories accordingly.

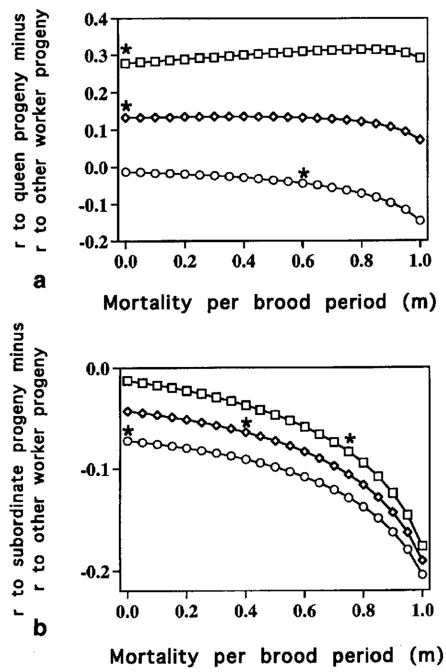

Fig. 5a, b Change in average worker preferences with mortality of the older age classes. **a** Worker relatedness to queen progeny minus worker relatedness to the progeny of other workers. **b** Worker relatedness to subordinate foundress progeny minus worker relatedness to the progeny of other workers. Three age categories of future workers were genotyped in the brood stage. By the time all have emerged as adults, an unknown number of the older and middle-aged workers (genotyped as pupae and larvae) would have already died. The fraction that die during each period is assumed to be *m*. Therefore $1-m$ of the middle-aged workers survive (they have been adults for one brood period) and $(1-m)^2$ of the old workers survive (they have been adults for two periods). The three separate *lines* depict relatedness differences to female brood, to broods with equal numbers of each sex, and to male brood. The *asterisks* mark the lowest values of *m* for which the relatedness difference is statistically different from zero. All values of *m* higher than these are also significant. (□ females, ◇ 1:1, ○ males)

Figure 5a plots the results, showing that while workers continue to gain if the queen lays the female eggs, their preference for worker-laid male eggs increases with worker mortality and becomes statistically significant when *m* is at least 0.6.

Are collective preferences in accord with queen succession rules?

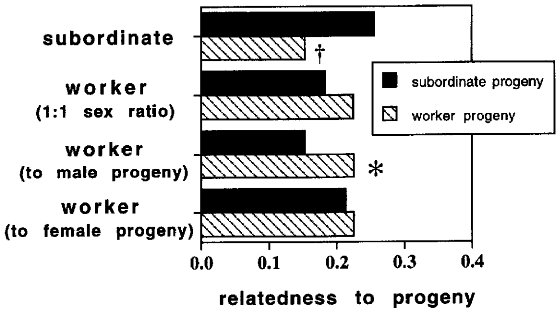

Fig. 6 Relatedness consequences of succession by a subordinate foundress versus a worker. Subordinates and workers are each considered to have the choice of raising the progeny of a subordinate or a worker. The actual queen successor is a subordinate (*solid bars*). Worker relatednesses are shown for a 1:1 brood sex ratio, and for male and female brood. *Asterisks* denote significant differences between the two bars (two-tailed test): * $P < 0.05$, † $P < 0.05$ for one-tailed test. Precise relatedness for the eight bars, *top to bottom*: 0.26, 0.15; 0.18, 0.22; 0.15, 0.22; 0.21, 0.22

Do workers collectively prefer succession first by a subordinate and then by an older worker? The relatednesses relevant to subordinate versus worker succession are shown in Fig. 6. Subordinates appear to be considerably more related to the progeny of other subordinates than to worker progeny, though the difference is significant only in a one-tailed test. The worker choice again depends on the sex of the progeny to be produced by the successor, because workers are sometimes the daughters of subordinates. If only male progeny were to be produced, then workers gain a significant relatedness advantage if the successor is another worker. If only female progeny were to be reared, the direction of preference is the same, but it is small and not statistically significant. A 1:1 sex ratio among the progeny gives an intermediate result, also not significant. The relatedness preference for another worker is most pronounced for young workers (eggs in Table 3) who are most highly related. Thus, as the older workers die off, the preference for a worker successor increases. Figure 5b shows this effect, using the same mortality model as above, with mortality rate *m* per brood period. For $m = 0.4$ or higher, the worker preference for a worker successor becomes statistically significant for a 1:1 progeny sex ratio. Even if broods were to consist entirely of females, there can be a significant preference for a worker successor, if mortality is very high ($m = 0.75$ or higher).

The choice of age of worker successor is analyzed only for worker preferences, because worker successors appear only after no foundresses remain. Figure 7 shows

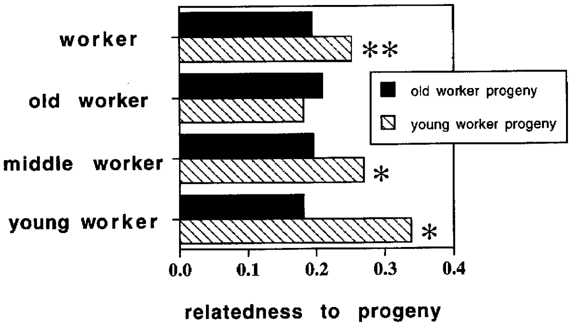

Fig. 7 Relatedness consequences of succession by an old worker versus a young worker. Only workers are considered as having this choice, since foundresses are all dead before workers have an opportunity to succeed. The actual successor is an old worker (*solid bars*). *Asterisks* denote significant differences between the two bars (two-tailed test): * $P < 0.05$; ** $P < 0.01$. Precise relatedness estimates for the 8 bars, *top to bottom*: 0.19, 0.25; 0.21, 0.18; 0.20, 0.27; 0.18, 0.34

that the average worker is significantly more related to the progeny of a worker successor who is young than to the progeny of an older worker. There is no disagreement among the three age categories of workers, although older workers have no preferences one way or the other (see Table 3). This means the preference for a younger worker becomes even stronger as older workers die off.

Discussion

How is reproduction in social insect colonies controlled, and often monopolized, in the face of the temptation of individuals to lay their own eggs in the colony? Our data show that, as expected, this temptation to reproduce is real in *P. annularis*. An individual's best option, other things equal, is usually to lay her own eggs. Each individual should prefer to be the queen. This was certainly expected for subordinate foundresses. Since a subordinate comes from the same generation as the queen, her relatedness to the queen's progeny is half of her relatedness to the queen, and this must be less than her relatedness to sons and daughters. The same argument holds for workers only for male production; a worker might prefer the queen's daughters to her own because of the high relatedness among full sisters. Our relatedness data confirm that a worker prefers her own sons, and show that she is, on average, indifferent between her own and the queen's daughters. The lack of a preference for the queen's daughters arises because the current queen is not always the worker's mother, so the queen will not always produce full sisters.

So why is there generally monarchy instead of anarchy? Who has the power, either individually or collectively to control reproduction? *Polistes* societies have traditionally been viewed as controlled by individual dominance, and this likely forms part of the answer, particularly for foundress associations. In foundress associations, dominance interactions are often common and severe, and it may take a long time before one foundress establishes real reproductive dominance. Body size is often an important determinant of who becomes the dominant foundress in *Polistes* (Reeve 1991), including *P. annularis* (Sullivan and Strassmann 1984). However, individual dominance seems unlikely to provide the whole answer for several reasons. First, the extent of dominance interactions varies widely, with some species being comparatively peaceful (Reeve 1991). This is not logically inconsistent with dominance, but it does at least suggest an additional element of conventional settlement. Order of arrival at the nest site is sometimes correlated with foundress dominance (Strassmann et al. 1987), and may play the role of a conventional cue. Second, additional considerations come into play when workers emerge. A queen need only suppress a few foundresses, but even though *Polistes* colonies are towards the small end of the social insect colony-size spectrum, the queen is still greatly outnumbered by workers on the larger colonies. Can she really physically dominate and control dozens of workers by herself? We suspect that the answer is no, given the fact that the queen often seems to have trouble establishing dominance over a much smaller number of foundresses (although it could be argued that workers, unlike foundresses, may be subject to some pre-imaginal suppression). Third, for *P. annularis*, and two other Texas *Polistes* (*P. exclamans* and *P. instabilis*) older workers usually succeed to the queenship over younger workers (Strassmann and Meyer 1983; Hughes and Strassmann 1988; Hughes et al. 1989; this is not a universal pattern in other *Polistes*; reviewed by Reeve 1991). The first workers in *Polistes* colonies are often smaller than later workers (Reeve 1991). If this pattern holds for *P. annularis*, then older workers may be taking over in spite of a physical disadvantage, something which has been directly demonstrated in *P. instabilis* (Hughes and Strassmann 1988).

Policing theory (Starr 1984; Woyciechowski and Lomnicki 1987; Ratnieks 1988) provides an alternative dominance-based explanation for reproductive control. In this view, the power lies not with one individual, but with some larger collective, usually workers. Its counterintuitive result is that this collective power is not necessarily applied to increasing the reproduction of members of the collective. Workers may suppress other workers in favor of the queen. In *P. annularis*, we find that the queens reigning at the time of our collections would generally be expected to be supported by other colony members (Fig. 4). That is, if an individual could not reproduce directly, its next best option would generally be to support the queen.

There are two partial exceptions to this generalization. First, it is not clear that subordinate foundresses gain by supporting the queen over another foundress. The significance is marginal, and if the effect were real, it would seem to require abilities to distinguish full sibs from other colonymates, abilities that *P. annularis* foundresses do not show in another context (Queller et al. 1990). Moreover, of the 40 foundresses, there are only 4 (one on each of four nests) whose genotypes make them inconsistent as full sisters of the other foundresses on their nest. If the remainder truly are full sisters, which admittedly is not certain, then most of the variation in relatedness to queens versus other foundresses is simply statistical noise around the true value of 3/4. Therefore, though the finding is intriguing, we would not accept it as definitive without additional data.

The second exception to the support-the-queen rule concerns the classic worker policing decision: should workers rear the sons of the queen or of other workers (Starr 1984; Woyciechowski and Lomnicki 1987; Ratnieks 1988)? When all workers are weighted equally, both choices yield about the same relatedness to males (Fig. 4). However, as the older workers die off, a significant preference for worker-laid males may emerge (Fig. 5a). This is not surprising given that *P. annularis* is singly-mated and that queen dominance increases with time (Peters et al. 1995). In the limit, when all colony members are the progeny of one singly mated queen, workers should strongly prefer the sons of other workers ($r = 3/8$) over those of the queen ($r = 1/4$). Therefore worker policing theory suggests that workers should not prevent each other from laying male eggs in *P. annularis*. This could be accomplished, without also allowing worker laying of female eggs, because workers are unmated until they succeed to the queenship. We do not yet know who lays the male eggs in this species.

Thus, the dominance of the current queen is generally consistent with collective worker control, though we do not yet know if her dominance extends to male production. Of course, the current queen has attained her position without any help from the workers (we collected workers as brood), so at best, we might argue that collective worker support helps her maintain her position as the colony becomes larger and harder to control. But if collective worker control is effective here, one might also expect it to be effective in the context of queen succession, and it is not.

Workers tend to favor a worker successor (Fig. 6) rather than the subordinate foundress who actually does succeed, though the degree of confidence in this conclusion depends on worker mortality and on the sex of the brood the successor will produce. With a 1:1 brood sex ratio, the worker preference for another worker becomes statistically significant when worker mortality per brood period (larval or pupal stage) exceeds 40% (Fig. 5b). Though there are no data for *P. annularis*, data from Texas *P. exclamans* (the closest relative of *P. annularis* in the area) suggest that worker mortality may exceed this figure. If we consider each brood period to be about 12 days long (for a total of 36 days to adulthood; see Strassmann and Orgren 1983) then worker mortality over one time period, m, approaches 50% (data from Strassmann 1985, averaged over 3 study years; see Queller 1996, Fig. 1).

This preference for a worker successor is strongest if successors produce males rather than females. It might be argued that a 1:1 sex ratio is unrealistic because workers may control sex ratios, making them more female biased. However, worker control of sex ratios might actually have the opposite effect, by causing the successors to specialize on males. Split sex ratio theory predicts that, if workers control sex investment ratios, colonies with lower than average relatedness asymmetry will specialize on males (Boomsma and Grafen 1991; Pamilo 1991a). Queen replacement is one of the factors affecting relatedness asymmetry (Strassmann 1984; Mueller 1991). The asymmetry arises from the presence of full sisters, related by 3/4. The workers in a colony with a new worker replacement queen will have no full sisters among the brood, hence no relatedness asymmetry. Workers in a colony with a new subordinate replacement queen may have some full sisters, but not as many as a colony with its original queen. Therefore, if workers control sex investment, colonies with replacement queens, particularly worker replacement queens, may specialize on males. In a study anticipating the later development of formal split sex ratio theory, Strassmann (1984) tested this prediction in *P. exclamans*. Colonies with worker replacement queens tended to produce a higher fraction of males than colonies with a foundress queen (original and replacement foundresses were not distinguished in this study). This trend is consistent with the theory. It approaches, but does not reach, statistical significance if colonies with very poor sex ratio samples (1 or 2 sexuals) are eliminated (Strassmann 1996). If *P. annularis* replacement queens specialize on males, as predicted by theory, it strengthens the conclusion that workers do not choose the successor who will provide the most related offspring.

It is clear that there is no support for the hypothesis that workers prefer a subordinate successor. The only uncertainty, which hinges on the degree of worker mortality, is whether we can meet the much more severe standard of showing a statistically significant preference in the opposite direction. This strict standard is clearly met in the other succession context. Workers would gain a significant relatedness advantage if a young worker takes over, but an old worker succeeds instead. The model developed above suggests that a preference for young workers would arise from increasing reproductive dominance of the queen, and that is indeed the general pattern in this species (Strassmann 1981; Peters et al. 1995).

The failure of worker collective interests to predict the queen succession rules falsifies the general hypothesis of total worker reproductive control in this species. Why are worker collective interests not enforced even though they are the most numerous class of individuals? One possibility is that colonies are too small; there may be a continuum ranging from large colonies with collective worker control down to small colonies without it. But this should be viewed as a hypothesis in need of testing rather than an automatic escape from refutation. Even if there are only 25 workers, this represents a 25-fold numerical advantage over the queen. Support for worker control of sex ratios has been found in some species with small colonies (Mueller 1991; Boomsma 1991; Packer and Owen 1994).

Sometimes workers must oppose more than just the queen. Succession by a subordinate seems to be advantageous to other subordinates, so they may collectively oppose the worker preference for a worker (a possibility suggested by Hughes and Strassmann 1988). However, workers often greatly outnumber subordinates, particularly as the season progresses. More crucially, opposing subordinate preferences cannot account for the rule that an old worker succeeds over a young worker, because all subordinates have died by that time.

Another hypothesis is that workers, even though they have an average preference, are too divided in their interests to enforce it effectively. However, with respect to the queen succession rules, there are no real disagreements between age categories of workers (old workers are relatively indifferent but they do not disagree with middle-aged and young workers; Table 1, Fig. 7). There could be strong disagreements if workers could identify their own mother or their own full sisters, but there is no conclusive evidence that social insects make this kind of distinction (Carlin and Frumhoff 1990; Alexander 1991; Breed et al. 1994), and there is good evidence against it in *P. annularis* (Queller et al. 1990).

It might also be argued that worker preferences are not met because the individuals they prefer are insufficiently fertile to be queen. However workers do become apparently effective queens on many colonies after the subordinates have died (Queller and Strassmann 1988; Strassmann 1989b) so they ought to be able to do so when subordinates are alive, provided workers are in control. Even if a worker queen has somewhat lower fecundity, workers could make up for this disadvantage by allowing more than one of their number to lay eggs.

Possible limitations of this study include the small number of colonies and the fact that worker interests were assessed for only one time window, but neither of these appears serious. Although we scored microsatellites for only nine colonies, the statistics take this into account by using eight degrees of freedom. When the result is statistically significant, that means we are entitled to make inferences about the population at large, in spite of having only nine colonies. The choice of colonies to use was haphazard, except that, being interested in foundress relations, we deliberately avoided single-foundress colonies. However, such colonies are rare in this species and would not necessarily have been included in a sample of nine anyway (Strassmann 1989a). In any event, with respect to the crucial results on worker queen succession preferences, such colonies are irrelevant. First, there is no opportunity to choose a subordinate foundress. Second, since all females on single-foundress colonies will be full sisters, workers will be equally related to old or young worker queen candidates. Such colonies would therefore slightly dilute any overall preference of young or old worker successors, but they would not affect the direction of preference.

Strictly speaking, our worker-preference results pertain to the preferences of only the first generation of workers on their colonies, because that is who we genotyped. However, it appears that the most interesting results also apply to later generations. Our data show a temporal trend towards increasing queen dominance (Peters et al. 1995) and the fact that queens are highly related (0.62) suggests that this pattern is never reversed (queens are colonymates from the end of the previous season). Increasing monogyny increases all of the following effects on worker preferences: the queen as the best source of female eggs, self or other workers as the best source of male eggs, worker succession over subordinate foundress, and young worker succession over old worker.

In conclusion, our results bolster the view that *P. annularis* employs conflict-reducing conventional cues to settle who should reproduce, and that the convention does not necessarily reflect the optimal interests of the dominant individual or class. This conclusion derives from the rules of queen succession, where age acts as the conventional cue, with subordinate foundresses succeeding first, and then older workers. Self-restraint alone does not explain who gets to be queen, because each individual would gain from producing its own offspring rather than those of any other class of successor. Physical domination by the strongest individual may play some role, but the age convention seems to override it when older smaller workers succeed before younger larger ones. Finally, collective dominance by the largest class, workers, is not the answer because the succession rules go contrary to worker interests.

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