Colony genetic structure in a facultatively eusocial hover wasp

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The degree of genetic heterogeneity among the individuals in an animal society depends on the society's genetic structure. Genetic heterogeneity, in turn, means that group members will differ in their reproductive objectives and conflicts over reproduction may arise. The resolution of these conflicts may be reflected in the way that reproduction is partitioned between potential reproductives. We used 5 microsatellite loci to investigate genetic structure and reproductive skew in 17 nests of the Malaysian hover wasp, *Parischnogaster alternata*. *Parischnogaster alternata* colonies are small (1–10 females), and all adult colony members are capable of mating and producing offspring. We found that colonies tended to consist of closely related individuals and that at any one time the production of both female and male offspring was nearly always monopolized by a single dominant female, despite considerable variation between nests in parameters predicted to affect skew. Subordinate females that remained in their natal colonies obtained indirect fitness benefits by helping to raise offspring to which they were related. Subordinate females also appeared to be positioned within an age-based queue for inheritance of the dominant egg-laying position. We suggest that the high skew in *P. alternata* may result from strong ecological constraints on solitary nesting, high relatedness, and a relatively high probability that subordinates will eventually inherit the position of dominance. *Key words:* eusociality, *Parischnogaster*, relatedness, reproductive skew, Stenogastrinae. *[Behav Ecol 17:873–880 (2006)]*

K in selection theory (Hamilton 1964) predicts that animals will act in ways that tend to maximize their inclusive fitness. Whenever there is genetic heterogeneity among the individuals in an animal society, relatedness asymmetries between group members and potential offspring may lead to conflicts of interest as different individuals simultaneously attempt to maximize their genetic profit (Ratnieks and Reeve 1992). Examples include conflicts over sex allocation and over the parentage of reproductive offspring. The genetic structure of an animal society, and hence the degree of heterogeneity within it, depends on factors such as the sex determination mechanism (e.g., haplodiploidy), the number of reproductive individuals and their relatedness, the number of times that each female mates, and whether there is inbreeding (Ratnieks and Reeve 1992).

Reproductive skew, the way in which reproduction is shared among the members of a society, is one aspect of genetic structure that has recently attracted particular attention. The distribution of reproduction among group members may range from complete equality (low skew) to monopolization by a single breeder or dominant (high skew). Over the past decade it has become increasingly appreciated that explaining reproductive skew will greatly advance our knowledge of the evolutionary causes and consequences of sociality (Sherman et al. 1995; Johnstone 2000). This realization has led to the formulation of evolutionary models that examine how the partitioning of reproduction among group members may be affected by genetic, ecological, and behavioral factors: for reviews of skew theory, see Johnstone

(2000), Magrath and Heinsohn (2000), and Reeve and Keller (2001).

Two basic types of model, known as concession and tug-of-war models, have customarily been considered as possible contenders for explaining reproductive skew in facultatively eusocial wasps (Field et al. 1998; Reeve et al. 2000; Seppa et al. 2002; Sumner et al. 2002). Concession models are built on the assumption that groups contain a single dominant breeder that has complete control over subordinate reproduction (Vehrencamp 1983; Reeve and Ratnieks 1993; Kokko and Johnstone 1999; Ragsdale 1999). This dominant individual has the option of yielding a reproductive concession to a subordinate in exchange for its cooperation. The concession may represent a staying incentive when given to prevent a subordinate from leaving the association or a peace incentive when given to prevent it from fighting the dominant for control of the group. In contrast to concession models, tug-of-war models are built on the assumption that dominants have incomplete control of subordinate reproduction (Reeve et al. 1998). In these models, group members channel resources into an intragroup competition over reproduction. Dominants differ from subordinates in that they either have access to a greater proportion of the group's resources or use those to which they have access more efficiently. The competition over reproduction has a cost that is reflected in the productivity of the group. The concessions and tug-of-war models make different predictions about the relationship between skew and other variables such as dominant–subordinate relatedness and relative fighting ability, the relative productivity of groups versus independent-nesting females, and ecological constraints on independent nesting (e.g., Reeve and Ratnieks 1993; Field et al. 1998). To date, there have been few empirical investigations of reproductive skew in primitively eusocial wasps and bees (Field et al. 1998; Hogendoorn and Velthuis 1999; Reeve et al. 2000; Paxton et al. 2002; Seppa et al. 2002; Sumner et al. 2002; Langer et al. 2004). In this paper, we present data on colony genetic structure and reproductive skew in the tropical hover wasp *Parischnogaster alternata* Sakagami (Hymenoptera: Stenogastrinae).

Parischnogaster alternata inhabits the rain forests of South-East Asia, where it nests in damp sheltered locations such as caves, cement pipes, and the undersides of bridges and rocky overhangs. In these locations, nests are often found in dense clusters and are often interspersed with those of another hover wasp, *Liostenogaster flavolineata* (Turillazzi 1986). *Parischnogaster alternata* nests are delicate tubular structures that reach a maximum length of approximately 6.5 cm and consist of around 35 cells. A protective envelope surrounds the cells, forming a corridor that has a single opening at the bottom of the nest. Colonies are small, usually consisting of between 2 and 4 individuals, although nests with up to 15 individuals have been reported (Turillazzi 1986). Nesting and nest initiation occur throughout the year, and colonies may be long lived. In this respect, *P. alternata* differs from temperate wasps that have annual colony cycles, for example, *Polistes*. Nests are often founded by single females, although associative founding has also been reported (Turillazzi 1985a). Typically, a foundress constructs a few cells out of vegetable matter and then lays a single egg into each of these. When the eggs hatch, she proceeds to feed the resulting larvae progressively on prey that she captures. After pupation, newly hatched adults may disperse to found new nests or may become helpers on their natal nests, where they have the potential to become reproductives. All adults are physiologically capable of mating and producing both male and female offspring (Turillazzi 1985b). Conflicts over reproduction therefore include all colony members.

Liostenogaster flavolineata is the hover wasp that has been best studied to date in terms of colony genetic structure and behavioral ecology (e.g., Samuel 1987; Shreeves and Field 2002; Sumner et al. 2002). In *L. flavolineata*, female nest mates are close relatives ($r = 0.52 \pm 0.05$), and a single female, known as the dominant, lays almost all the eggs at any one time. The dominant rarely leaves the nest, never forages for larval provisions, and is more aggressive than other group members (Samuel 1987; Field and Foster 1999). The remaining females in the group help by foraging for larval provisions and form an age-based queue to inherit the dominant position. When the dominant dies, she is nearly always replaced by the next oldest female, and more than 50% of nests contain immature offspring of both the current dominant and her predecessor (Samuel 1987; Shreeves and Field 2002; Sumner et al. 2002; Bridge 2005). Nesting occurs continuously throughout the year in both *L. flavolineata* and *P. alternata*. Successive dominant replacements mean that as in *L. flavolineata*, *P. alternata* colonies are unlikely to comprise simple mother–daughter families but will often comprise mixtures of sisters, cousins, aunts, nieces, etc.

METHODS

Sample collection

The study population consisted of approximately 150 *P. alternata* nests that were situated in a culvert carrying a stream under a 4-km stretch of road between Raub and Bukit Fraser in Malaysia, northeast of Kuala Lumpur. All adult females from 141 nests were individually marked with enamel paint spots between 20 June and 15 July 1998. Starting on 17 July, all nests were censused every few days during the daytime to determine which individuals spent the most time on their nests. On 7 days, nests were censused between 2 and 7 times throughout the day. In total, nests were censused on 17 separate days. To determine colony size and to detect residents that are rarely on their nests during the day, 14 additional nighttime censuses were performed. All surviving adults and brood from 47 colonies were collected at 06.30 AM on 6 September.

Ovarian development and insemination status

Due to sample storage problems, the ovaries of the monitored females were unmeasurable. Therefore, the number of females capable of laying eggs was determined using an additional sample of 20 unmonitored colonies that were collected at 09.30 AM on 13 May 2002. The average length of a laid egg was 1.39 ± 0.025 mm with the smallest being 1.15 mm. Female abdomens were dissected and measured using a dissecting microscope. Females containing eggs at least 1.15 mm long in length are defined as potential egg layers in order to distinguish them from females that are unlikely to have been ovipositing at the time of nest collection.

The spermathecae of the monitored females were located and their insemination status determined. In total, spermathecae were located for 66 of the 77 females, 60 of which were found to be inseminated. Sperm DNA was extracted from the inseminated spermathecae and then genotyped.

Molecular techniques

Seventeen of the monitored colonies were selected for genetic analysis. These were chosen on the basis that colonies contained more than one adult female and that the majority of females seen on the nests at least 2 weeks prior to collection were available for genetic analysis. These colonies contained a total of 77 adult females and 132 brood. Brood were classified as eggs, small larvae (<20 mg), large larvae (>20 mg), or pupae. We extracted DNA from all adults, brood, and inseminated spermathecae and attempted to amplify 5 microsatellite loci: PA74, PA154, PA168, PA180, and PA195 (A Bolton and J Field, unpublished data). Products were separated on 6% polyacrylamide gels using the methods of Strassmann et al. (1996). Alleles were scored independently by 2 of the authors. There were between 11 and 16 alleles at each locus, with observed heterozygosities of 0.71–0.8. Using the computer programe Genepop (Raymond and Rousset 1995), loci were found to be unlinked and at Hardy–Weinberg equilibrium (Bolton 2002).

Genetic structure analysis

All 77 adult females were genotyped at at least 4 loci and 91% (70 females) at all 5 loci. Of the 132 brood, 5 eggs failed to amplify at 4–5 of the loci and were removed from the analysis. The probability of a diploid being homozygous at all 5 loci was 2.66×10^{-5}. Even using only the 2 least heterozygous loci (PA74 and PA154), this probability was 0.021, so that only one in 47 individuals would be wrongly assigned as a male. Therefore, brood that were found to be homozygous at 2 or more loci (and heterozygous at none) were sexed as males (haploids). Of the 127 brood used in the analysis, 84 (66.2%) were females and 43 (33.8%) males. Seventy (83.3%) female brood were successfully genotyped at all 5 loci and 82 (97.6%) at 2 or more loci. Thirty-one (72%) male brood were successfully genotyped at all 5 loci and 43 (100%) at 2 or more loci. We used Relatedness 5.07 (Goodnight 2000) to estimate the mean regression relatedness between various categories of colony members. Colonies were weighted equally and standard errors were obtained by jackknifing over nests. Comparisons of relatedness values were performed using *t*-tests. In statements such as "female to male relatedness," the first category (males) is taken as the notional actors and the second category (females) the notional recipients when calculating relatedness. When multiple comparisons were performed, the probability of making a type I error was controlled using Bonferroni tests by the Dunn–Sidak method (Sokal and Rohlf 1995).

Brood maternity assignment

Parentage analysis was used to analyze the production of female eggs and small larvae, with the dominant on each nest being defined as the female that produced the largest number. Sperm were successfully genotyped at loci Pa195 and Pa168 for 44 (73.3%) and 24 (40%) of inseminated females, respectively. Sperm was not successfully genotyped at any other loci. Only one female showed evidence of multiple mating (nest 64). Therefore, when assigning maternity, we assumed that *P. alternata* mates only once. We did not use parentage analysis to assign older brood because of the increased chance that mothers had died before nest collection (Field et al. 1998).

As already noted, groups of *P. alternata* females are unlikely to comprise simple mother–daughter associations. In this situation, haploid male offspring cannot be assigned individually to mothers with a high degree of certainty (Field et al. 1998). Therefore, the production of male eggs and small larvae was analyzed using a maximum likelihood approach: see below for details.

Female brood

The duration of brood development is unknown in *P. alternata* and was assumed to be approximately 50 days as in *Parischnogaster nigricans serrei* (Turillazzi 1985b). This brood development time was used in conjunction with census data and insemination status to identify candidate mothers for the female brood. The genotypes of the candidate mothers plus those of their mates, if available, were used to determine whether they could have produced the progeny genotypes at all loci. In nests where all candidates were excluded bar one, the unexcluded individual was assigned as the parent (nests 33, 40, and 123).

For the remaining nests and for older brood, we identified brood sibgroups using the program Kinship 1.5b4 (Goodnight and Queller 1997; Sumner et al. 2002). Female brood were placed in the same sibgroup when 1) they were more likely to be sisters ($r = 0.75$) than aunt–niece ($r = 0.375$) at the $P < 0.05$ significance level, 2) their combined genotypes constituted no more than 3 alleles at each locus, and 3) all individuals shared at least one allele in common at each locus, assumed to be the paternally inherited allele. Progeny that fitted into more than one sibgroup were assigned to the largest group. Potential mothers of sibgroups containing eggs and small larvae were identified using their genotypes, brood development times, census data, and insemination status. Mothers were assigned to those sibgroups for which all candidates bar one could be excluded.

Male brood

When analyzing male brood, the reproductive dominant within each colony was defined as the adult female revealed through maternity assignment to have laid the largest proportion of female eggs and small larvae. A maximum likelihood analysis was then carried out for each nest that contained 2 or more young male brood (eggs and small larvae). This approach calculates the most likely proportion of male offspring produced by the dominant as opposed to all the subordinates combined, given their respective genotypes and the genotypes of the male offspring themselves. We followed the methods of Arévalo et al. (1998), with the exception that brood genotypes were not pooled: for each colony the probability that a given male was produced by the subordinates was calculated as the average of the probabilities for the individual subordinates on the nest. Colony-specific likelihoods were multiplied together to give a likelihood for the overall population.

Measuring skew

Several indices of reproductive skew have been published, and there has been much debate concerning which index is best (Keller and Krieger 1996; Nonacs 2000; Tsuji and Kasuya 2001). To date no consensus on the merits and defects of the various indices has been reached (Tsuji and Kasuya 2001). We used the program Skew 1.1.1 to calculate the corrected S index, which adjusts for offspring number (Keller and Krieger 1996; Krieger and Keller 1997). As the probability of assigning mothers accurately to male progeny was low, skew indices were calculated only for female brood. Furthermore, skew indices were calculated only for young female brood because the probability that all potential mothers have not been collected increases with older brood (Field et al. 1998). Young brood were defined as eggs and larvae smaller than 20 mg. Due to the small number of young female brood that were present in nests, it was not possible to test whether the observed skews for individual nests differed from expected skews under the assumption of random reproduction. A maximum likelihood analysis was therefore performed, using the same procedure as in the analysis of male brood, to determine the most likely proportion of females produced by dominants in the population. This analysis was performed for 7 nests that contained a minimum of 2 young female brood and for which the genotypes of dominants were known (nests 33, 40, 50, 62, 70, 83, and 123).

Explanatory variables

Four variables were quantified to determine whether they were correlated with skew: relatedness, relative fighting ability, group size, and per capita productivity. The relatedness variable used in the concession and tug-of-war skew models is subordinate–dominant relatedness (Vehrencamp 1983; Reeve et al. 1998). However, this parameter could not be estimated for all *P. alternata* nests as not all dominants were collected. For the 7 nests from which dominants were genotyped, average subordinate–dominant relatedness did not differ from average female nest mate relatedness: ($t_{(0.05(2)7,7)} = 0.75$, $P > 0.1$). Therefore, average adult nest mate relatedness was used as the relatedness variable because it could be estimated for all nests. Body size appears to reflect competitive ability in several species of polistine wasps (Reeve 1991). Therefore, wing size was used as an index of relative size and fighting ability. The index used was (dominant wing size − average subordinate size)/(mean for all females on nest), where our measure of forewing size was the distance between the inner edge of the discoidal cell and the outer edge of the marginal cell. This measure controls for between-nest variation in size, which is often a major component of size variation in primitively eusocial wasps (Sullivan and Strassmann 1984). Wings were flattened between 2 slides and then measured using an eyepiece graticule on a dissecting microscope set at 20× magnification. The smallest measurement was 94 graticule units. Twenty randomly selected wings were remeasured, giving a measurement error of 0.31%. Group size was defined as the number of collected females plus uncollected females that could potentially have produced the eggs and small larvae. Uncollected females were individuals that had been marked and monitored but were not present when nests were collected. Per capita productivity was calculated for each colony as the total number of brood present at collection divided by group size.

Indirect fitness benefits

To determine whether subordinates gain indirect fitness benefits from helping, total productivity (total number of brood

per colony) was tested for an association with group size using the generalized linear modeling package GLIM (Crawley 1993). For this analysis, the 17 nests (sample 1) used to examine skew were combined with 18 colonies (sample 2) collected in May 2002. The sample consisting of 17 nests was also analyzed separately to determine whether relatedness had an effect on total productivity.

Determinants of dominance

Wing size and census data were used to examine whether dominance is likely to be determined by size or by the duration of an individual's colony membership: both tug-of-war and concession models assume, when considering the effect of aggression on skew, that the dominant is the individual in the group with the highest competitive ability (Reeve and Ratnieks 1993; Reeve et al. 1998).

RESULTS

Genetic structure

Kinship among adult females
The mean number of adult females present on 45 randomly chosen nests was 3.07 ± 0.283 (range 1–10). Average within-colony relatedness among adult females from 17 nests was 0.46 ± 0.054. This estimate was not significantly different from a previously published estimate based on allozymes ($r = 0.56 \pm 0.19$: Strassmann et al. 1994—Welch's approximate 2-sample t-test with unequal variances: $t\text{'s}_{(22,17)} = 0.52$, $P > 0.05$). Female nest mates were not all sisters (one sample t-test for deviations from the full-sister value of 0.75: $t_{(0.05(2)17)} = -5.47$, $P < 0.01$). There was no evidence to suggest that females were inbred: $F_{is} = 0.028 \pm 0.022$, not significantly different from zero ($t_{(0.05(2)17)} = 1.27$, $P > 0.05$).

At the colony level, relatedness estimates varied from 0.016 to 0.703. In 8 of the 17 nests, average female nest mate relatedness was significantly greater than 0, and 12 of the 17 nests had relatedness values that were not significantly different from 0.75. Paternally inherited alleles among the brood suggested that in 2 nests (nests 44 and 74), adult subordinates were daughters of the dominant. In nest 44, a female was probably the mother of 3 of its 4 nest mates. In nest 74, a female was probably the mother of 4 of its 5 nest mates. In nests 85 and 109, it was not possible to determine whether individuals were mother–daughter. We used Kinship 1.5b4 (Goodnight and Queller 1997) to determine whether the remaining pairs of adults were more likely to be sisters ($r = 0.75$) than aunt–niece ($r = 0.375$). Using the 4 least heterozygous loci and $\alpha = 0.05$, the power of this analysis was 79%, so that as many as 21% of real sisters may not have been detected. The kinship analysis suggested that while the adult females in some colonies were all sisters (nests 40, 50, 70, and 122; Figure 1), this was not always the case: there were between 1 and 4 adult female sibgroups per colony (Figure 1).

Brood
There was an average of 7.76 ± 1.06 brood per nest (17 nests). The average relatedness among female brood of all ages within nests was 0.55 ± 0.070 ($n = 15$ nests), significantly different from 0.75 ($t_{(0.05(2)15)} = -2.89$, $P < 0.05$). Relatedness estimates for individual age groups suggested that females within a particular age cohort were mainly sisters: within-nest relatedness among female eggs and small larvae, $r = 0.69 \pm 0.068$ ($n = 9$ nests, 29 brood); large larvae, $r = 0.69 \pm 0.082$ ($n = 9$ nests, 25 brood); and pupae, $r = 0.78 \pm 0.084$ ($n = 6$, 15 brood) (one sample t-tests for deviation from 0.75: $P > 0.05$ in each case). Female progeny were not inbred:
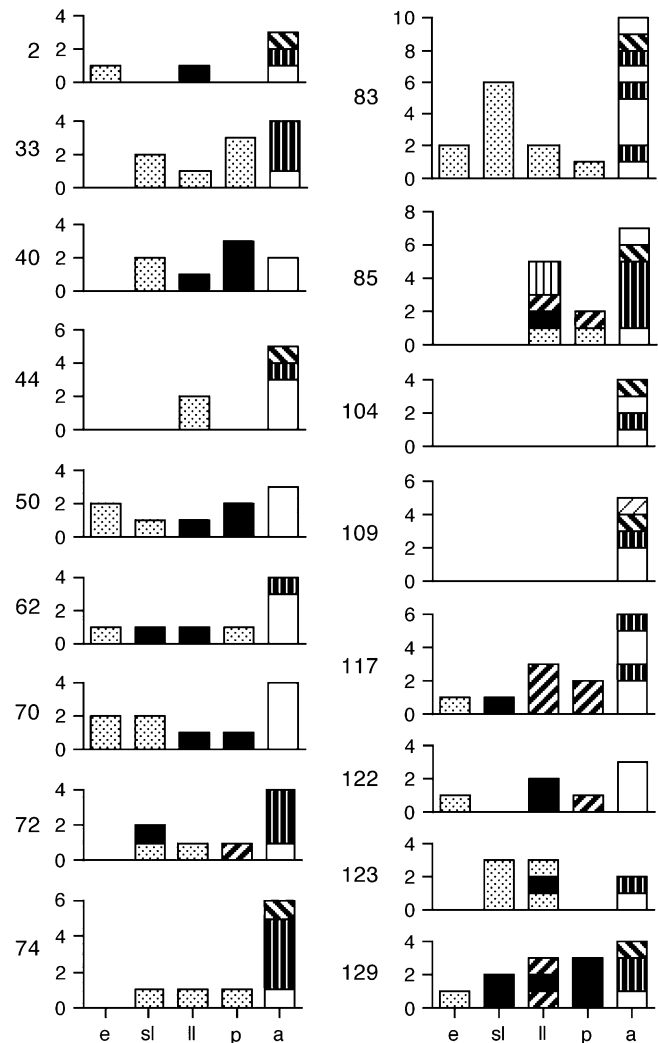

Figure 1
Sibgroup membership for female brood and adults in 17 nests of *Parischnogaster alternata*. Colony identification numbers are shown to the left of each graph. e = eggs, sl = small larvae, ll = large larvae, p = pupae, and a = adult. The *y* axis indicates the number of individuals. Shadings indicate sibgroup membership, for example, on nest 74, all the brood are sisters, whereas on nest 70, the brood comprise 2 sibgroups. Within brood stages, heavier (presumably older) individuals are stacked on lighter individuals. Brood and adults were analyzed separately.

$F_{is} = 0.022 \pm 0.018$ ($t_{(0.05(2)15)} = 1.25$, $P > 0.05$). The average within-nest relatedness for male brood of all ages combined was 0.34 ± 0.056 ($n = 12$, 40 brood), significantly different from brother–brother relatedness of 0.5 ($t_{(0.05(2)12)} = 2.8$, $P < 0.05$).

Maternity assignment

Female eggs and small larvae
A single nest was found to contain no brood (nest 104) and another only male brood (nest 109). Kinship analysis of the remaining 15 nests indicated that the female brood formed between 1 and 4 sibgroups per nest (2.34 ± 0.270, mean ± standard error; Figure 1).

Maternity assignment was successful for the youngest sibgroup in 13 of the 15 colonies. Nest 85 was an exception, and nest 117 was excluded because a dominant turnover occurred just before nest collection. Of these colonies, 12 contained

Table 1

Reproductive skew and putative correlates for 9 *Parischnogaster alternata* nests

Nest	Skew	Group size	Size ratio	Relatedness $\pm$ SE	Per capita productivity
123	1	2	0	0.258 $\pm$ 0.299	3.5
129	0.64	5	ND	0.295 $\pm$ 0.067	2.4
33	1	5	−0.0033	0.345 $\pm$ 0.137	2.4
72	0.67	6	ND	0.430 $\pm$ 0.077	1.17
62	1	4	−0.0051	0.483 $\pm$ 0.123	1.5
83	1	10	0.0424	0.521 $\pm$ 0.054	1.7
40	1	2	−0.0106	0.691 $\pm$ 0.176	3.5
70	1	5	−0.0220	0.703 $\pm$ 0.047	1.6
50	1	3	0.0103	0.755 $\pm$ 0.087	4
Mean $\pm$ SE	0.92 $\pm$ 0.05	4.67 $\pm$ 0.82	0.002 $\pm$ 0.05	0.50 $\pm$ 0.0613	2.42 $\pm$ 0.34
CV	16.95	53.95	ND	37.62	43.62

Means $\pm$ standard errors and coefficients of variation are given for each category. Coefficients of variation were corrected for bias (Sokal and Rohlf 1995). Colonies are ranked according to their relatedness estimate. ND = no data available.

female eggs and small larvae. In 10 of the 12 colonies, all the young female brood were assigned to a single mother, including 3 nests where the youngest sibgroup included only a single egg or small larva: nests 2, 74, and 122. Within these colonies the dominant females (assigned mothers) and subordinates (excluded candidates) were related on average to the female eggs and small larvae by 0.59 $\pm$ 0.104 and 0.28 $\pm$ 0.072, respectively. The estimate for subordinates was significantly different from mother–daughter relatedness of 0.5 ($t_{(0.05(2)10)} = -3.14$, $P < 0.05$), whereas the estimate for dominants was not ($t_{(0.05(2)10)} = 2.05$, $P > 0.05$), consistent with expectation.

Reproductive skew was calculated for the 9 nests that contained 2 or more female eggs and small larvae (mean skew = 0.92 $\pm$ 0.051, 29 brood). Reproductive skew among the young brood was 1.0 in 6 of the 9 nests, that is, a single female on each nest had produced all the young female brood (Table 1). In 3 colonies, skew was less than 1. We performed an analysis to determine the maximum likelihood proportion of females produced by dominants as opposed to subordinates. The analysis was performed for the 7 nests that contained a minimum of 2 young female brood and for which the genotypes of the dominants were known (nests 33, 40, 50, 62, 70, 83, and 123). Unfortunately, 2 of the nests for which skew was less than 1.0 could not be used in the analysis because the genotypes of the dominants were not known (nests 72 and 129). The likelihood curve shows that the small female brood were most likely to have all been produced by a single dominant female on each nest (Figure 2).

Colonies were examined to determine whether or not sibgroups overlapped temporally. For example, if a sibgroup is composed of an egg and a small larva that weighs 10 mg, whereas another sibgroup within the same nest contains a small larva of 6 mg and a pupa, then these sibgroups would be considered to overlap temporally. In 11 nests, there was no temporal overlapping of sibgroups, suggesting that only one female had been laying eggs within these colonies at any one time (nests 2, 33, 40, 44, 50, 70, 72 74, 83, 117, and 122). Another nest contained 2 sibgroups that overlapped temporally (nest 62). These sibgroups combined could only have been produced by a single female as all candidate mothers were excluded except one: this female's genotype was consistent with her being the mother of both sibgroups, suggesting that they were the result of double mating. Therefore, on 12 of the 15 nests examined (80%), only one female had been producing female offspring at any one time. The 3 remaining nests contained overlapping sibgroups, suggesting that the production of female offspring was not completely monopolized by a single female at all times (nests 85, 123, and 129).

Male eggs and small larvae
A maximum likelihood analysis of young male brood was performed on those colonies for which a dominant had been identified, and male eggs and small larvae were genotyped (nests 33, 44, 50, 62, 70, 83, and 122). These colonies contained between 1 and 3 male eggs and small larvae per nest (total of 12 brood). As sample sizes were small, colony-specific likelihoods were multiplied together to give an overall population likelihood. The maximum likelihood curve suggests that all male eggs and small larvae were laid by the female that had laid most or all of the female eggs (Figure 2). This is consistent with the relatedness data. First, average relatedness of male small larvae and eggs to female small larvae and eggs within nests was 0.29 $\pm$ 0.04 ($n = 8$), consistent with females and males of the same age cohort being siblings (one sample t-test for deviation from brother–sister relatedness of 0.25: $t_{(0.05(2)8)} = 1.05$, $P > 0.05$). Second, relatedness of dominants and subordinates to male eggs/small larvae was $r = 0.94 \pm 0.06$ and 0.56 $\pm$ 0.04, respectively, consistent with high skew.

Explanatory variables

As skew was uniformly high, it was not possible to test whether variation in skew was correlated with variation in the potential explanatory variables (relatedness, group size, per capita productivity, and relative fighting ability). However, we could test whether the lack of variation in skew was associated with a lack of variation in the explanatory variables. Coefficients of variation were calculated for skew and the explanatory variables (Table 1). Z-tests were then used to test for differences between the coefficient of variation for skew and the coefficients of variation for the explanatory variables (Zar 1999). The Z-test assumes that the coefficients of variation are from normal distributions. Because skew was nearly always 1.0, this test was not entirely appropriate, so the results are only approximate. Relatedness, group size, and per capita productivity were all significantly more variable than skew ($Z_{(0.05(2)9,9)} = -1.99$, -2.64, -4.48, respectively, $P < 0.05$ in each case). The coefficient of variation is an inappropriate statistic for comparing variability between skew and the size ratio (relative fighting ability). This is because the size ratio functions in such a way that its sample mean approximates to zero, so that the coefficient of variation will inevitably be large. Size ratios could be calculated for 7 of the 9 nests for which skew was measured.
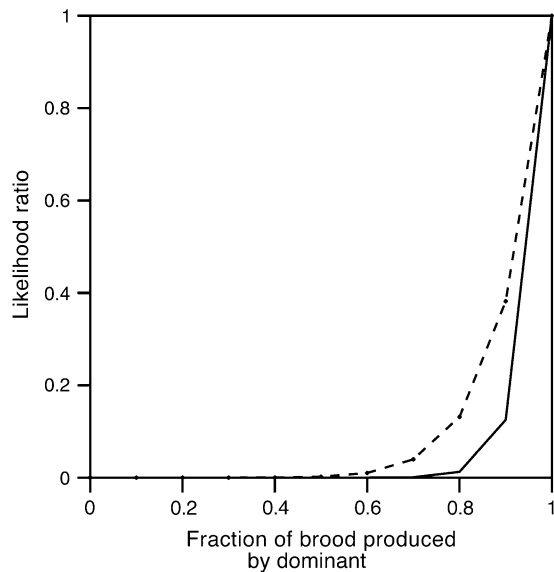
Figure 2
Likelihood curves for the proportion of females (solid line) and males (dashed line) produced by the dominant, averaged over nests. Curves are scaled so that the maximum likelihood is 1.0.

On 4 of these 7 nests, the dominant was not the largest individual present.

Indirect fitness benefits

Group size (the number of females on a nest when it was collected) had a significant effect on total productivity, indicating that subordinate females gain indirect fitness benefits from helping: $F = 8.269$, $P = 0.007$, df = 1,31 (Figure 3). Adding a quadratic term did not improve the fit of the model. The slopes of the 2 samples were not significantly different, but there was a significant difference between the intercepts: $F = 4.990$, $P = 0.033$, df = 1,31. The difference between the
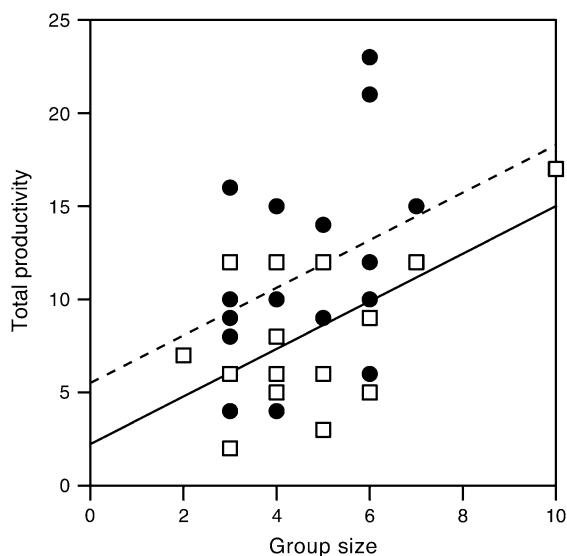

Figure 3
The relationship between group size (number of adult females) and total productivity. Open squares indicate sample 1 ($n = 16$), and filled circles indicate sample 2 ($n = 18$). The solid and dashed lines are the lines of best fit for samples 1 and 2, respectively.

intercepts was probably because sample 1 (1998) was collected early in the morning (06.30 AM) when all resident females were likely to have been on their nests, whereas sample 2 (2002) was collected during daylight (09.30 AM) when some females would have been off the nest. Within-nest adult female relatedness had no effect on total productivity: $F = 1.52$, $P = 0.24$, df = 1,13.

Determinants of dominance

On 10 of the 12 nests, the dominant was the female that had been resident for longest. Dominants were the largest (nest 83) or joint-largest (nests 74 and 123) females on only 3 of 12 nests, and there was no difference in average size between dominants and subordinates (dominants mean wing length = 5.83 ± 0.034 mm, subordinates mean = 5.83 ± 0.020 mm: $t_{(0.05(2)41,12)} = 0.72$, $P > 0.05$). This suggests that in general, dominance is not determined by size. Interestingly, however, on 2 of the 3 nests on which the dominants were the largest or joint-largest females (nests 74 and 83), they were not the oldest serving residents. Although only correlative, these data are compatible with the hypothesis that females enter a primarily age-based queue for the inheritance of reproductive dominance, as occurs in *L. flavolineata* (Field et al. 1999; Shreeves and Field 2002). Other factors, such as size, might sometimes outweigh age in determining dominance, but further data are required to demonstrate this.

On 6 of the 10 nests where the dominant was the longest serving resident, 2 or more nonoverlapping offspring sibgroups were present. If the brood development period is approximately 50 days, as in *P. nigricans serrei* (Turillazzi 1985b), this suggests that the tenure of dominants can be quite short. On one nest, the dominant was a female that had joined the group during the monitoring period (nest 40), indicating that a joiner can become the dominant on its adopted nest.

DISCUSSION

We have presented data on genetic structure and partitioning of reproduction in colonies of the stenogastrine wasp *P. alternata*. The population we studied is similar in terms of demography to those examined by Turillazzi (1986) and Strassmann et al. (1994) and is probably therefore representative of *P. alternata* as a species. The results indicate that nest mates are outbred and tend to be close relatives such as sisters, daughters, mothers, and cousins. Similar results have been reported for another hover wasp *L. flavolineata*, which is often found nesting in the same locations (Sumner et al. 2002). The relatedness estimate for *P. alternata* female nest mates of $r = 0.46 \pm 0.054$ was not significantly different from Sumner et al.'s (2002) corresponding relatedness estimate for *L. flavolineata* of $r = 0.52 \pm 0.053$: $t_{(0.05(2)17,27)} = -0.44$, $P > 0.05$. Within *P. alternata* colonies, the production of female offspring is nearly always monopolized by a single dominant female at any one time. This has also previously been reported for *L. flavolineata*: all female eggs on 11 of 13 *L. flavolineata* nests had been laid by a single dominant female (Sumner et al. 2002). However, about 10% of male eggs are produced by *L. flavolineata* subordinates (Sumner et al. 2002). In contrast, we found no evidence to suggest that *P. alternata* subordinates produce males, although the power of our analysis was low as there were only 7 nests that contained a total of 12 male brood. Theory predicts that mutual policing of male production by subordinates may occur when subordinates are more closely related to males produced by the dominant than they are to the sons of other subordinates (Hammond and Keller 2004). *Parischnogaster alternata* subordinates were related to

Table 2

Colony structure of *Parischnogaster alternata* compared with *Liostenogaster flavolineata*

	P. alternata	*L. flavolineata*
Adult female relatedness	0.46 ± 0.05	0.52 ± 0.05
Reproductive skew	Uniformly high	Uniformly high
Modal mating frequency	1	1
Age-based queue for inheritance of dominance	Data are consistent with this hypothesis	Yes

male eggs and small larvae by $r = 0.56 \pm 0.04$. Each subordinate should therefore have preferred her own sons ($r = 1.0$) to those actually being reared, but subordinates may be indifferent between the dominant's sons and those of other subordinates. In contrast, the dominant should strongly prefer her own sons ($r = 1.0$) to those of subordinates. In *P. alternata*, where subordinates are generally not the offspring of dominants, a similar argument applies to female offspring. The small group sizes and small physical nest size in *P. alternata* suggest that queen policing of subordinate oviposition is feasible.

Parischnogaster alternata nests often consist of 2 or more nonoverlapping brood sibgroups with the older sibgroups having been produced by the previous dominants. Therefore, although the tenure of a dominant may be short, when it dies the surviving colony members continue to raise its brood. Overall, colony genetic structure in *P. alternata* is very similar to that previously reported for *L. flavolineata* and may be representative of hover wasps in general (Table 2). The main difference between *P. alternata* and *L. flavolineata* appears to be colony size. *Parischnogaster alternata* colonies have fewer adult females than *L. flavolineata* nests and also contain fewer brood, 7.76 ± 1.06 (this study) compared with 16.92 ± 1.44 (Sumner et al. 2002): $t_{(0.05(2)17,13)} = 5.24$, $P < 0.001$).

At least 78% of the females used in our genetic analyses were inseminated, and 55% of those collected in 2002 were potential egg layers, similar to the data of Turillazzi (1986). This indicates that a large number of subordinates are capable of laying both male and female eggs, although they do not do so (see also Richards et al. 2005). This begs the question of why so many subordinates have fully developed ovaries. It is unlikely that subordinates with developed ovaries were laying trophic eggs as there are no known examples of facultatively eusocial insects doing so (Crespi 1992). Furthermore, the laying of trophic eggs would not necessarily require insemination because uninseminated females can still lay haploid eggs. It is possible that subordinates have developed ovaries so that they can step immediately into the egg-laying position as soon as the reproductive tenure of the current dominant ends. Females that can begin ovipositing as soon as they achieve dominance are likely to gain greater fitness returns than unprepared females. Although at least 78% of females were inseminated, only 1.6 females per colony carried eggs of at least the minimum length laid. This suggests that potential egg layers may consist of dominants plus their probable successors.

In *P. alternata*, reproduction is nearly always monopolized by a single female regardless of the values of the putative explanatory variables, genetic relatedness, group size, and per capita productivity, which were all significantly more variable than skew. This high skew could reflect additional factors that apply to all colonies, such as strong ecological constraints on independent nesting, and that subordinates have a high probability of eventually inheriting the position of dominant (Kokko and Johnstone 1999; Ragsdale 1999). No females that nested

alone during the monitoring period were observed to survive or remain alone on their nests for longer than 3 weeks. This suggests that ecological constraints may be strong and that solitary nesting may be a viable reproductive strategy only when lone foundresses are joined by other females within a few weeks of nest initiation. Although we have no experimental data, the finding that dominants on 10 out of 12 *P. alternata* nests were the longest serving residents is consistent with findings in *L. flavolineata*, where subordinates form an age-based queue for inheritance of dominance (Shreeves and Field 2002; Bridge 2005). *Parischnogaster alternata* colonies are small, usually consisting of 2 or 3 females, so that queues are relatively short. In addition, the presence of more than a single brood sibgroup in 11 of the 15 nests suggests that the average reproductive tenure of a dominant may be relatively short. If this is the case, then subordinates may have a fairly high probability of inheriting the egg-laying position. A high probability of inheritance coupled with strong ecological constraints on solitary nesting may explain why *P. alternata* subordinates are prepared to accept the monopolization of reproduction by dominants (Kokko and Johnstone 1999; Ragsdale 1999): in terms of lifetime reproductive success, the partitioning of reproduction may actually be more equitable. Furthermore, *P. alternata* subordinates obtain some indirect fitness benefits through helping because there was a significant positive correlation between total group productivity and group size.

Sumner et al. (2002) contrasted the small group sizes, extended colony cycles, and absence of seasonal constraints on breeding in tropical hover wasps with the larger group sizes and more seasonally restricted annual nesting cycles of temperate wasps. They suggested that the uniformly high skew in *L. flavolineata*, compared with the lower, more variable skews that have been observed in temperate wasps (Field et al. 1998; Reeve et al. 2000; Seppa et al. 2002), may be because female hover wasps can afford to wait longer to inherit dominance than can seasonally restricted temperate wasps. The results presented in this paper for *P. alternata* are consistent with this idea. In fact, prospects of future breeding are likely to be important in explaining skew in most species as all organisms face a trade-off between present and future reproductive effort. For example, in all species of temperate wasps for which skew has been studied, skew has been found to increase as the colony cycle progresses and the chance of direct reproduction presumably decreases for subordinates (Field et al. 1998; Reeve et al. 2000; Seppa et al. 2002). Skew models that do not consider future breeding prospects may therefore have limited application.

More empirical studies using genetic markers are clearly needed to provide greater understanding of the factors that may affect genetic structure and the partitioning of reproduction in small-colony eusocial insects. Social wasp colonies are convenient systems for investigating skew. However, the uniformly high skews observed in the tropical stenogastrines *P. alternata* and *L. flavolineata* suggest that temperate species such as paper wasps, which have lower and more variable skews, may be better suited for testing the predictions of the skew models.

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