

Original Article

Reproductive skew is highly variable and correlated with genetic relatedness in a social apoid wasp

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Our knowledge of primitively eusocial societies is focused particularly on cooperatively breeding vertebrates and vespid wasps, whereas numerous taxa representing independent origins of social behavior have been largely overlooked. The lineage of apoid wasps including the genus *Microstigmus* represents a relatively neglected independent origin of eusociality. We present the first use of modern hypervariable genetic markers, in combination with behavioral observations, to investigate reproductive division of labor and cooperative brood care in an apoid wasp, the Brazilian *M. nigrophthalmus*. *Microstigmus nigrophthalmus* is unusual because, although there is cooperative brood care, reproductively dominant females carry out at least as much risky foraging as their subordinate nest mates. Empirical studies of reproductive skew are often hampered by a lack of variation in skew. We find that reproductive skew is highly variable between nests in *M. nigrophthalmus*. There was no correlation between skew and either body size or group size. The absence of an effect of body size is typical of studies of skew in insects and may indicate that body size is a poor measure of an individual's ability to control a group. However, skew was positively correlated with genetic relatedness. This provides rare support for concession models based on "social contracts" between dominant individuals and their subordinates. *Key words:* cooperative brood care, Crabronidae, microsatellites, relatedness, reproductive skew. [Behav Ecol 22:337–344 (2011)]

Eusociality is classically associated with reproductive division of labor (a monopolization of reproduction within a colony by a small number of individuals) and cooperative brood care (where individuals in the colony help raise the brood of others) (Michener 1969; Wilson 1971). To a large extent, the form that sociality takes in a given species can be described by these characteristics and the extent to which they vary between nests. In many social lineages, however, these characteristics remain little studied, severely limiting our appreciation of the range of social systems that exist.

The extent to which reproduction in animal societies is monopolized by one or a few individuals is termed "reproductive skew." Even in solitary animals, however, some individuals will be more successful at producing offspring than others. Reproductive skew is therefore used to describe a situation in which reproductive dominance reflects social superiority, not just greater reproductive potential. A large family of models predict how reproductive skew might be affected by various characteristics, such as relatedness, individual power, and group size (e.g., Reeve and Ratnieks 1993; Reeve and Keller 1995; Reeve 1998; Reeve et al. 1998; Johnstone and Cant 1999a, 1999b; Reeve and Shen 2006). For a review of most of these models, see Johnstone (2000). However, tests of these models have often been hampered by low variation in skew, greatly reducing their statistical power (Field and Cant 2009).

Most studies of cooperative brood care and reproductive division of labor have focused on advanced eusocial taxa (such

as ants and bees) or on a restricted group of primitively eusocial lineages (societies of vertebrates or vespid wasps). *Microstigmus* Ducke is a little-studied genus of neotropical apoid wasps that, despite representing an independent origin of sociality in the Hymenoptera (Ross and Matthews 1989), has largely escaped the attention of evolutionary biologists. Their small size and enclosed nests make it difficult to obtain behavioral data, and this has limited work on their social biology until the advent of molecular methods for relatedness estimation.

Only a single species, *M. comes*, has been subjected to a thorough analysis of social structure. Matthews (1968) found that multifemale nests of *M. comes* usually contained one female with ovaries far more developed than those of her nest mates, though several females engaged in foraging. Later, using allozyme markers, Ross and Matthews (1989) found that the genetic composition within nests was usually consistent with a single mother for all the immature brood. Their analysis was, however, not powerful enough to consistently identify reproductive individuals.

Microstigmus nigrophthalmus also lives in social groups, with nests containing up to 6 adult females with overlapping generations (Melo and Campos 1993; Melo 2000) but lacks the simple matrifilial structure described for *M. comes* (Lucas et al. 2010). *Microstigmus nigrophthalmus* nests are up to 2 cm in diameter and constructed from an amalgam of external organic material, such as particles of wood or bark, held together in a matrix of silk produced by the adult females. These nests are suspended by a silk petiole from the underside of leaves. The brood are provisioned progressively (i.e., food is provided gradually as the offspring develop) in contrast to many other species in the genus, such as *M. comes*, which are mass provisioners (Richards 1972;

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West-Eberhard 1977; Melo 1992). Melo (2000) reports that female nest mates tend to all have developed ovaries in contrast to what has been found in *M. comes*. There is no clear breeding season, and reproduction appears to occur all year round, with nests being perpetuated through repeated inheritance (Melo 2000), creating a great variety of social structures (Lucas et al. 2010).

In this paper, we have 2 aims. First, we use behavioral observations and genetic analysis of kinship to examine cooperative brood care and reproductive division of labor in *M. nigrophthalmus*. Second, we investigate reproductive skew in relation to 3 theoretically important factors: genetic relatedness, variance in body size, and group size.

MATERIALS AND METHODS

Field methods

Fieldwork was conducted at the Mata do Paraiso, Viçosa, Minas Gerais, Brazil (lat 20°48'S, long 42°51'W), from 27 March 2008 to 11 September 2008. This field site is a reserve of inland Atlantic forest (Oliveira-Filho and Fontes 2000), covering around 200 ha at approximately 600 to 700 m elevation, belonging to the Universidade Federal de Viçosa. The forest is around 80 years old and was developed on what had originally been farmland. The surrounding area contains several patches of Atlantic forest of varying sizes separated by farmland.

Nests were located by searching the underside of leaves in the forest. In order to conduct behavioral observations, females were marked for recognition using enamel paint and fine-meshed marking cages (Lucas et al. 2010).

Video recordings

Behavioral observations were conducted on 14 nests from which genetic data were later obtained. Sony DCR SR32E digital camcorders were placed at a distance of approximately 30 cm from the nest, positioned so that the entrance faced the lens as much as possible. Nests were videoed for 1 day each week over the course of at least 4 weeks, with each day's videoing lasting around 6 h. Each nest was videoed for 2 consecutive days immediately prior to collection, and videoing on the final day was extended to approximately 8 h in order to last until the moment of collection.

After observations were complete, nests were collected after nightfall to ensure that all wasps were on the nest. Each nest was surrounded with a small ziplock bag, and the petiole was pulled or cut off the supporting leaf so that the whole nest and its contents were captured. Adults and brood were stored by killing them in 100% ethanol. If it had been raining before nightfall, it was considered possible that some individuals may have been unable to return to their nests and no collections would be performed that night.

Molecular genetic analysis

Maternity and relatedness were analyzed using microsatellite markers Mni001–003, Mni005, Mni007, Mni008, Mni011–014, Mni016, Mni019, Mni020, Mni023, Mni027–035, Mni038, Mni042–044, Mni047, Mni048, and Oni001 (Lucas et al. 2009). DNA was extracted and amplified using the methods described in Lucas et al. (2010). Polymerase chain reaction products were analyzed using an Applied Biosystems 3730 sequencer at the Natural Environment Research Council Biomolecular Analysis Facility —Sheffield (NBAF-S), Sheffield, UK. Alleles were scored using the software Genemapper 3.7 from Applied Biosystems.

Brood were sexed, and offspring were assigned to their mothers using the software KINGROUP (Konovalov et al.

2004) as described in Lucas et al. (2010). All tests were conducted with a significance level of $\alpha = 0.05$.

Calculation of reproductive skew

Because the concept of reproductive division of labor cannot be applied to single female nests, these were excluded from this analysis. Of the 14 nests that were videoed for behavioral observations, 9 contained more than 1 female. A further 11 nests, which were collected using the same method as the videoed nests, were also included in this analysis for a total of 20 nests.

Reproductive skew was calculated among all female nest mates using the index Q suggested by Ruzzante et al. (1996). A value of $Q = 0$ indicates that the variance in brood number is equal to that expected by random chance. $Q \geq 0$ indicates overdispersion of brood numbers, whereas $Q < 0$ indicates that brood are more equally distributed among potential mothers than would be expected by random chance (Ruzzante et al. 1996). The maximum value of Q is 1, which occurs when one female is the mother of all the brood. Q can be calculated only if the female nest mates are between them the mothers of at least 2 offspring. This excluded 1 of the 11 nonvideoed nests from the calculation, leaving 19 nests for which the skew index could be obtained.

Nest cells of *M. nigrophthalmus* are occasionally found containing more than one egg. There is good evidence that, in these cases, only one egg survives to the larval stage (Melo 2000; Lucas 2009). The value of cell-sharing eggs therefore needs to be devalued according to the egg's probability of being the one that survives, which can be estimated as $1/n_e$, where n_e is the number of eggs sharing the cell. So, if a female was assigned as the mother of 2 larvae and of 1 egg, which was found in the same cell as 2 other eggs, she was estimated to have 2.33 offspring for the purpose of reproductive skew calculations.

Q was calculated for each nest, and the mean, \bar{Q} , was calculated across the 19 nests. In order to test whether skew was significantly different from 0 in our sample, 10 000 randomizations were performed in which, within each nest, each brood item was randomly reallocated among the females in that nest. The observed value of \bar{Q} was then compared with those obtained in the simulations.

The 9 nests that had been subjected to video observations were used to examine the potential effect of age on whether a female was assigned as the mother of any brood (the method for estimating female age is detailed below). A further 10 000 randomizations were performed based on the hypothesis that females below the age of 10 days were incapable of reproducing but that brood were allocated randomly between the remaining females.

Group size, relatedness, and individual power

Group size was calculated as the number of females present in the nest on collection. Life-for-life relatedness (r) (Hamilton 1972) was calculated between female nest mates using the software RELATEDNESS v.5.0.8 (Queller and Goodnight 1989). Correlations were performed between Q and group size and between Q and relatedness.

Resource-holding potential could not be estimated directly. Body size (calculated as wing size) was therefore used as a proxy for this. Wing size was measured as the distance between the distal tip of the marginal cell and the junction of the media and cubitus veins on the right forewing, following the terminology of Bohart and Menke (1976). Measurements were taken with a binocular microscope with a graticule fitted inside the

eyepiece and calibrated against a 1-mm standard. We also measured head width as an alternative proxy for body size. However, measurements were harder to take consistently, and we therefore considered them less reliable. As the results regarding head width were qualitatively similar to those regarding wing size, we report only the latter here.

If size is indeed related to resource-holding power and if resource-holding power is in turn related to reproductive fitness, then the size of a female should affect the number of offspring she has in the nest. Within each nest, a Spearman's rank correlation was performed between size and the number of immature offspring assigned to each female. The number of positive and negative correlation coefficients were compared using a sign test. To examine whether the extent of the difference in resource-holding power had an effect on reproductive skew, a Spearman's rank correlation was performed between Q and the within-nest variance in wing size.

As there were no significant pair-wise correlations between any of these potential explanatory variables (relatedness, variance in wing size, and group size, $P \geq 0.4$ in all cases), the correlations between reproductive skew and each of these factors were considered to be independent.

Estimation of female age

For the 9 nests that were subjected to video observations, we could estimate the minimum age of individual females in the nest. The day when a female was first observed was considered to be her estimated date of "arrival." The time between this estimated date of arrival and the day she was collected was used as an estimate of the minimum age of the female. Although the possibility that the female had arrived from a different nest could not be excluded, we have no evidence that females of this species ever immigrate from foreign nests. Individuals present on the first day of video observations were given this date as their estimated date of arrival. This estimate of female age is not precise, but it is sufficient to crudely differentiate between females that appeared early or late in the observation period.

In order to examine the effect of female age on reproductive dominance, correlations were performed between female age and the number of offspring assigned to a female. In each of the 9 nests, Q , relatedness, variance in body size, and group size were recalculated, with all females below the age of 10 days excluded. Q was then once again correlated against each of the explanatory variables described in section 'Group size, relatedness, and individual power'.

Statistical tests

All statistical analyzes not requiring KINGROUP or RELATEDNESS were conducted in R (R Development Core Team 2008). Data were tested for normality of residuals with an Anderson-Darling test and for constancy of variance with a Levene's test. Data, which did not violate the assumptions of normality and constancy of variance, were treated with parametric tests. Those that did were analyzed with nonparametric tests. All correlations with Q were performed nonparametrically as several points were equal to the maximum value of 1, indicating a nonnormal distribution.

RESULTS

Reproductive skew

Reproduction was significantly skewed but was nevertheless often shared between group members. The average proportion of females on multifemale nests that could be identified

as the mother of at least one offspring was 55%, both with nests weighted equally ($n = 20$ nests, $\sigma = 17$) and with individuals weighted equally ($n = 56$ individuals). Q , the measure of reproductive skew, was highly variable. The mean value of Q across the 19 nests was $\bar{Q} = 0.62$ ($\sigma = 0.46$). Of 10 000 simulations performed, assuming the null hypothesis where each individual in a nest had an equal chance of being allocated a given offspring, the highest observed value of Q was 0.33.

In 2 of the 9 videoed nests, it was not possible to test for a correlation between female age and number of offspring as there was no variation in the estimated age of the females on the nest. In the remaining 7 cases, correlation coefficients were always positive (sign test, $n_1 = 7$ positive correlations, $n_2 = 0$ negative correlations, $P = 0.02$). Older females may have more offspring purely by virtue of having had more time to produce them. This can be controlled for by considering only eggs in the calculation of skew as these will have been laid recently. Only 6 of the 7 nests produced a positive correlation coefficient when female age was correlated against number of eggs assigned to her. The remaining nest produced a negative correlation coefficient. This pattern is nonsignificant (sign test, $n_1 = 6$ positive correlations, $n_2 = 1$ negative correlations, $P = 0.12$). However, only if all 7 nests had produced a correlation in the same direction would the result have been significant. This test would therefore have been powerful enough to pick out a significant difference 80% of the time only if the true probability of obtaining a positive correlation coefficient in a given nest was greater than 0.97 or smaller than 0.03.

None of the 5 adult females estimated to have been less than 10 days old on collection were found to be the mother of any brood on the nest. The youngest females to have been assigned brood were 20 days old (no individuals between 10 and 19 days of age were present in this analysis). If we assume that the 5 individuals younger than 10 days were not reproducing because they were too young, then we estimate that 19% of females in the population are too young to reproduce. Of 21 females that were at least 20 days old, 5 (24%) could not be assigned as mothers to any brood in their nest. Thus, females do not always become reproductive once they reach a certain age. Similarly, nests in which one individual had produced all the offspring sometimes contained other females at least 20 days old.

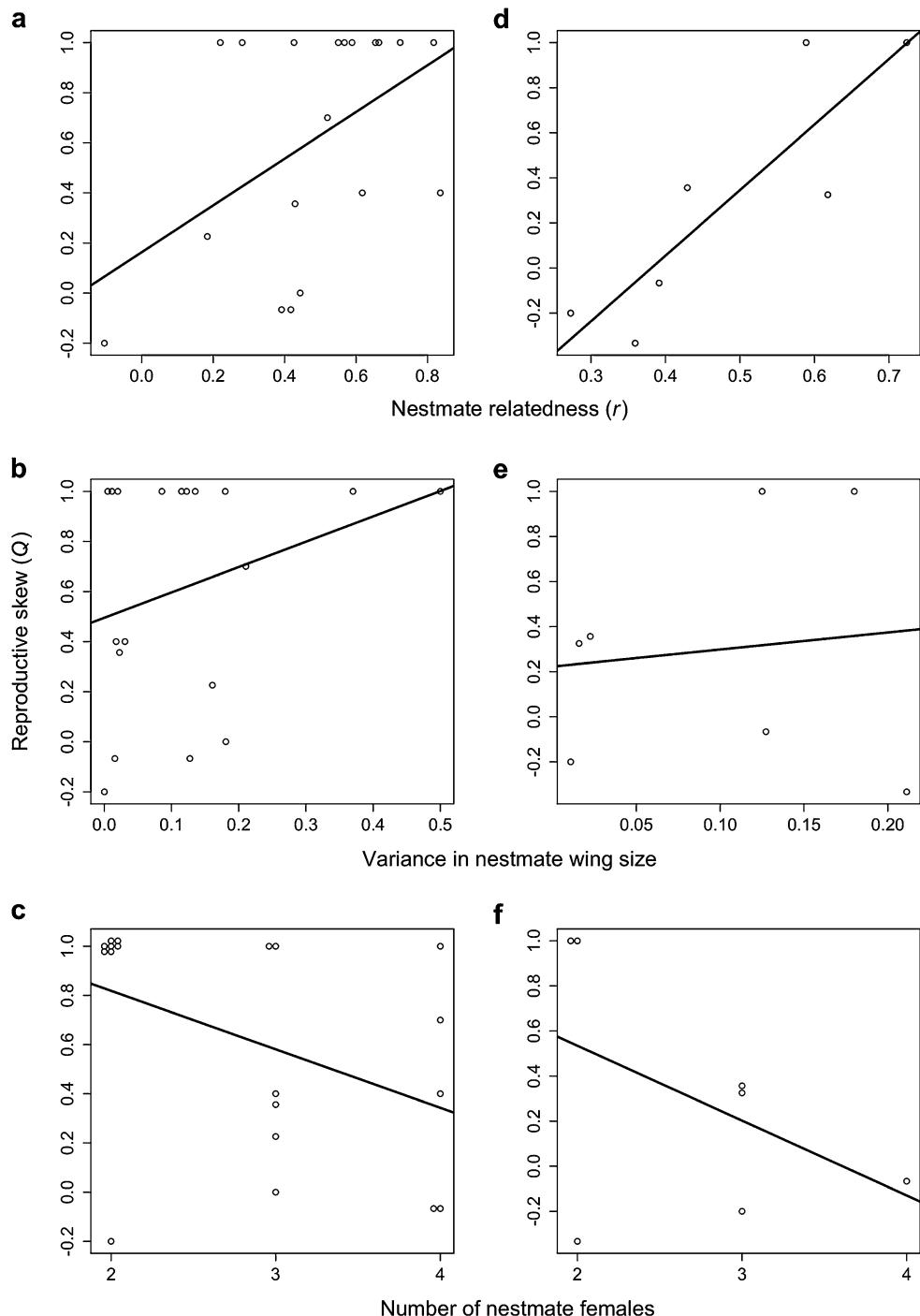
The mean value of Q across the 9 nests that had been videoed was $\bar{Q} = 0.51$ ($\sigma = 0.49$). Of 10 000 simulations performed according to the hypothesis that skew was 0 among individuals that were at least 10 days old but that younger individuals could not reproduce, 0.26% had a value of $Q_{9,0}$ larger or equal to 0.51. The P value for this test was therefore $P = 0.0026$.

In most nests, only 1 or 2 adult females had immature offspring in the nest at the time of collection. There were 2 exceptions in which 3 females had offspring in the nest. Usually, one female was found to be the mother of the majority of the brood, but this was not always the case.

Factors affecting reproductive skew

Reproductive skew and relatedness

Reproductive skew was positively correlated with relatedness (Figure 1a,d). There was a barely significant positive correlation between relatedness (r) and reproductive skew as measured by Q (Spearman's rank correlation, $r = 0.45$, degrees of freedom (df) = 17, $P = 0.05$). The correlation remained significant when young females were excluded, despite the large reduction in the number of nests included in this analysis (Spearman's rank correlation, $r = 0.83$, df = 5, $P = 0.02$).

**Figure 1**

Best-fit regression lines of reproductive skew against nest mate relatedness (a and d), variance in nest mate wing size (b and e), and group size (c and f). Figures a–c represent all 19 nests for which Q was calculated. Figures d–f represent the 9 nests for which estimates of female age were available, with females younger than 10 days removed from the calculation of skew. Points are jittered to show overlapping data.

Reproductive skew and body size

Within nests, wing size was correlated with the number of immature offspring assigned to a female but, surprisingly, it was smaller females that tended to have more offspring. Of 19 nests that displayed variation in wing size, Spearman's rank correlation coefficients between wing size and number of brood assigned to a female were positive in 2 nests, negative in 16 nests, and 0 in 1 nest (sign test, $n_1 = 2$ positive values, $n_2 = 16$ negative values, $P = 0.001$).

There was no correlation between the variance in female wing size within a nest and Q (Figure 1b: Spearman's rank correlation, $r = 0.18$, $df = 17$, $P = 0.46$). This was still the case when the data set was reduced so that only older females

were included (Figure 1e: Spearman's rank correlation, $r = 0$, $df = 5$, $P = 1$).

Among the nests for which females could be assigned an approximate age, wing size was negatively correlated with age. Of 9 nests, 2 showed no variation in estimated age between nest mates, and no correlation coefficient could be calculated. Among the remaining 7 nests, all the correlation coefficients of wing size with age were negative (sign test: $n_1 = 0$ positive values, $n_1 = 7$ negative values, $P = 0.02$).

Reproductive skew and group size

The relationship between reproductive skew and group size was ambiguous. There was a significant negative correlation

Table 1

Number of mothers and nonmothers observed to be foraging during the last few days before nest collection

	Mothers	Nonmothers
Forager	12	4
Nonforager	8	12

between group size and Q (Figure 1c: Spearman's rank correlation, $r = -0.47$, $df = 17$, $P = 0.04$). However, this was not the case for the reduced data set in which younger females were excluded (Figure 1f: Spearman's rank correlation, $r = -0.31$, $df = 5$, $P = 0.50$). It should be noted that, in the reduced data set, the correlation coefficient is closer to zero than for the larger data set. The lack of significance when considering the reduced data set is therefore not only due to a reduction in statistical power associated with a smaller sample size. Instead, the proportion of the variance explained by the explanatory variable decreased when a more refined test was conducted.

Cooperative brood care

Foraging tended to be performed by individuals who were the mothers of some of the brood, but other females also participated. Twelve of 16 (75%) individuals observed to have been foraging in the 2–3 days prior to nest collection were identified as mothers of current brood. This proportion becomes 81% if nests are weighted equally ($n = 12$ nests, $\sigma = 36$). This is higher than the 55% observed if all adult females are considered (see above). Table 1 shows the number of individuals recorded as being mothers or nonmothers depending on whether they had been observed foraging or not. As the data from the table are not all independent (some females are nest mates), it was not appropriate to perform a χ^2 test on these data. However, given the strong bias toward mothers among the foragers, it seems very unlikely that nonmothers are in fact the principle foragers.

DISCUSSION

Reproductive division of labor

The level of reproductive skew in multifemale nests, as measured by the index Q (Ruzzante et al. 1996), was significantly greater than 0. This was still the case even when recently emerged individuals were removed from the analysis. Older females tended to have more offspring in the nest than younger ones, except in the case where only eggs, but not larvae and pupae, were considered. The latter case, however, still produced a relatively small P value (0.12) despite very low statistical power. We consider that the accumulated evidence suggests that age does have an effect on reproductive dominance in *M. nigrophthalmus*.

Caution must nevertheless be exercised before concluding that there is a reproductive division of labor in *M. nigrophthalmus*. Even nonsocial organisms will exhibit some level of reproductive skew because some individuals are typically more successful at reproducing than others. Overall, over half of all adult females were assigned as mothers to at least one offspring in the nest at the time of collection. There is also good evidence that, in this species, almost all females are inseminated and have at least slightly developed ovaries (Melo 2000).

The best evidence that there is positive reproductive skew in *M. nigrophthalmus* comes from the significant correlation between skew and the purely social characteristic of relatedness.

It therefore appears that, although most females in this species are prepared to reproduce, a form of social dominance exists, which limits the actual reproductive success of some individuals.

Cooperative brood care

There was no evidence for any behavioral worker caste in *M. nigrophthalmus*. In general, it was found that females returning to the nest with prey tended to be the mothers of brood in the nest. Due to the enclosed nature of the nests, it was not possible to observe which brood were provisioned by a given female. However, there were cases of individuals foraging despite not having their own offspring to feed, indicating that foragers do not provide only for their own brood. Therefore, although there is no evidence of a distinct worker caste, it appears that females of *M. nigrophthalmus* do invest costly effort in the brood of others. This combination of traits is unusual. In some groups of allodapines, the reproductive individuals forage, whereas remaining nest mates provide cooperative brood care (Schwarz et al. 2010), but the brood care in question comprises within-nest activities not participation in foraging.

To our knowledge, *M. nigrophthalmus* presents the first substantiated example of a nest-sharing wasp or bee with cooperative brood care in which skew is not consistently high. That there are few other convincing examples is not necessarily a sign of the rarity of this condition. Most molecular genetic work in the Hymenoptera has focused on highly social species. A few studies of less socially advanced species have looked at overall relatedness between nest mates (e.g., McCorquodale 1988; Kukuk and Sage 1994; Danforth et al. 1996), but more powerful analyses are required to assign brood to mothers. Without knowing which adults are mothers of larvae, it is difficult to determine whether a forager is providing for her own brood.

Eusociality in *M. nigrophthalmus*

Wilson (1971) and Michener (1974) defined eusociality as the combination of overlap of generations, reproductive division of labor, and cooperative brood care because they recognized these as important characteristics of social behavior. For this reason, and because an overlap of generations has already been established (Melo 2000), we have focused on the latter 2 characteristics in *M. nigrophthalmus*. However, several factors make it difficult to obtain the level of detail required for confident determination of the exact extent of these characteristics.

Microstigmus nigrophthalmus lives in a small enclosed nests within which behavioral observations are very difficult. Lifetime reproductive success cannot satisfactorily be estimated because ovipositions are not visible, meaning that individuals can be assigned to mothers only if they are collected. Therefore, reproductive skew data can be collected only as instantaneous measurements on collection. In some species, this problem can be addressed by nonlethal sampling of emerging adults (e.g., Liebert et al. 2005). Studies have shown that DNA can be collected from some social insect species by removing a piece of leg without significantly affecting the behavior of the individuals involved (Starks and Peters 2002; Holehouse et al. 2003). In *M. nigrophthalmus*, however, it appears that this is not the case (Lucas 2009). Equally, as the allocation of prey within the nest cannot be followed, it cannot be determined whether a forager will preferentially provision her own brood if she has any or whether prey is effectively distributed randomly with respect to kinship.

From this study, we can conclude that *M. nigrophthalmus* displays all 3 characteristics identified by Wilson (1971) and Michener (1974) to at least some extent. For example, reproductive skew was greater than 0 but was variable and was less extreme than in most "eusocial" species. This puts *M. nigrophthalmus*, along with many other species, in a rather gray area with regards to eusociality. Overall, however, we consider it more useful to concentrate on the individual behavioral characteristics rather than to dwell on the definition of eusociality (Wcislo 1997).

Implications for models of reproductive skew

Relatedness

Different models of reproductive skew make very different predictions about the relationship between skew and relatedness. Skew may be predicted to decrease or increase with relatedness or not be affected by relatedness at all. In *M. nigrophthalmus*, reproductive skew increases with increasing relatedness, thus supporting the predictions of models by Reeve and Ratnieks (1993), Johnstone et al. (1999), Kokko and Johnstone (1999), Ragsdale (1999), and Reeve and Emlen (2000). These are all so-called "transactional" models, which follow the logic of the original models by Vehrencamp (1983) and Reeve (1991), where a dominant female controls reproduction by subordinates and permits them to reproduce only as much as is necessary to keep them from leaving and nesting independently. As a more closely related subordinate can obtain greater indirect fitness through raising the dominant's offspring than can a less related one, the amount of subordinate reproduction which the dominant needs to allow decreases with increasing relatedness.

Although transactional models predict a positive association between relatedness and skew, few experimental studies have found evidence of this. We do not include studies that find simply that both mean skew and mean relatedness are low/high in a given population (e.g., Richards and Packer 1998; Fournier and Keller 2001). In the social Hymenoptera, negative correlations between relatedness and skew have been found in ants (Rüppell et al. 2002; Hannonen and Sundström 2003), bees (Langer et al. 2004, 2006), and possibly wasps (Field et al. 1998) (in the latter study, relatedness was correlated with the proportion of reproduction achieved by the dominant wasp but not with the index of skew). Positive correlations, to our knowledge, have been found only in the wasp *Polistes fuscatus* (Reeve et al. 2000), though queens of the sweat bee, *Lasioglossum malachurum*, may allow unrelated workers to reproduce more than related workers (Paxton et al. 2002). Over half the studies we have found that investigated the relationship between relatedness and skew in social insects found no evidence of a correlation (in ants: Evans 1996; Heinze et al. 2001; Fournier et al. 2004; Hammond et al. 2006 and in wasps: Seppä et al. 2002; Sumner et al. 2002; Fanelli et al. 2005). However, these results must be considered with caution. Most of these studies found very little variation in relatedness and/or skew, so tests for an association between the 2 had low power (Heinze et al. 2001; Seppä et al. 2002; Sumner et al. 2002; Fournier et al. 2004; Fanelli et al. 2005), whereas the study by Evans (1996) had large error margins for the estimates of relatedness. Lack of correlations with relatedness is therefore more closely associated with low statistical power than anything else.

Too few studies have been performed to provide an accurate impression of how reproductive skew tends to be associated with relatedness across the Hymenoptera. However, both negative and positive associations have been found, and there does not appear to be a clear taxonomic divide behind these results. Several parameters may affect the predicted

association between relatedness and reproductive skew. The model of Johnstone (2000), for example, could not be tested in this study because its predictions are complex and require accurate quantification of parameters such as constraints on independent founding and the extent to which some individuals are better able to utilize group resources as well as knowing the range of relatedness found in the study organism. Whether the contrasting results among different studies of relatedness and reproductive skew are due to variation in such parameters, or in the extent to which it is possible for a single individual to control reproduction, is not known.

Body size

Models, which examine the effect of competitive ability, assume that it has a positive effect on reproductive success and therefore predict a positive correlation between variance in competitive ability and reproductive skew. There was no evidence of such an effect in *M. nigrophthalmus*. One possible explanation for our results is that body size is not a good indicator of competitive ability in this species.

Surprisingly, body size was in fact negatively correlated with a female's reproductive success within nests of *M. nigrophthalmus*. A similar result was discussed by Hogendoorn and Velthuis (1999) in 2 species of *Ceratina* bees, though this was not statistically significant. In these dyadic associations, the smaller bee is consistently the forager, whereas the larger one guards the nest, yet the foragers seem able to claim more than 50% of the reproduction.

We found that older *M. nigrophthalmus* females tend to have smaller wings than younger ones. It seems unlikely that this is due to some unprecedented kind of shrinking with age. More probably, this trend could be caused by changes in environmental conditions over our 6 month collection period. Conditions may have improved over this period, either seasonally or by chance, so that younger females were reared in better conditions than older ones. As older females tended to have more offspring in the nest than younger ones, this could lead to an illusory association between wing size and offspring production.

The finding that differences in female size do not correlate with reproductive skew is in accordance with other results from the literature (Field and Cant 2009). Field et al. (1998), Reeve et al. (2000), Rüppell et al. (2002), Seppä et al. (2002), Sumner et al. (2002), and Fanelli et al. (2005) all found no effect of size difference on skew. Several studies have found that size is not even associated with dominance (e.g., Field et al. 1998; Rüppell et al. 2002; Seppä et al. 2002; Fanelli et al. 2005). It seems improbable that reproductive skew is not associated in any way with some kind of competitive ability. We consider it more likely that, in the social Hymenoptera at least, size may be a poor indicator of competitive ability with regards to competing for direct reproductive success in an established nest. It is quite possible that size is related to survival or to nest-founding success but not to the ability to gain a high share in reproduction in the nest. Greater success at nest founding is predicted to influence reproductive skew in transactional models (Reeve 1991; Reeve and Ratnieks 1993), but this may be confounded by an associated increase in the help an individual can provide as a subordinate, which should influence skew in the opposite direction.

Group size

The relationship between reproductive skew and group size was inconclusive but was either significantly or nonsignificantly negative. Only one model attempts to predict the effect of group size on reproductive skew, but the direction of the predicted correlation depends on other parameters (Reeve and Emlen 2000). Conclusions can therefore only very tentatively be drawn. A negative correlation is predicted when

relatedness is high and solitary founding success is low. As relatedness has indeed been found to be high in *M. nigrophthalmus*, it may be that this possible negative correlation of skew with group size indicates a low success of independent founding in the species. Cant and English (2006) model the effect of reproductive skew on group size, but the nature of their predictions depends on quantifying the relative mortalities of breeders and nonbreeders and therefore could not be tested here.

Reproductive skew and social contracts

Reproductive skew has attracted much attention in the 2 decades since it was first highlighted as a potentially unifying concept (Sherman et al. 1995) and remains an active focus of research in animal societies from vertebrates to insects (Hager and Jones 2009). However, advances in the field have come mostly from theoretical insights and models, whereas the collection of field data has lagged far behind. In particular, there has been a lack of diversity in the taxa that have been investigated. To our knowledge, this study represents the first investigation of reproductive skew in a species of apoid wasp.

This study also provides rare evidence for the positive correlation between reproductive skew and relatedness predicted by most transactional models of skew. Such models have formed the basis for the bulk of theoretical work on skew so far, yet there has been little support for this key prediction. Our results may indicate a social hierarchy in *M. nigrophthalmus* governed by so-called "social contracts" with regards to reproductive effort. Further studies on other *Microstigmus* species are needed to determine whether such contracts are typical in this genus. The absence of correlations between reproductive skew and relatedness in other taxa might reflect differences in the factors that govern skew so that an important next step is to understand the basis of this variation. However, as we have discussed, the data available for many social insect groups do not allow sufficiently powerful tests to conclude whether such correlations truly exist. In-depth study of a wider range of systems that exhibit variation in reproductive skew, at either the intraspecific or interspecific level, will be required.

Microstigmus nigrophthalmus displays overlap of generations, cooperative brood care, and reproductive division of labor, though, as discussed above, the exact extent of the latter 2 characteristics remains unclear. The existence of highly variable reproductive skew in a species with cooperative brood care is not only highly unusual in wasps and bees but also provides desirable variation for the testing of models.

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