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Social and genetic structure in colonies of the social wasp *Microstigmus nigrophthalmus*

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Abstract *Microstigmus* (Hymenoptera: Crabronidae) is a genus of social apoid wasps which represents an origin of sociality independent from vespoids, but which has so far received little attention. Though group-nesting is widespread in *Microstigmus*, genetic relatedness has so far been studied in only one species, *M. comes*. We report on the social biology of *M. nigrophthalmus*, drawing from behavioural observations and molecular genetic analyses of relatedness and kinship. There was no evidence of distinctive behavioural suites that distinguished reproductive and non-reproductive individuals. Females could mate more than once, but mating frequency was low. Mean relatedness within nests was high, particularly between females (close to 0.5), but pairwise relatedness values were very variable, as nestmates displayed a wide range of relationships. Such high levels of relatedness should be a factor promoting social nesting and cooperative brood care in this species, as females gain only a slight genetic advantage through rearing their own offspring rather than those of nestmates. This study provides the finest analysis of genetic structure so far in an apoid wasp, and indicates that the form of sociality varies greatly between species of *Microstigmus*.

Keywords Relatedness · Kinship · Social structure · Crabronidae

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Introduction

The super-family Apoidea (Hymenoptera) comprises the socially diverse and species-rich family Apidae (bees), as well as several lesser-known families of principally solitary or communal wasps. These latter groups, loosely termed "apoid wasps", display a wide range of nesting and foraging strategies (O'Neill, 2001) and there is evidence that social altruism does exist in at least the lineage containing the genus *Microstigmus* (Matthews, 1991). However, very few studies have so far been conducted to examine the genetic structure of nesting groups in social apoid lineages.

Microstigmus Ducke (Crabronidae) is a genus of neotropical wasps which, despite representing an independent evolutionary origin of sociality in the Hymenoptera (Ross and Matthews, 1989a), has largely escaped the attention of evolutionary biologists. Their small size and enclosed nests make it difficult to obtain behavioural data, and this has limited work on their social biology.

Microstigmus species range in size from about 2.5 to 5.5 mm (Melo, 1992; Richards, 1972). Prey varies according to species and has not been identified in every case, but is usually Collembola (springtails), Thysanoptera (thrips) or Cicadellidae (leafhoppers) (Melo and Campos, 1993a; Richards, 1972; West-Eberhard, 1977). The nests are enclosed and hang from a silk petiole with only a small opening to the outside (Matthews, 1991; Richards, 1972).

Nest location and building material also vary between species, though the material is always bound with silk produced by adult females. Examples include *M. myersi*, which nests under mud banks and mixes silk with mud (Melo and Campos, 1993a), *M. comes*, which suspends its nests from the underside of leaves and uses leaf fibres (Matthews and Starr, 1984) and *M. brasiliensis*, which builds nests under tree buttresses and uses lichen and wood material (Melo, 1992). In *M. myersi* and *M. puncticeps*, the petiole is formed from a root tip rather than silk (Melo and Campos, 1993a; Melo and Matthews, 1997).

Until now, most work on *Microstigmus* has focused on describing their biology, demographics or aspects of nesting behaviour (Asís, 2003; Carithers, 1997; Grajales and Wcislo, 1998; Marsh and Melo, 1999; Matthews and Starr, 1984; Melo, 1992; Melo, 1997; Melo and Campos, 1993a; Melo and Campos, 1993b; Melo and Evans, 1993; Melo and Matthews, 1997; Richards, 1971; Richards, 1972; West-Eberhard, 1977). Only a single species, *M. comes*, has been subjected to a thorough analysis of social structure. Matthews (1968) found that multi-female nests of *M. comes* usually contained one individual with ovaries far more developed than those of its nestmates, despite the observation that several females engage in foraging. Later, using allozyme markers, Ross and Matthews (1989a) found that the genetic composition within *M. comes* nests was usually consistent with the brood having been produced by a single female.

The phylogenetic position of *Microstigmus* (Melo, 1999) makes these findings particularly interesting, as *M. comes* is the only known example of dedicated workers in the apoid wasps. This should encourage researchers to further investigate the extent and variability of social behaviour in *Microstigmus*. So far, however, data regarding other species in the genus are sparse.

Melo and Campos (1993b) and Melo (2000) have presented mainly anecdotal behavioural data on *M. nigrophthalmus*, one of the larger species. *M. nigrophthalmus* preys on Cicadellidae nymphs (Melo, 1992) and is a progressive provisioner (that is, it lays its eggs in empty cells, then collects prey as and when the developing larvae require it). This contrasts with many other species in the genus, such as *M. comes*, which are mass provisioners (Melo, 1992; Melo and Campos, 1993a; Richards, 1972; West-Eberhard, 1977). Melo (2000) reports that female nestmates all tend to have developed ovaries, in contrast to what has been found in *M. comes*. Using an otoscope to look through the nest entrances, he also observed an apparent division of labour in a nest containing two adult females. However, he points out that only one nest showed such a clear distinction between roles. Using the same method, Melo and Campos (1993b) found evidence of trophallaxis between adults within the nest.

Other than this, *M. nigrophthalmus* has thus far received no attention, despite the potential for contrasts with apparently more socially advanced congeners. In this paper we present more extensive information on the biology and social structure of *M. nigrophthalmus*, drawing from genetic analyses of relatedness and kinship and medium term behavioural observations.

Methods

Field methods

Field work was conducted at the Mata do Paraiso, Viçosa, Minas Gerais, Brazil (20°48′S, 42°51′W), over the course of two field seasons, one from 22/11/2005 to 10/01/2006 and the other from 27/03/2008 to 11/09/2008. The first field season consisted of nest collections, while the second field season was used for behavioural observations as well as further nest collections.

The field site is the type locality for *M. nigrophthalmus.* It is a reserve of inland Atlantic forest (Oliveira-Filho and Fontes, 2000), covering around 200 ha and approximately 600–700 m in 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. We found that *M. nigrophthalmus* may build nests on almost any plant, provided that the leaf is sturdy enough to hold them. In order to conduct behavioural observations, females were individually marked (see Supplementary Material for details). Individuals were collected by placing a small ziplock bag around their nest and gently tapping the leaf from which it was suspended. The wasps would then evacuate and become caught in the bag. On some occasions, wasps were also collected by placing a pooter to the entrance of the nest and aspirating its contents. After marking, each wasp was released in good weather next to its nest, or directly onto it if possible.

Males were marked if caught, but no effort was made to ensure that all males on a nest were marked. This did not hamper behavioural observations of females, as it is easy to distinguish between the sexes with the naked eye. Males were marked with a common colour (with one exception), but distinct from the colours used for females.

Video recordings

Behaviour on the nest exterior was videoed on 17 nests in the second field season (see Supplementary Material for details). Nests were videoed for 1 day each week over the

course of at least 4 weeks, with each day's videoing lasting around 6–8 h. Fourteen out of the 17 nests were videoed on two consecutive days immediately prior to collection. One nest was videoed on the day of collection, but not the previous day. Videoing on the day of collection always lasted until the moment of collection (around 18.00), this allowed us to determine whether all individuals had returned to the nest for collection. Finally, two nests failed (all adults disappeared) before the end of the videoing period, and were collected after it had been confirmed that they had failed. Total observation time for a nest observed during the second field season ranged from 31 to 87 h.

Collections

After observations were complete, entire nests with all contents were collected before dawn or after nightfall in a ziplock bag to ensure all wasps were in the nest. If it had been raining before nightfall, it was considered possible that some individuals could have been unable to return to their nests and no collections would be performed that night. A further 26 nests from the second field season and 41 nests from the first field season, not used for behavioural observations, were collected in the same way. Adults and brood were stored by killing them in 96–100% ethanol.

Molecular genetic analysis

Social structure and relatedness in nests from the second field season were analysed 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 using an ammonium acetate method and amplified using multiplex PCR (see Supplementary Material). PCR products were analysed using an Applied Biosystems 3730 sequencer at the NERC Biomolecular Analysis Facility–Sheffield (NBAF-S), Sheffield, UK. Alleles were scored using the software Genemapper 3.7 from Applied Biosystems. Adult females had either one or two alleles at each locus, while all adult males had only one. Brood were sexed by genetic analysis: individuals that were homozygous at every locus were considered to be males. Excluding the possibility of typing error, the probability of a male being wrongly assigned as a female was therefore 0 and the probability of a female being wrongly assigned as a male was calculated as 2.8×10^{-15} based on the observed allele frequencies.

Group size and genetic structure

Group size was calculated as the number of adult females found in the nest upon collection. Brood size was calculated as the number of eggs, larvae and pupae found in the nest upon collection. Cells were sometimes found containing more than one egg. As there is good evidence that only one egg survives to become a larva (Lucas, 2009; Melo, 2000), the estimate of brood size used only a single egg from such cells.

Thirty-two nests from the second field season were included in the analysis of genetic structure. For each of these nests, we attempted to reconstruct a family tree of the individuals within it. The relationships between pairs of individuals in a nest were determined using the software KINGROUP (Konovalov et al., 2004). This uses a maximum likelihood approach to compare hypothesised relationships between individuals and determine whether one hypothesis can be significantly rejected based on these likelihoods. The threshold likelihood values on which the significance tests are based are calculated from the population allele frequencies and apply only to pairs of individuals which have been successfully typed at all loci in the analysis. Individuals for which data was missing at certain loci were therefore analysed independently, with the missing loci removed from the analysis, in order to generate unbiased p values.

This type of analysis can in some cases generate seemingly contradictory results. It is important to note that if one is testing between alternative hypotheses A and B, it is possible for A to be rejected when A is set as the null hypothesis and B rejected when B is the null hypothesis. This occurs when the observed genotype is intermediate between what would be expected according to hypotheses A and B, either by chance or because the true relationship is indeed an intermediate one. We therefore performed tests only between hypotheses that have no natural intermediates, such as between full-sisterhood and a mother–offspring relationship, and accepted hypothesis A only when null hypothesis B was rejected without the opposite being true (i.e., without A being rejected when B was the primary hypothesis and A the null hypothesis). If neither or both hypotheses could be rejected, we did not assign either relationship to the pair. All tests used a significance level of $\alpha = 0.05$.

This method was used to sort all the individuals in a nest to groups of full-sisters and to assign offspring to their mothers. A pair was assigned full-sisterhood if a mother–offspring relationship could be rejected without the opposite being true. A pair was assigned a mother–daughter relationship if both full-sister and aunt–niece relationships could be rejected, without the opposite being true. If these tests were non-significant, a pair could still be assigned a given relationship if this relationship was not significantly rejected, but fitted with other relationships in the nest. For example, if individual 1 was assigned as the daughter of individual 2 and as the full-sister of individual 3, then individual 3 would be assigned as the daughter of individual

2 as long as this relationship was not itself rejected. Examples of how this method is applied are provided in Supplementary Materials.

The significance thresholds from KINGROUP cannot be applied to haploids. An alternative method was used to assign parent–offspring relationships to male–female pairs. A pair of individuals, in which one was male and one was female, was assigned a parent–offspring relationship if the two individuals shared an allele at every locus. The probability of an aunt–nephew pair being wrongly assigned as parent–offspring in this way was calculated as $p = 0.024$; this is the product of the probabilities that an aunt–nephew pair would by chance share an allele at each locus. Relationships other than parent–offspring were not assigned to female–male pairs. No relationships were assigned to male–male pairs.

For the calculation of sex ratio, the same nests were used as for the analysis of social structure, with the addition of two nests that contained no adult females. Brood sex ratio, rather than adult sex ratio, was used as this gives a better impression of investment into each sex, unaffected by differential adult mortality.

Relatedness and kinship

Thirty-three nests from the second field season were included in this analysis. Life-for-life relatedness values (Hamilton, 1972) were calculated with the software RELATEDNESS v5.0.8 (Queller and Goodnight, 1989). Relatedness between females and males was calculated as the value of the male (as a potential beneficiary) to the female (as a potential altruist), and relatedness between adults and brood were calculated as the value of the brood to the adult. Nests were weighted equally and data were jack-knifed over loci and nests to obtain 95% confidence intervals.

Statistical tests

All statistical analyses 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 analysed with non-parametric tests.

Results

Group size and composition

Group size tended to be small. Of the 58 nests that contained at least one female, 21 contained only a single female (with varying numbers of males). This figure may be slightly biased, as some nests were assigned for use in other experiments according to the number of females estimated to be inside them, and thus were not included in this analysis. The largest number of females found in a single nest was 6, the largest number of males was 8, and the largest total adult group size was 13. Many nests contained only males, but these were not collected. Such nests could in some cases persist for at least several months, gradually deteriorating in physical structure, without the males vacating.

We found no significant difference in number of females (Wilcoxon ranked sum test, $n_1 = 25$, $n_2 = 33$, $W = 442$, $p = 0.64$), total brood size (2 sample t test, $t = 0.593$, $df = 56$, $p = 0.56$) or brood per female (Wilcoxon ranked sum test, $n_1 = 25$, $n_2 = 33$, $W = 359$, $p = 0.40$) between the two field seasons. For the purposes of statistical tests, these data were therefore pooled.

The largest observed brood size was 15. Mean brood size was 5.9 ($n = 58$, $\sigma = 2.9$) and mean number of brood per female was 3.0 ($n = 58$, $\sigma = 1.7$). All combinations of brood developmental stages were found in the collected nests. More than one individual at the same developmental stage were commonly found together.

Number of brood was positively correlated with number of adult females in the nest (Pearson's correlation, $r = 0.46$, $df = 56$, $p < 0.001$, Fig. 1a). After a log transformation to deal with unequal variance, there was a significant negative correlation of per capita brood size with number of adult females (Pearson's correlation, $r = -0.55$, $df = 56$, $p < 0.001$, Fig. 1b).

Behavioural repertoire

The typical behavioural repertoire of *M. nigrophthalmus* on the nest exterior involved foraging, inspecting (an individual exits the nest and walks around it before re-entering), silking (a female dabs her abdomen on the nest surface, often on or around the petiole, presumably adding silk to reinforce the physical structure of the nest) and cleaning (ejecting material from the nest). Behaviours observed much more rarely were nest defence (usually against a fly or parasitoid wasp, but always small arthropods), foraging for nesting material and mating (a male and female exit the nest, joined by their genitalia and walk around the nest exterior, before re-entering; this behaviour was observed three times).

There did not appear to be distinctive behavioural repertoires performed by different classes of individuals. Of the common behaviours, all were performed both by females that were later identified as the mothers of brood and those that were not [though the task of foraging may not be equally shared (Lucas, Martins and Field, submitted)]. Interestingly, males engage in inspection and cleaning

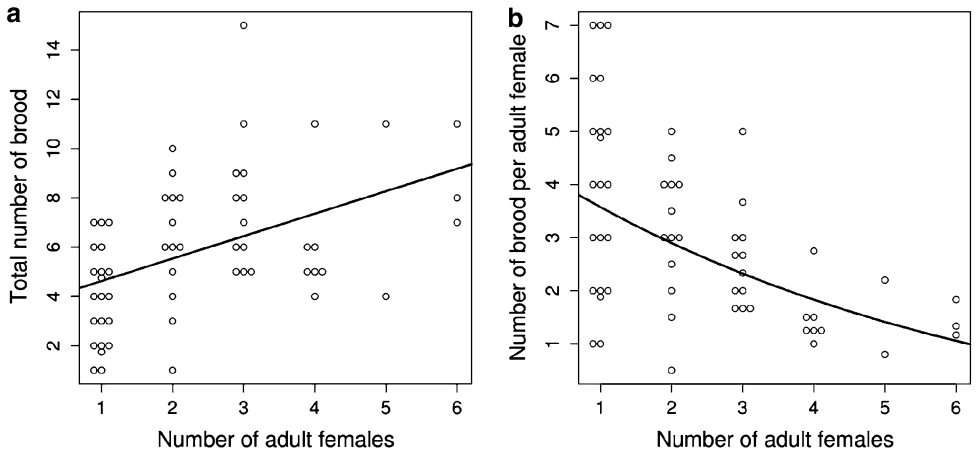

Fig. 1 Total (**a**) and per capita (**b**) brood size plotted against number of adult females in a nest. The trend line in **a** is the best fit regression line through the data. The trend line in **b** is obtained by back-transforming the regression through log-transformed data. Points are jittered in order to show overlapping data

behaviour apparently identical in nature to these behaviours in females (described above). Furthermore, on one occasion, we observed a male performing defensive behaviour.

Behaviour at night

Night time observations revealed little activity after around 16.00, and very little after around 17.00. The latest we ever observed a female return to the nest was 17.03, the latest that a male was seen returning was 17.33. This suggests that our collection time of around 18.00 almost certainly allowed us to capture all resident females, and probably all resident males. In some cases, females were observed displaying a distinctive (and consistent) behaviour, which involved being absent from the nest for most of the day and not always returning to the nest at night. However, it is likely that this was associated with independent nest-founding (Lucas, 2009).

Relatedness and genetic structure

Relatedness within nests was generally high (Table 1). In summary, relatedness was quite high between adult female nestmates (0.49), and lower between adult females and their nestmate males (0.24). However, 5 out of 22 multi-female nests contained pairs of adult females that were significantly more likely to be unrelated than cousins. Relatedness was also high between adult females and the brood present in their nest (Table 1). Relatedness between female brood was the same as between adult females (0.49). Relatedness did not differ depending on whether all brood or only dependent brood (larvae and eggs) were considered (Table 1).

Most of the relatedness estimates in Table 1 were not significantly correlated with the number of adult females in the nest. The exceptions were the relatedness of adult females to male brood and to male non-independent brood, which were negatively correlated with group size (Spearman's rank correlations: $r = -0.62$, $df = 30$, $p < 0.001$; $r = -0.70$, $df = 27$, $p < 0.001$ for all male brood and non-independent male brood, respectively).

Table 1 Population-wide values for within-nest relatedness

	r	*n* (nests)	*L*	*N*
Ad Fem–Ad Fem	0.49	22	±0.04	±0.10
Ad Fem–Ad Mal	0.24	20	±0.02	±0.06
Ad Fem–Br Fem	0.50	30	±0.03	±0.07
Ad Fem–Br Mal	0.34	32	±0.02	±0.04
Ad Fem–NBr Fem	0.49	28	±0.03	±0.08
Ad Fem–NBr Mal	0.34	29	±0.02	±0.04
Br Fem–Br Fem	0.49	28	±0.03	±0.12

Fem female, *Mal* male, *Ad* adult, *Br* brood, *NBr* non-independent brood, *r* life-for-life relatedness, *n* sample size (number of nests), *L* 95% confidence interval generated by jack-knifing over loci, *N* 95% confidence interval generated by jack-knifing over nests

There was no consistent pattern of kinship between adult females in a nest. Some females were full-sisters, mother–daughter, had a lower level of relatedness or were apparently unrelated. Six out of 22 multi-female nests contained at least one co-habiting confirmed adult mother–daughter pair, while 15 of these contained at least one pair of adult full-sisters.

Sex ratio

In total, 95 male and 121 female brood were identified in the 34 nests used in this analysis, which equates to a 1.26:1 female:male numerical sex ratio. We cannot reject the null hypothesis that the sex ratio is in fact 1:1 ($\chi^2 = 3.12$, $df = 1$, $p = 0.08$).

Mating frequency

Analysis of parentage indicated that *M. nigrophthalmus* females can mate more than once. In several cases, female brood that could all be assigned to the same mother could not all be placed into the same full-sister group. Relatedness between these individuals was roughly what would be expected for half-siblings. Of 18 females that were assigned more than one daughter (and who therefore could in theory have been identified as having mated several times), 6 had daughters in more than one full-sib group. We can therefore estimate that a minimum of 33% of females in our sample mated with more than one male. However, full-sister groups as large as six were found, and there were never more than two confirmed full-sister groups assigned to any one mother. Therefore, either mating frequency is low, or sperm from the same male is usually used repeatedly before that of another male takes priority.

Discussion

Group size and genetic composition

Group size in *M. nigrophthalmus* was small, with nests containing no more than six adult females. This is smaller than in the congeneric *M. comes*, where as many as 13 adult females have been found in the same nest (Ross and Matthews, 1989b). The largest number of adult females recorded in *Microstigmus* was from a nest of an undescribed species which contained 26 females (Melo, 2000).

Nests could also contain large numbers of males. This may be explained by the unbiased sex ratio of the brood and the fact that males appear to reside in nests rather than being nomadic. As most females are inseminated (Melo, 2000), it is unlikely that these males are the result of nest-founding by unmated females. Nests containing only males are most likely the result of the death of all resident females. As the number of females in a nest can be small, it would not require a large number of deaths for this to occur.

It was not unusual to find more than one immature offspring in the nest at the same developmental stage, in marked contrast to what has been found in *M. comes* (Ross and Matthews, 1989b) and in the closely related *Arpactophilus mimi* (Matthews and Naumann, 1988). In these two species, this regular spacing of brood age has been used as evidence that, unless females adopt a strict turn-based system of egg-laying, reproduction is likely dominated by a single female. That this feature is absent in *M. nigrophthalmus* may therefore be of significance. *M. comes* is a mass provisioner: it lays each egg on a pre-formed mass of prey which provides sufficient food to raise the larva to pupation. The lack of age-overlap in the brood of this species may be a result of this, as there is little benefit in provisioning two cells at once when eggs cannot start developing until a cell has been fully provisioned (Field, 2005). *A. mimi* and *M. nigrophthalmus*, however, both provision their brood progressively, each larva receiving a new item of food as and when it is required. Whether or not several offspring are found at the same developmental stage in these species may therefore be an indicator of the number of egg-laying females.

Within-nest relatedness between females in *M. nigrophthalmus* is relatively high (around 0.5), whereas relatedness of adult females to males, whether nestmate or brood, is lower (Table 1). This is not surprising as, even if the genetic structure is the same for both sexes, the high relatedness of 0.75 expected between full-sisters will boost mean relatedness between females. Therefore, if for every full-sister relationship in a nest there exists a sister–brother relationship, relatedness will still be lower between females and males than between females.

Relatedness of adult females to brood in their nest therefore tends to be close to that expected to their own offspring, despite the lack of a simple matrifilial structure in most nests. High relatedness should promote within-nest altruism, as individuals may gain indirect fitness benefits by helping their nestmates and by raising brood that are not their own. The relatedness cost of raising the brood from one's natal nest rather than one's own offspring will be small, and therefore only slight ecological advantages should be required for this strategy to be evolutionarily selected. These advantages may come from a high cost of nest-founding, as *Microstigmus* females must invest silk, time and energy to found a nest (Matthews, 1991), or from a safer return on their investments in an established nest in which future nestmates are already developing (Gadagkar, 1990; Queller, 1994; Lucas and Field, submitted).

All levels of relatedness were observed between individual nestmates. It is not necessary to invoke the exchange of individuals between nests to explain this. Melo (2000) found that nests of *M. nigrophthalmus* were usually re-used by the females emerging within it. As individuals one by one become reproductive and/or die, communities within a nest eventually contain both very closely and very distantly related individuals. After only three generations, relatedness between two adult females could be as low as 0.05, even if the nest was founded by one singly mated female and is populated only by her descendents. High mean relatedness within the nest appears to be maintained by low mating frequency (leading to large groups of full-sisters).

In the congeneric *M. comes*, Matthews (1968) found that nests usually contained only one female with developed ovaries, while Ross and Matthews (1989a), using allozyme markers, found a high level of within-nest relatedness ($r = 0.6$–0.7). This suggested that many nests of *M. comes*

are composed of one singly mated female and her daughters. These values of relatedness are higher than in *M. nigrophthalmus*, in which only a few nests conform to the simple family structure suggested by Ross and Matthews. If nests of *M. comes* really do conform to this simple structure, it suggests a significant difference in the mode of life of the two species. In *M. comes*, females remain on their natal nest primarily to help raise their relatives, and perhaps to have a base from which to found their own nest (Matthews and Starr, 1984). In contrast, *M. nigrophthalmus* females might not only help raise relatives, but also have a chance to obtain some direct reproduction themselves, both while their mother is still on the nest, and after she has died.

What happens when the main reproductive dies in *M. comes* is unclear. Some nests studied by Ross and Matthews (1989a) had a lower relatedness than expected for a simple mother–daughter structure. Perhaps these were nests in which the mother had died and the reproductive position was taken over by one of the daughters. The resulting aunt–niece associations between this new reproductive's sisters and her brood would then bring down the mean relatedness in the nest. Alternatively, however, sisters which fail to inherit the breeding position might choose to leave the nest, as they benefit less from raising their nieces and nephews than their sisters and brothers.

Behavioural repertoire

Reproductive and non-reproductive females did not appear to have distinctive behavioural repertoires in terms of behaviours outside the nest. It thus appears that there is no strict queen–worker divide in this species (but see Melo, 2000). Several of the elements of the females' behavioural repertoire were also observed in males. Male contributions to colony life are unusual but have been recorded in some polistines (O'Donnell, 1999; Pardi, 1977; Sen and Gadagkar, 2006; Steiner, 1932). On one occasion, we observed defensive behaviour on the part of a male. This defensive behaviour will be discussed more extensively in a forthcoming paper (Lucas and Field, in prep.).

Relatedness correlations and sex-biased dispersal

Relatedness of adult females to male brood was correlated with group size. One possible explanation is that the highest possible relatedness between a female and a male comes from a mother–son relationship ($r = 0.5$). As group size increases, the frequency of these mother–son relationships as a proportion of all relationships must decrease, and so the mean relatedness of females to males also decreases. Between females, the equivalent fall in the number of mother–daughter relationships may be compensated for because as group size increases, the number of full-sister relationships ($r = 0.75$) can also increase.

The absence of such a correlation between adult female–adult male relatedness and group size may be explained if males disperse more than females, as there would then be few mother–son relationships between adult nestmates in the first place. It may therefore be that males tend to be the dispersing sex in *M. nigrophthalmus*. This would not be unexpected. As females tend to make use of their natal nest to reproduce (Melo, 2000), and as mating can occur within the nest, lack of dispersal by males could lead to a high level of inbreeding. As females are limited in their reproduction by access to a nest in which to lay, a dispersing female would have to construct her own nest, or usurp an existing nest. Both are likely to be costly and risky activities. Males, on the other hand, need only find an unrelated nest within which they can mate.

In *M. comes*, Ross and Matthews (1989b) found that males are almost always related to their female nestmates, even when the nest was too recent for the males to have emerged from them. Their estimate of inbreeding in the population, however, was low and not significantly different from 0. It is possible, then, that *M. comes* avoids inbreeding by mating away from the nest, with males returning to their natal nest after mating.

Conclusion

Despite the absence of obligate single mating and of the simple matrifilial social structure found in *M. comes*, nests of *M. nigrophthalmus* tend to display high levels of relatedness, close to that which a female could expect to her own offspring. This is presumably due to low mating frequency and nest inheritance. Under such circumstances, only slight ecological advantages are needed for social altruism to be favoured by selection.

This study provides the first microsatellite analysis of relatedness, and one of the rare studies of genetic structure, in an apoid wasp. The extent to which this social structure is reflected in related groups is therefore currently hard to determine. Similar studies in other species, along with an accurate phylogeny of the Spilomenina including details of the relationships between groups of *Microstigmus*, are needed to appreciate the scope of social evolution in this group.

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