Absence of within-colony kin discrimination in behavioural interactions of swarm-founding wasps

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SUMMARY

Within-colony kin discrimination has not been demonstrated conclusively for any social insect, perhaps partly because highly polymorphic genetic markers necessary to assess within-colony relatednesses have only recently become available. We use microsatellite loci to investigate within-colony kin discrimination in behavioural interactions in the neotropical multiple-queen wasp, *Parachartergus colobopterus*. Within-colony kin discrimination would be particularly advantageous in this species since average genetic relatedness among colony members overall is low (0.32 ± 0.06), compared to the relatedness value between full sisters of 0.75. Using seven colonies of individually marked females, we recorded behavioural interactions that were cooperative (222 grooming, 2438 feeding), aggressive (511 body or wing biting, 240 mandible biting) or neutral (1676 antennating). We expected cooperative behaviours to favour closer kin and aggressive behaviours to be directed towards more distant kin, but found that none of the behaviours we investigated showed discrimination on the basis of relatedness. We could have detected a difference in relatedness values of as little as between 0.03 and 0.12, depending on the behaviour being analysed. Thus, we found no evidence for kin discrimination in within-colony behaviour in this species.

1. INTRODUCTION

Early applications of Hamilton's theory of kin selection (Hamilton 1964*a,b*) focused on its prediction that aid would be directed towards kin other than progeny when the benefit measured in genes passed on outweighs the cost (e.g. Sherman 1977). More recently, Hamilton's rule has been used to explore conflicts of interest that arise when groups contain differently related individuals with some opposing interests (Crozier & Pamilo 1996). The most complex of these groups occur in the social insects, particularly those with multiple queens, or multiply mated queens. Hamilton's rule has been applied to conflict between queens and the collective interests of workers in sex allocation (Trivers & Hare 1976; Boomsma & Grafen 1991; Pamilo 1991), and male production (Starr 1984; Ratnieks 1988; Woyciechowski & Lomnicki 1987). In these cases, females must often be able to assess average relatednesses among colony members, or relatedness-lowering events, but not relatednesses to specific individuals within the colony. If social animals could discriminate closer from more distant kin within cooperating groups, then the nature of that cooperation would be affected. Hamilton's rule would predict that

closer kin would be favoured over more distant kin in a variety of ways, including some which might disrupt the group itself.

The possible impact of within-colony kin recognition and subsequent discriminatory behaviour on social organization has been appreciated for some time (e.g. Michener & Fletcher 1987). The most intensive efforts to identify within-colony kin discrimination have used honeybees (Breed *et al.* 1994; Keller 1997). Initial positive results (e.g. Getz *et al.* 1982; Getz & Smith 1983; Visscher 1986) were criticized on a number of grounds, including artificiality of colony composition and possible observer bias when visible genetic markers were used (Carlin & Frumhoff 1990; Alexander 1991; Frumhoff 1991; Breed *et al.* 1994). The most recent reviews tend towards the view that within-colony kin discrimination is not an important factor organizing honeybee societies (Breed *et al.* 1994; Keller 1997). Studies of ants and wasps likewise fail to demonstrate within-colony kin discrimination, though the power of the allozyme-based assays these studies used make detection more difficult (Queller *et al.* 1990; Snyder 1993; DeHeer & Ross 1997). The methodological difficulties of early studies combined with the potential importance of within-colony kin discrimination make it desirable to conduct further studies in a wider variety of species using natural colonies, sensitive genetic markers, and blind observations.

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Table 1. *Study colonies and their characteristics*

(All adults are females. Queens are inseminated while workers are not.)

colony ID	V19-30	V19-42	V20-7	V20-8	V20-13	V20-38	V20-45
colony age	<1 month	<1 month	2–6 months	>6 months	2–6 months	>6 months	>6 months
no. of combs	4	6	6	9	6	4	7
no. of cells	278	285	435	314	486	164	518
no. of wasps	130	146	110	64	120	69	297
no. of queens— developed ovaries	3	4	6	5	1	1	91
no. of queens— undeveloped ovaries	0	0	3	0	20	0	3
no. of microsatellite loci used	6	10	10	6	10	10	6
worker $r \pm$ s.e.	0.20 $\pm$ 0.043	0.195 $\pm$ 0.039	0.185 $\pm$ 0.082	0.322 $\pm$ 0.085	0.686 $\pm$ 0.109	0.314 $\pm$ 0.06	0.287 $\pm$ 0.058
no. of workers for relatedness	85	142	98	57	82	68	197
queen $r \pm$ s.e.	0.225 $\pm$ 0.153	0.834 $\pm$ 0.043	0.400 $\pm$ 0.106	0.889 $\pm$ 0.082	0.587 $\pm$ 0.10	—	0.380 $\pm$ 0.089
no. of queens for relatedness	2	4	9	5	21	1	94

The recent development of highly polymorphic DNA microsatellites for use as genetic markers should allow studies that are precise enough to detect within-colony kin discrimination if it occurs (Queller *et al.* 1993a). The first such study involved swarming in honeybees using microsatellite markers and it failed to detect any discrimination (Kryger & Moritz 1997). Here we use DNA microsatellites to investigate within-colony kin discrimination in *Parachartergus colobopterus*. Numbers of queens per colony in this species average 27 ± 6 (s.e.), high enough to reduce within-colony relatedness, but low enough that full sisters are encountered frequently (Strassmann *et al.* 1991). *P. colobopterus*, along with other species in the tribe Epiponini, has a colony cycle called cyclical oligogyny (Strassmann *et al.* 1991, 1997; Queller *et al.* 1993b). Under cyclical oligogyny, new queens are not produced until queen number in the colony has been reduced to one, or nearly one, queen (West-Eberhard 1978; Strassmann *et al.* 1991; Queller *et al.* 1993b). Then a large new cohort of highly related queens is produced by the remaining queen. Relatedness among the new queens is particularly high because queens mate only once (Goodnight *et al.* 1996). Thus, during most of the queen cycle, there are multiple matrilineal lines present in the colony, but ultimately the next generation of queens is most closely related to one of them, a circumstance that should favour within-colony kin discrimination. New colonies are produced by swarming; a number of workers and queens leave the colony to begin a new one (Strassmann *et al.* 1997). Swarming is most frequent in the rainy season (June to October) and is independent of the queen cycle (Strassmann *et al.* 1998).

A number of other advantages make *P. colobopterus* an attractive choice. We have ten polymorphic DNA microsatellite loci for this species which will allow us to investigate relatedness within colonies. Since the nest envelope is not attached to the combs, it can be moved to make videotape recording and marking all individuals feasible. *P. colobopterus* is common in much of Venezuela and abundant at our study site. Another

advantage to this species is that we have observed frequent instances of both very cooperative behaviours such as social grooming, and extremely aggressive fighting. For this study we chose seven colonies that varied in age and where they were in the queen cycle (table 1). We focused on smaller colonies to facilitate individual identification of all interactants.

2. MATERIALS AND METHODS

(a) *Field marking and videotape recording of the study colonies*

We studied *P. colobopterus* in Maracay, Venezuela ($10^{\circ}16' N$, $67^{\circ}36' W$, altitude 445 m), at Hotel Maracay in 1992 (colonies V19-30 and V19-42), and at the Universidad Central de Venezuela, Facultad de Agronomía in 1993 (colonies V20-7, V20-8, V20-13, V20-38, and V20-45). All colonies were on man-made structures: poles supporting a roof or exterior walls of quail cages.

We marked all individuals on the seven colonies. To do this we visited each colony for two or three mornings and teased many of the wasps out onto the nest envelope by stroking it gently. We marked these wasps freehand on the thorax, abdomen, and wings with about ten colours of paint pens or Testor's glossy enamel. The latter gave a more permanent mark which was applied by dipping the tip of an insect pin (stuck into a pencil eraser) wrapped in cotton fibres into the paint and then touching it to the wasps. After as many wasps as possible had been teased onto the envelope and marked, we opened the envelope and marked additional wasps directly on the combs of the nest.

We video record each colony for 6–8 h per day for 1–3 d between 24 July and 6 August, in 1992 and 1993. We used Sony Hi8 camcorders and Hi8 evaporated metal or metal particle videotape. We obtained resolution sufficient for clear identification of individual marks by focusing close enough that between two and four combs filled the camcorder field of view. When we changed tapes we focused on a different pair of combs so we could obtain behavioural samples from all, or nearly all, combs.

After we completed all videotaping of a colony, we collected it at dusk by surrounding the nest with a zip-shut plastic bag and knocking the combs off the substrate with forceps. Ethyl acetate was introduced onto the nest envelope

or into the bag to facilitate the capture of all wasps. We visited the nest sites the following day to collect any stragglers that had not arrived at the nest even at the late hour we collected. The wasps were then stored in liquid nitrogen for transport back to our laboratory, where we stored them in a -60°C freezer.

(b) Behavioural observations of videotapes and individual identifications

We photographed all wasps, five at a time, with associated individual and colony identification. We used these photographs (sorted by colour mark) to identify interacting individuals on the videotapes.

We recorded all interactions of the five types noted below, between identifiable individuals for a given period of videotape (that was not the same for all nests), though we did not use all the videotapes. Interactions among unmarked or uncollected individuals were not included since these individuals could not be identified.

We took detailed data on interactions, including the succession of acts, length of time of the interaction and the response of the recipient. For this study, we counted five types of interactions. (1) *Social grooming* is common in *P. colobopterus*, particularly if the recipient is wet from rain. In social grooming the actor uses the mandibles and mouthparts to 'lick' the recipient gently and continuously. The actor's body does not jerk while performing this action and antennal motions are slow. (2) *Biting* is an aggressive interaction characterized by chewing on a victim's body part as the actor opens and closes her mandibles and moves rather jerkily. This behaviour is directed towards the wings, thorax, legs, abdomen or head of the recipient. The wasp being chewed upon sits motionless, with her body hunched close to the nest, or curls over on its side, bending the gaster tip towards the aggressor. In the most aggressive biting, the biter climbs on the victim. (3) *Mandible biting* is a biting interaction directed towards the mandibles of the recipient. The actor opens and closes her mandibles which sometimes causes the recipient to give her food. Because of its association with food solicitation, we separated biting directed towards the mandibles from biting other parts of the body. (4) *Feeding* is a behaviour initiated by a female with food in her mandibles or crop. She approaches another wasp and offers food. This is distinct from mandible biting where the initiator is forcefully soliciting food. (5) *Antennating* is a behaviour that involves one wasp touching another with her antennae. We scored behaviours as antennate alone if no further escalation occurred. If the antennation resulted immediately in another interaction, then we called the whole episode the latter behaviour. For example, an antennate that escalated into a bite would be scored as a bite.

If within-colony kin discrimination occurs, we expected that social grooming and feeding would be preferentially directed towards closer relatives while biting and biting mandibles would be preferentially directed towards less close relatives. We viewed antennating as a neutral, possibly kin identifying, behaviour for which we did not have directional predictions with respect to relatedness. Because of these predictions, we ran two-tailed tests for antennate and one-tailed tests for the other behaviours. We used one-tailed tests to increase the chance that we would detect a significant difference. Using two-tailed tests would not have changed the outcome.

We did not analyse the behaviours that workers directed towards queens separately, because queens were seldom involved in behavioural interactions. The 111 queens with developed ovaries in the seven colonies received only 54

actions (4 grooms, 4 feeds, 25 antennates, 15 bite mandibles, and 6 bites), which is only 1% of all recorded acts. There are too few acts for meaningful analyses to determine whether queens are favoured by their matriline. In fact, the paucity of such behaviours suggests that they are not.

(c) Assessment of sex and caste

All adults in these colonies were females. We dissected them and evaluated their ovarian development and insemination status. We divided them into workers and queens according to whether or not they had sperm in their spermatheca, calling those with sperm in their spermatheca the queens, and those lacking sperm, the workers. We divided queens into those with substantial ovarian development and mature or nearly mature eggs in their ovaries, and those with slight or no ovarian development (table 1). Very few of the workers had any ovarian development (51 of 1932), so they were not further subdivided.

(d) Genotyping individuals using DNA microsatellite loci

We genotyped all 864 adults collected, at between six and ten DNA trinucleotide repeat microsatellite loci (table 1). These loci were Paco3434AAT, Paco3155TAG, Paco3436AAT, Paco3417AAT, Paco3457AAT, Paco3107TAG, Paco41TAG, Paco3305CAT, Paco3304CAT, and Paco3117TAG (Strassmann *et al.* 1996a). These loci had between 4 and 22 alleles (average = 10) and had heterozygosities of 0.50 to 0.94.

We ran 10 μl polymerase chain reactions (PCR) that incorporated ^{35}S labelled dATP nucleotides into the PCR products (see Strassmann *et al.* (1996b) for complete methods and protocols). We ran the PCR products on 6% denaturing sequencing gels and visualized length polymorphisms in PCR products by exposing the dried gel to X-ray film. We ran M13 sequencing reactions as size standards. We loaded a mix of A, G and T of the sequencing reaction in one lane, and C in its own lane.

All genotypes were scored by two people, independently, for size of product and this genotype was entered into a spreadsheet. Discrepancies between the individuals doing the scoring were resolved by re-evaluating the autoradiogram and re-running the sample if necessary.

(e) Assessment of genetic relatedness among interactants

We analysed data from each behavioural act separately, using a test of paired relatedness differences based on that of Queller (1994). To obtain relatednesses between actors and the recipients of the action, we created a matrix of pairwise relatednesses among all colonymates generated with a prototype of the program Kinship 1.0 (Goodnight & Queller 1997) using the relatedness measure of Queller & Goodnight (1989). We also created a matrix for each of the five behaviours with the number of times an interaction occurred between each pair of interactants. From these matrices we obtained the average relatedness between each actor and the recipients of a given action, weighted by the number of times that interaction occurred between the pair (this is relatedness value *A*). To see if relatives were favoured or disfavoured for specific acts, we compared this value to that likely if behavioural acts were random with respect to relatedness. For this value, we chose to use the relatedness of the actor to all other females (excluding the actor herself) who were recipients of the same behavioural act by all actors combined, again weighted by frequency (relatedness value *B*). This creates a

set of recipients of the behaviour who are the individuals that might have received it from the actor in question. We felt this to be a better control set than all females on the nest, because certain females may be more likely than others to either perform or receive certain behaviours due to age or other factors. In that case, using all females on the nest might give a false positive (as discussed by Snyder (1993)). We then took the average difference between value *A* and value *B* as the measure of kin discrimination for each behaviour. We evaluated whether or not these values were different in each colony using as degrees of freedom the number of actors for the behaviour minus one. In the colony level analyses, we applied the Bonferroni procedure (Zolman 1993) to reduce possible type I errors due to multiple tests. Results were considered significant at the α/c level where α is alpha (0.05) and c is the total number of comparisons made. In the overall analysis where we combined colonies we could not assume that interactions in a colony were independent because even if a given behavioural interaction occurs at a separate time and part of the nest from another, the interacting wasps share the physical, social and genetic environment of the colony. Therefore we combined the analyses for the seven colonies by averaging values for each colony, and used six degrees of freedom (seven colonies minus one).

3. RESULTS

(a) Opportunity for within-colony kin discrimination

For kin discrimination to occur among matriline in the same colony, it is first necessary to demonstrate that there are multiple matrilines in each colony. We assessed this with the DNA microsatellite genotypes by estimating average relatednesses and by looking at pedigrees. Inspection of the pedigrees is made easier since females mate with only a single haploid male (Goodnight *et al.* 1996). This means that a single

matriline can have no more than three alleles per locus, one of which is shared by all daughters (the paternal allele). All colonies had multiple matrilines as assessed by genetic relatednesses (table 1). Relatednesses averaged 0.315 ± 0.060 (903 individuals in seven colonies) and varied from 0.670 ± 0.107 to 0.192 ± 0.045 . We also inspected the genotypes of all colonies and confirmed that multiple matrilines were present in all colonies including colony V20-13 where relatedness was highest. Clearly, all of these colonies had an opportunity for kin discrimination favouring members of the same matriline over that of other matrilines.

(b) Within-colony kin discrimination

We analysed behavioural interactions among females in the seven colonies, counting a total of 222 grooming, 2438 feeding, 1676 antennating, 511 biting, and 240 mandible biting interactions (table 2). Not a single behaviour was significantly directed towards or against closer kin within the colony (table 2; figure 1). Given the estimated standard errors, we could have detected a relatedness difference of as little as 0.03 to 0.12, depending on the behaviour being considered. We found no differences in genetic relatednesses for any of the behavioural categories either within individual colonies or for the colonies pooled. We conclude that kin discrimination is absent from within-colony behaviour in this species.

4. DISCUSSION

If kin discrimination were occurring in these behavioural interactions, it is highly likely that we would have detected it for several reasons. (1) We studied a

Table 2. Sample sizes and relatedness differences by colony for each behaviour

(We give 95% confidence intervals for relatednesses between actors and recipients. A is actor and R is recipient.)

colony ID	V19-30	V19-42	V20-7	V20-8	V20-13	V20-38	V20-45
<i>r</i> between groom							
A & R	0.036 ± 0.188	-0.014 ± 0.049	0.022 ± 0.106	-0.018 ± 0.101	-0.018 ± 0.101	-0.013 ± 0.340	-0.026 ± 0.175
no. of grooming actors, recipients, and acts	13, 12, 17	57, 60, 106	22, 15, 31	3, 3, 3	26, 23, 42	6, 7, 8	9, 13, 15
<i>r</i> between feed							
A & R	-0.01 ± 0.031	-0.020 ± 0.101	0.004 ± 0.032	-0.037 ± 0.062	0.005 ± 0.024	-0.012 ± 0.065	0.03 ± 0.071
no. of feeding actors, recipients and acts	81, 83, 375	15, 13, 16	78, 80, 1669	39, 45, 191	80, 75, 268	48, 37, 160	55, 43, 87
<i>r</i> between antennate							
A & R	-0.004 ± 0.027	0.112 ± 0.115	-0.024 ± 0.032	0.023 ± 0.055	0.004 ± 0.042	0.034 ± 0.104	0.005 ± 0.043
no. of antennation actors, recipients, and acts	83, 75, 373	11, 14, 16	65, 66, 487	17, 16, 244	63, 49, 118	23, 23, 48	107, 94, 399
<i>r</i> between bite A & R	0.036 ± 0.125	-0.014 ± 0.061	0.027 ± 0.102	$0.273 \pm 0.160^*$	-0.038 ± 0.216	-0.006 ± 0.143	0.022 ± 0.073
no. of biting actors, recipients, and acts	10, 13, 18	40, 53, 220	17, 38, 77	15, 18, 25	6, 6, 6	13, 18, 33	64, 52, 132
<i>r</i> between bite mandible A & R	0.084 ± 0.119	-0.059 ± 0.10	0.042 ± 0.100	-0.103 ± 0.134	0.012 ± 0.027	-0.104 ± 0.186	-0.026 ± 0.175
no. of bite mandibles actors, recipients, and acts	9, 11, 16	14, 17, 21	12, 17, 20	5, 5, 6	68, 66, 155	5, 6, 7	93, 84, 225

*Bonferroni multiple comparisons correction for this 95% confidence interval includes zero.

Figure 1. Relatedness differences between an actor's own recipient minus other recipients of the action. Given are average differences for the seven colonies combined with 95% confidence intervals.

species that has multiple matriline for much of its colony cycle. This means that females can choose between aiding full sisters to whom they are related by 0.75 and aiding cousins (daughters of their mother's sisters) to whom they are only related by 0.1875, a very large difference. (2) We used a very sensitive genetic marker to assess relatednesses. The 6–10 DNA microsatellite loci that we used were capable of giving very accurate relatednesses between actors and recipients. This level of analysis has not been possible with any other kind of marker. (3) The behavioural observations were conducted blind with respect to relatedness among interactants. We either genotyped the individuals after analysing their behaviour or had different researchers conduct the DNA analyses and the behavioural observations. (4) Our study was of natural colonies that were not artificially put together from different populations or colour races. In this natural context, kin discrimination likely to be occurring naturally should have been revealed. (5) We chose behavioural acts to analyse that were clearly favourable or antagonistic. Feeding and grooming are clearly favourable, while biting and mandible biting are clearly antagonistic. (6) Though the large numbers of individuals per colony make large sample sizes tedious to obtain, we did observe large numbers of behavioural acts in seven different colonies, and genotyped all individuals. The large sample sizes and colonies at different stages increase the likelihood of detecting a kin-favouring behaviour even if it only occurs in a minority of colonies. (7) We used appropriate controls for the behaviours we examined. Using the set of other individuals receiving the action controls for any matriline differences in the tendency to receive particular behaviours.

It is clearly unlikely that undetected kin discrimination occurs in the behavioural context which we examined. Furthermore, favouring of one matriline over another has an important connection to the reproductive success of that matriline since ultimately, only one queen produces the next generation of queens, after all the other queens have died (Strassmann *et al.* 1991; Queller *et al.* 1993b). Any behaviours favouring one matriline could result in the queen of that matriline producing all the future queens (Queller *et al.* 1993b). This is less true for male production since males are

produced by queens at a multiple-queen stage (Queller *et al.* 1993b).

We have also looked at other contexts for within-colony kin discrimination in this species where it might be even more likely to occur since it would be less likely to increase within-colony conflict. The most likely candidate is a behaviour very closely connected with fitness, e.g. who the workers join when new colonies are initiated by swarms. Other work in our laboratory analysed kin discrimination in the context of swarming in this species, and did not detect any sorting on the basis of relatedness in that context either (Solís *et al.* 1998).

This study adds to the growing number of negative results for within-group kin discrimination (reviewed in Keller (1997)). A number of hypotheses have been put forward to explain the lack of within-colony kin discrimination (Keller 1997). Perhaps selection does not favour discrimination that would reduce overall colony fitness (Page *et al.* 1989). Recognition errors may also make within-colony kin discrimination unlikely to evolve (Reeve 1989, 1998). Individuals likely to be discriminated against within colonies may even be selected to scramble kin recognition signals (Reeve 1998).

Though to date there have been no studies conclusively demonstrating within-group kin discrimination, the study of this topic has been hampered by the lack of genetic markers. It is an important enough question that we should not assume within-group kin discrimination to be absent in all species until more species have been studied in a variety of situations using sensitive genetic markers like microsatellites.

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