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Genetic relatedness in early associations of *Polistes dominulus*: from related to unrelated helpers

LORENZO R. S. ZANETTE* and JEREMY FIELD†
*School of Biological Sciences University of East Anglia, Norwich, Norfolk NR4 7TJ, UK, †Department of Biology and Environmental Science, School of Life Sciences, John Maynard Smith Building, University of Sussex, Brighton BN1 9QG, UK

Abstract

Indirect benefits obtained through the reproduction of relatives are fundamental in the formation and maintenance of groups. Here, we examine the hypothesis that females of the temperate paper wasp *Polistes dominulus* preferentially form groups with close relatives. Genetic relatedness data were obtained for 180 groups of females collected at the early stages of the nesting cycle of a large population of *P. dominulus* in two sites in southwestern Spain. Average within-group relatedness values ranged from 0.189 to 0.491. Foundresses on early nests were significantly more closely related than females in winter aggregations or in stable groups (just before workers emerged). Within-group relatedness values were independent of group size. The vast majority of worker-producing nests (*c.* 85%) had one or more females that were unrelated (or distantly related) to the remaining members of the group. These results provide further support to the hypothesis that indirect fitness benefits alone are unlikely to explain why *P. dominulus* foundresses form cooperative associations.

Keywords: group formation, kin selection, kinship, microsatellites, paper wasps, sociality

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Introduction

Kin-selection theory has provided a fundamental framework for the study of cooperative behaviour and altruism (Grafen 1984; Frank 1998; Griffin & West 2002). Paper wasps of the cosmopolitan genus *Polistes*, one of the most extensively studied groups of wasps, have been at the forefront of the application and testing of kin selection theory since its conception (Hamilton 1964; Reeve 1991; Turillazzi & West Eberhard 1996). These wasps display a primitive form of eusociality, with no permanent morphological caste differentiation between group members. Consequently, all individuals in a group are potentially capable of independent reproduction (Reeve 1991; Pardi 1996). Such behavioural flexibility has provided a rare opportunity to accurately measure the costs and benefits associated with cooperative and noncooperative reproductive strategies within the same population (Queller 1996).

Central to kin-selection theory is the idea that individuals can obtain indirect fitness benefits through boosting the reproduction of relatives. Consequently, reliable estimates of relatedness are essential to test predictions derived from this theory (Strassmann *et al*. 1989). The advent of relatively low-cost high-resolution genetic markers has greatly facilitated this task, allowing accurate estimates of genetic relatedness to be obtained for a myriad of species (review of methods in Blouin 2003). For the paper wasps, early studies using allozymes indicated that nest-mates of at least eight species of *Polistes* had relatedness values close to the haplodiploid full sister value of three quarters (Strassmann *et al*. 1989). More recently, with the use of microsatellite markers, these high values of foundresses relatedness were confirmed for at least three temperate and subtropical species: *Polistes bellicosus* (Field *et al*. 1998); *Polistes fuscatus* (Reeve *et al*. 2000); *Polistes carolina* (Seppa *et al*. 2002).

In contrast, unexpected low levels of genetic relatedness have been found in an Italian population of *Polistes dominulus*, where 35% of nest-mate foundresses were nonrelatives (Queller *et al*. 2000). Cooperation between unrelated individuals occurs in other communal (Kukuk & Sage 1994) and eusocial Hymenoptera (Bourke & Franks 1995; Bernasconi & Strassmann 1999). However, in *P. dominulus* associations,

Correspondence: Lorenzo R.S. Zanette, Fax: +44 (0) 1603 592250; E-mail: l.zanette@uea.ac.uk

the dominant female produces most or all the eggs (Queller *et al*. 2000; Liebert & Starks 2006). This almost complete reproductive skew in groups of unrelated individuals is uncommon in social insects (Queller *et al*. 2000).

The majority of *P. dominulus* females in the Italian population do nest with close relatives (56% full-sisters; Queller *et al*. 2000). In addition, evidence that foundress associations might be composed mainly of full-sisters has been found for another European *P. dominulus* population (south of Spain; Cant *et al*. 2006b). Here, we test the hypothesis that foundresses of *P. dominulus* preferentially form groups with their full-sisters by analysing the changes in intra group relatedness at successive stages of the nesting cycle, before group composition becomes stable and workers start to emerge.

Methods

Natural history of Polistes dominulus

Polistes dominulus has a colony cycle typical of polistine wasps in temperate regions (Reeve 1991). Mated females overwinter in aggregations ranging from a few to more than a hundred individuals. Winter diapause refuges, known as hibernacula, are often re-used by females of different years and although their location varies considerably, they are commonly observed behind large nests of the previous year (Pardi 1942; Turillazzi *et al*. 2006). New nests start to appear in late winter (late February–March, in Spain), soon after the first foundresses have left their winter refuges. The nest foundation period ends in late spring, when all winter refuges are completely empty and the first workers are about to emerge (Reeve 1991). Nests are founded either singly (monogyny) or jointly with auxiliary foundresses (polygyny; 2–23 females in our studied population) (Pardi 1942). In southern Spain, the founding period is relatively long, usually ending in mid-May.

All foundresses are potentially capable of reproducing (Röseler 1991), but in polygynic nests, one individual is behaviourally dominant and lays most of the eggs, while the subordinates take on most of the riskier activities away from the nest: foraging to feed the offspring and collecting material for nest construction (Pardi 1942; Reeve 1991). Towards the middle of the founding period (early spring: March–April), changes in group composition are frequent, with new foundresses (joiners) arriving at established nests. Nest switching and usurpation are also relatively common in this period (but see Queller *et al*. 2000). Usurpers usually destroy the previous foundress's eggs and early instar larvae (Nonacs & Reeve 1995; Starks 2001). In the same period, at our study site, *P. dominulus* is subject to attack by a social parasite, *Polistes semenowi* (see Zacchi *et al*. 1996; Shreeves *et al*. 2003). The nest foundation period is considered to be finished in early summer (late April and May in southern Spain), just before the first brood (workers) starts to emerge, and changes in group composition then become less frequent (i.e. groups become stable). In mid-summer, new workers stop emerging and the first reproductives start to emerge. They do not participate in the activities of the nest and generally mate away from their natal nests (Pardi 1942). The reproductive phase lasts until the early fall when the colonies dissolve and gynes disperse to winter diapause refuges (Pardi 1942; Pardi 1996).

Specimen collection

Females of *P. dominulus* were collected from two nearby sites (720 m apart) in southwestern Spain (Conil de la Frontera, Cadiz, 36°15′N, 06°10′W). Seven hundred and four wasps from 180 groups were collected between February and May in 2004 and 2005. The habitat at each site consisted of pasture and arable fields, with nests and winter aggregations occurring on hedges of prickly pear cactus (*Opuntia* sp.). Nests and hibernacula were located by intensive searches during the day, then numbered and their location recorded. Nests are easy to spot on these plants, so that few groups remained undetected. Collections were made in the early morning (*c*. 7:00 AM), before wasps were active, by capturing all group members (on nests) or five random wasps (from the winter aggregations) with long forceps and placing them into plastic bags.

Random samples of groups were collected at four stages of the nesting cycle: (i) 23 winter aggregations (99 wasps), (ii) 27 early nests (84 wasps), (iii) 35 late nests (154 wasps), and (iv) 95 stable nests, that is, just before worker emergence (380 wasps). Winter aggregations were collected at the end of February (2004) and March (2005), before nest foundation started. Early nests were collected at the very end of February and in the week of March (in both years). Late nests were collected during mid/late March (in both years). Finally, stable nests were collected during April and May (in both years). All samples were subsequently stored at –80 °C (at the 'Laboratorio de Patologia e histofisiologia de species marinas ', University of Cadiz, Spain). Samples represented 15% of all detected nests at stages (ii) and (iii), and 40% and 35% at stage (i) and (iv), respectively.

DNA extraction, amplification and visualization

Total DNA was extracted from the anterior section of the thorax, from up to five wasps from each winter aggregation or nest collected. Extractions were made using 300 μL of grinding solution (0.1 M NaCl; 0.1 M Tris-HCl, h = 8.0, 0.05 M EDTA; 0.05% SDS), following Strassmann *et al*. (1996), with minor modifications. DNA extractions were diluted 1:10 with ultra-filtered distilled water.

Multiplex polymerase chain reactions (PCR) were performed using five previously described primers, fluorescently

Table 1 Maximum number of alleles and average heterozygosities (observed, H_O; and expected, H_E) for each microsatellite loci

Locus	Repeat*	Primers (5′–3′)*	No. of alleles	H_O	H_E
Pdom 127	$(AAT)_{13}$ ... $(AAT)_6$AA	F: TCCCCCGTTTTTGGTCCTTG R: GGGAGAGAATCGTGCCTTTTC	18	0.927	0.881
Pdom 139	$(AAC)_7(AAT)_2(AAC)$ $(AAT)_2(AAC)_2$	F: TGACAAAAGACAACAAAATATG R: AGCTTCGGTAGGGCTTCG	13	0.791	0.751
Pdom 140	$(TAG)_9$	F: GCTTTTCCCTTATTTTCCCG R: CGTGTTCGTATATTCCTGTAACG	8	0.769	0.731
Pdom 20	$(CAT)_{18}$	F: TTCTCTGGCGAGCTGCACTC R: AGATGGCATCGTTTGAAAGAGC	21	0.917	0.871
Pdom 7	$(AAG)CAG(AAG)_9$	F: CACTGTATTGTCCTACGGTGGTCC R: GCGAGAACCTGTACTCAAAACAAAC	8	0.749	0.711

*From Henshaw (2000).

labelled (Table 1; Henshaw 2000). PCR was carried out on a Peltier Thermal Cycler using 10-μL reactions with: 2 μL of DNA sample, 2 μL of reaction buffer $(NH_4)_2SO_4$, 0.6 μL of $MgCl_2$, 0.2 μL of each dNTP, 0.8 μL of each primer (Table 1) and 0.05 μL of *Taq* polymerase (annealing temperature = 54 °C). The PCR products were visualized using an Applied Biosystems 3100 sequencer. Allele sizes were scored against an internal size standard (Applied Biosystems GeneScan ROX 500) using ABI GENESCAN analysis software (version 3.7). Genotypes that appeared inconsistent with those of nest mates were rescored (through re-examination of the chromatogram) or retyped (with a repeat PCR). In addition, 40 random samples were retyped, of which all were confirmed correct. Scores that differed by 1 bp or less were considered to be equal. Moreover, 27 randomly chosen samples previously genotyped at four of the same loci using polyacrylamide gel electrophoresis (PAGE, Cant *et al*. 2006a) were re-genotyped. Scores differed between studies for only one allele in each of two samples [error rate = $2/(27 \times 2 \times 4) = 0.009$].

Relatedness estimation

Genetic relatedness (by site, by period, and within group) was estimated using the program RELATEDNESS 5.08 (www.gsoftnet.us/GSoft.html), which calculates regression relatedness values using population allele frequency data based on the formula described by Queller & Goodnight (1989). Groups were weighted equally, to account for differences in sample size between them. Confidence intervals for relatedness estimates were obtained by jackknifing over loci.

Pairs of foundresses were also assigned to sib categories using a likelihood-based method implemented by the program KINSHIP (Goodnight & Queller 1999). Assuming single mating, no inbreeding, and no linkage disequilibrium, the program uses population allele frequencies to estimate the likelihood that the genotypes of each pair of individuals would occur if they were full-sisters vs. the likelihood of the same genotypes arising if the females were maternal cousins. Cousins are the next likely relationship after sisters, since multiple mating has not been detected in *P. dominulus* and foundresses will generally be from the same generation (Queller *et al*. 2000; Strassmann 2001). The significance of the resulting likelihood ratio was obtained empirically, by simulating multilocus genotypes for 2000 random pairs of cousins using the observed population allele frequencies (i.e. the ratio above which 95% of simulated pairs of cousins were excluded). The rate of false–negatives (type II error) associated with this cut-off value was obtained by simulating 2000 random pairs of full-sisters (i.e. the proportion of simulated sister pairs with ratio values equal to or smaller than the cut-off; Goodnight & Queller 1999). At a significant value of $P \leq 0.05$ (ratio = 0.02), 99% of simulated pairs were correctly assigned to the full-sister category. Distinction between cousins and unrelated pairs requires at least 13 marker loci (Goodnight & Queller 1999), and therefore was not investigated here.

Statistical analysis

Generalized linear models (GLM), assuming normally distributed errors, were used to compare estimates of within-group relatedness between the four different periods of the nesting cycle considered. Relatedness values and proportions of full-sister pairs per group were arcsine transformed to improve the fit of residuals to assumptions of normality (Crawley 2005). Levene's test was used to examine the assumption of homogeneity of error variances across periods (Faraway 2004). For all GLMs, all potential explanatory variables (stage of the nesting cycle, group size, site and year) and their pairwise interactions were initially fitted. A minimally adequate model was found by the subsequent removal of explanatory terms (Faraway 2004). Starting with the interactions, terms were dropped until further removal led to significant ($P < 0.05$) increases in deviance, assessed from tabulated values of *F* (Crawley 2005). The

Table 2 Probability (*U*-test) of error when rejecting the hypothesis that heterozygote deficiency is not present in each sample set, for each of the five microsatellite loci (and respective standard errors)

	Locus									
	Pdom 127		Pdom 139		Pdom 140		Pdom 20		Pdom 7	
Site, year	*P*	±SE	*P*	±SE	*P*	±SE	*P*	±SE	*P*	±SE
Site 1, 2004	0.703	0.000	0.122	0.004	0.615	0.002	0.176	0.008	0.333	0.002
Site 2, 2004	0.538	0.010	0.297	0.005	0.224	0.004	0.598	0.005	0.241	0.001
Site 1, 2005	0.618	0.000	0.747	0.004	0.393	0.003	0.609	0.005	0.706	0.001
Site 2, 2005	0.091	0.001	0.139	0.002	0.379	0.002	0.448	0.009	0.543	0.003

significance of each term (or two–way interactions) is reported when adding them last to the minimally adequate model. Two–way interactions were not included in the results unless significant.

Independent comparisons (number of stages – 1) between the different stages of the nesting cycle were also performed to compare mean values of within-group relatedness. To correct for the unbalanced number of samples collected in each period, 23 randomly selected groups were used in these contrasts (23 = smallest sample size = no. of winter aggregations collected). All analyses were carried out using the statistical package R (version 1.9.1, www.r-project.org).

A subset of 20 random wasps (from different groups) from each site and year was used to examine the potential presence of null alleles, which could introduce biases in the relatedness estimates (Dakin & Avise 2004). Heterozygote deficiency, expected if null alleles are present, was tested for each locus and subset of samples using the score test (*U*-test) (Rousset & Raymond 1995). Fisher's exact test was used on pairwise comparisons of these subsets to test for population differentiation across loci, that is, differences between sites 1 and 2 (Raymond & Rousset 1995a). Exact *P* values for both analyses were estimated using a Markov chain method (Guo & Thompson 1992), with 10 000 dememorization steps, 500 batches and 10 000 iterations per batch, using the software GENEPOP 3.4 (Raymond & Rousset 1995b) (http://wbiomed.curtin.edu.au/genepop/). Average heterozygosity for each locus was calculated following Nei (1987).

Results

Null alleles and population differentiation

The five microsatellites used were highly polymorphic, with the number of alleles ranging from 8 to 21 (Table 1). No heterozygote deficiency was detected for any of the five loci (Table 2), indicating that null alleles were unlikely to be present.

Table 3 Pairwise comparisons between site 1 and 2 (2004 and 2005) for genetic differentiation across all five microsatellite loci

Site (year) pairs	χ^2_{10}	*P*
Site 1 (2004) × Site 2(04)	23.275	0.009
Site 1 (2004) × Site 1(05)	9.573	0.478
Site 1 (2004) × Site 2(05)	13.652	0.189
Site 2 (2004) × Site 1(05)	21.918	0.015
Site 2 (2004) × Site 2(05)	14.695	0.143
Site 1 (2005) × Site 2(05)	8.748	0.556

Differences in allele frequencies were found between samples collected from site 2 in 2004 and samples from site 1 (Table 3). Significant differences were observed for only two loci: Pdom140 and 127 (Fisher's exact test, $P = 0.030$ and $P = 0.013$, respectively). Because of these differences between the sites, relatedness values and likelihoods for pairwise relationship categories were estimated using the allele frequencies obtained for each site separately (not the overall frequencies).

Genetic relatedness between foundresses

Overall relatedness values for each site were relatively low and not significantly different ($r_{\text{site 1}} = 0.293 \pm 0.032$ SE and $r_{\text{site 2}} = 0.358 \pm 0.021$ SE; $t = 1.664$, $P = 0.098$). Considering all of the groups genotyped ($n = 180$), average within-group relatedness values for each stage of the nesting cycle ranged from 0.189 to 0.491 (Fig. 1). The distribution of group relatedness estimates (*r*) for each of the four stages exhibited peaks in three different positions: close to zero (e.g. late nests; Fig. 2), at intermediate values of relatedness (e.g. winter aggregations; Fig. 2) and near the full-sister value of three-quarters (e.g. early nests; Fig. 2). Although groups with intermediate values of relatedness are present in each stage (approximately 45% of all nests in each stage), clear differences between the four stages were observed

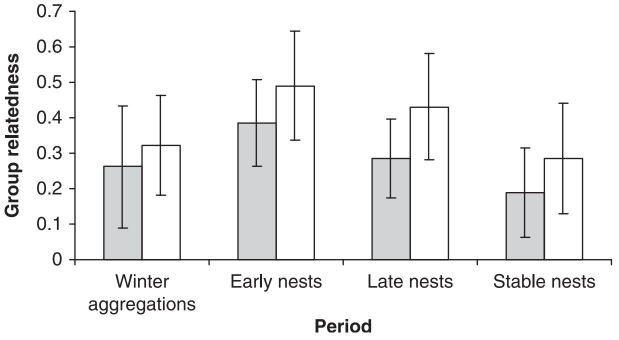

Fig. 1 Average within-group genetic relatedness estimates for the four stages of the nesting cycle at both sites (grey bars, site 1; white bars, site 2; whiskers indicate 95% CI). Significant differences were found between early nests and winter aggregations ($t = -2.035$, $P = 0.045$) and between early nests and preworker emergence stable nests ($t = -2.989$, $P = 0.003$).

at the extremes of the relatedness distribution (Fig. 2). In winter aggregations, groups of nonrelatives are as frequent as groups of full-sisters (27% and 26% of all aggregations, respectively). At the following stage (early nests), high relatedness values ($r \geq 0.6$) were obtained for 40% of the groups. At the two subsequent stages, this trend is reversed and larger peaks are observed close to zero, indicating the prevalence of groups of nonrelatives. Just before worker emergence, at the stable nest stages very low relatedness values ($r \leq 0.1$) were found for 36% of the groups.

The results of the GLM analysis confirmed these trends. Stage of the nesting cycle had a significant effect on within-group relatedness ($F = 3.018$, d.f. = 3, $P = 0.031$). Independent contrasts between the early nests stage and the remaining stages showed that within-group relatedness is significantly higher in early nests than in both winter aggregations and stable nests ($t = -2.035$, $P = 0.045$, and $t = -2.989$, $P = 0.003$, respectively).

Although group size varied considerably, ranging from 2 to 14 foundresses on nests and up to more than 100 wasps in winter aggregations, it had no significant effect on within-group relatedness ($F = 0.7870$, d.f. = 1, $P = 0.377$). The interaction term between group size and stage of the nesting cycle was also not significant ($F = 0.872$, d.f. = 3, $P = 0.459$), indicating that the differences in relatedness between stages were not affected by the number of wasps in each group. Site and year also had no significant effect ($F = 2.685$, d.f. = 1 $P = 0.105$ and $F = 2.726$, d.f. = 1, $P = 0.102$, respectively). Equivalent results were obtained in the GLM analysis when using the proportion of full-sisters pairs per group as a measure of relatedness instead of raw r values.

Estimated within-group relatedness values are highly correlated with the proportion of pairs within the group assigned to the category of full-sisters in the kinship analysis (Pearson's $R = 0.834$, $P \ll 0.001$). This indicates that the observed variation in relatedness values is primarily (70%, $R^2 = 0.696$) explained by changes in the number of full-sisters in each group. Since *P. dominulus* foundresses are known to be singly mated (Queller *et al.* 2000; Strassmann 2001), it can be assumed that groups with intermediate values of relatedness are a mixture of full-sisters, cousins and unrelated individuals, but not half-sisters.

Discussion

The results presented here show a surprising level of variation in within-group relatedness in foundresses associations of *Polistes dominulus*. Although full-sister groups could be found throughout the nest foundation period, the vast majority (*c.* 80%) of worker-producing groups, that is, stable nests, had one or more females which were unrelated (or

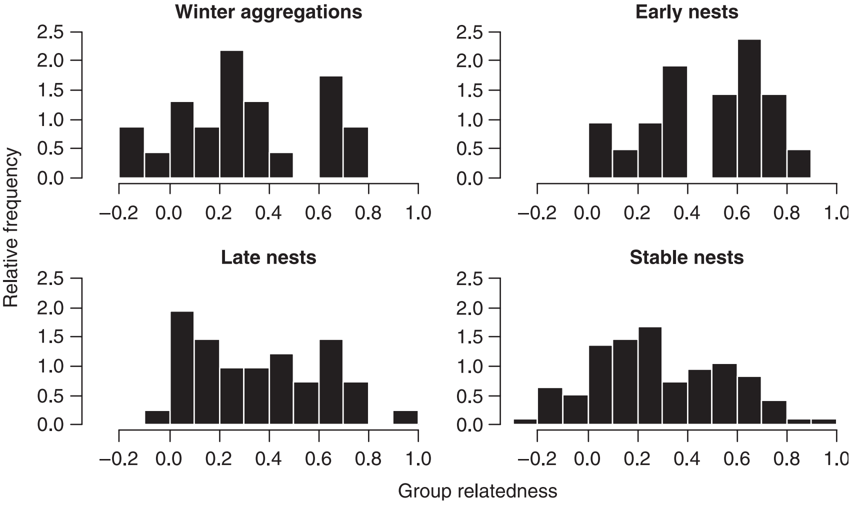

Fig. 2 Distribution of raw within-group relatedness estimates for the different stages of the nesting cycle. Overall number of groups considered: 23 winter aggregations (99 wasps), 27 early nests (84 wasps), 35 late nests (154 wasps) and 95 stable nests (350 wasps). Within-group relatedness values were calculated using the allele frequencies for the stage concerned as background population values.

distantly related) to the remaining members of the group. Associations between unrelated foundresses have been observed previously in other *P. dominulus* populations (Queller *et al*. 2000; Liebert & Starks 2006). However, the high frequency of within-group genetic asymmetries, that is different sibships in the same group, observed in our Spanish population has not been reported before.

The levels of within-group relatedness observed at the winter aggregations, where equivalent fractions of full-sister and non-sister pairs were found, indicate that winter aggregations tend to be mixtures of foundresses that emerged from different nests in the previous year. In addition, group size (i.e. number of wasps in the aggregation) had no significant effect on within-group relatedness, indicating that estimated values of within-group relatedness were not affected by the number of large aggregations (> 30 wasp) sampled. These results are in line with previous behavioural studies in semi-natural conditions which showed that winter aggregations are usually formed by gynes which emerge from different nests during the previous year (Pratte 1982; Starks 2003). *Polistes dominulus* foundresses have also been observed over-wintering with other wasp species (Pardi 1942; L. Dapporto, personal communication). Hence, it is not surprising that unrelated wasps are found in the same winter aggregation.

The mixing of foundresses from different natal nests in the winter aggregations has been correlated with a reduction in cuticular chemical differences between foundresses (Dapporto *et al*. 2004). This mixing of chemical cues could potentially explain mechanistically why unrelated foundresses end up nesting together (Dapporto *et al*. 2004; Gamboa 2004). However, since cofoundresses do not necessarily come from the same winter aggregation (L. Zanette and J. Field, unpublished), the effect of this winter mixing of cues on the formation of groups of unrelated wasps is uncertain. It has been experimentally shown that foundresses of *Polistes fuscatus* preferentially associate with full-sisters whether they hibernate with them or not (Post & Jeanne 1982). Despite it not being clear whether the associations observed in Post and Jeanne's experiment did represent stable groups or ephemeral associations, their results suggest that the effect of the winter mixing of chemical cues may not be strong enough to prevent foundresses from finding their close relatives.

The significant increase in relatedness observed when nests start to be formed (Fig. 2) could be the result of foundresses actively searching for their sisters, but there is no indication that *P. dominulus* females can recognize their close kin through chemical signals or otherwise at this early stage of the nesting cycle (Sledge *et al*. 2001; Sledge *et al*. 2004). The tendency of females to return to their original colony site (philopatry), when they leave their winter refuges, could explain why higher within-group relatedness values were more common on early nests (Klahn 1979; Jeanne & Morgan 1992; Queller 1994; Starks 2003; Sumana *et al*. 2005). Interestingly, philopatry alone may not necessarily lead to high levels of within-group relatedness as indicated by the lower levels of within-group relatedness observed in late and stable nests.

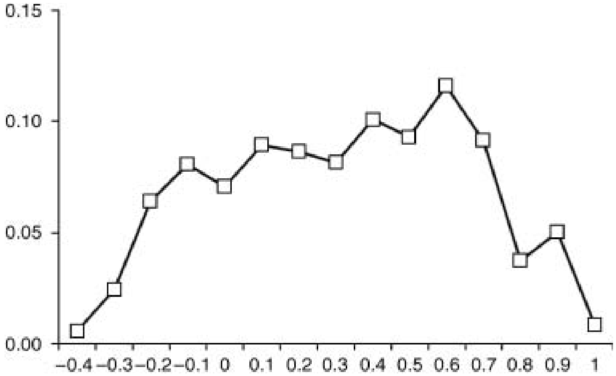

Fig. 3 Observed distribution of relatedness estimates of foundress nestmate pairs from all nests collected (116 pairs from 27 early nests, 274 pairs from 35 late nests and 629 pairs from 95 stable nests). Wasps from winter aggregations are excluded to facilitate comparison with Queller *et al*. (2000).

Apart from seasonal changes, the main differences between early nests and the subsequent two stages (late and stable nests) is the number of foundresses active at a site. Therefore, the significant decrease in relatedness observed in the stable nests could be a consequence of the arrival of unrelated joiners (potential usurpers) at established groups (L. Zanette and J. Field, unpublished). The within-group relatedness values observed in stable nests contrast with values found for other temperate *Polistes* species, where the average relatedness between foundresses is close to the haplodiploid full-sister value of three-quarters (Field *et al*. 1998; Reeve *et al*. 2000; Seppa *et al*. 2002). Our results also differ from the high average relatedness values previously found between pairs of dominant-subordinate foundresses in the same Spanish population (Cant *et al*. 2006a). However, this difference could be because nests where joiners arrived late in the foundation period were not utilized by Cant *et al*. (J. Field, personal observation).

The observed relatedness distribution in stable nests is similar to the pattern described by Queller *et al*. (2000) for *P. dominulus* in Tuscany (Italy), with a large peak of unrelated foundresses and a large peak of full-sisters. To directly compare our results, we plotted the distribution of relatedness estimates for foundress nestmate pairs from all nest collections combined, excluding winter aggregations (Fig. 3). As in the Italian population, a larger fraction of pairs had relatedness values close to the full-sisters three-quarters value than to zero. However, in our population a larger portion of nestmate pairs had intermediate values of relatedness ($0.2 < r < 0.4$) than in Italy, suggesting that nestmates are more commonly cousins or other relatives in our population.

Interestingly, our results show that a relatively small fraction (*c*. 10%, Fig. 2) of the stable nests, that is, groups that will produce the next generation, is formed exclusively by full-sisters. Thus, it can be assumed that most stable nests have one or more foundresses that are unrelated or distantly related to their cofoundresses. Direct fitness benefits through nest inheritance have been suggested as a possible explanation for these groups of unrelated foundresses (Queller *et al*. 2000). Testing this explanation, however, requires elucidation of fundamental aspects of the reproductive strategies of *P. dominulus*. Data on dominance turnover are scarce for most Polistinae (e.g. Field *et al*. 1998; Queller *et al*. 2000). Anecdotal evidences suggest that subordinate foundresses are evicted from worker-producing nests of *P. dominulus* (Pardi 1942), but this needs to be confirmed. Such information is crucial to examine the importance of direct benefits. In *Polistes*, other forms of direct benefit such as group augmentation are also likely to rely on nest inheritance (Field & Cant, 2007).

The very low frequency of monogynic nests observed in our study population (0.06% of all foundresses) suggests that nesting alone may not always be a viable option for *P. dominulus* foundresses. Hence, it may be that the cost of helping (c in Hamilton's rule) is close or equal to zero, so that helping distantly related individuals (e.g. cousins) may be the best strategy for many foundresses. Future tests of predictions derived from kin-selection models should consider this possibility. Nevertheless, low relatedness has also been found in *P. dominulus* populations where single foundress nests are more frequent and successful (Queller *et al*. 2000; Liebert & Starks 2006). This suggests that low c may not be a universal explanation for low r in *P. dominulus* groups.

In conclusion, the results present here show that low within-group genetic relatedness can be very common in foundresses associations of *P. dominulus*. Nevertheless, our results also show that a large fraction of foundresses preferentially nests with related individuals (full sisters or/and cousins). Local constraints on single nesting could explain why distantly related wasps associate, but not why many cofoundresses are completely unrelated (Fig. 2). Hence, indirect fitness benefits are unlikely to be the sole explanation for the occurrence of cooperative behaviour in these foundress associations.

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Lorenzo Zanette is currently a post-doctoral researcher at the University of East Anglia (UK), where he is investigating reproductive conflicts in bumble bees. This study is part of his PhD thesis on group formation in social wasps. Jeremy Field is Professor of evolutionary biology at the University of Sussex (UK). His research focuses on the behavioural and evolutionary ecology of social systems, using wasps and bees as model systems. His current work centres on environmental and genetic components of social plasticity; intra-group conflict; and the evolution of parental care strategies.
