Founders versus joiners: group formation in the paper wasp *Polistes dominulus*

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Within-group power asymmetries and the resulting reproductive skew, common in most social groups, may effectively be set at the very early stages of group formation, that is, when group membership is determined. Hence, groupmate choices can define an individual's future reproductive success. We examined how groups of *Polistes dominulus* formed under natural, unconstrained conditions, using data on the nesting history, kinship and morphology of individually marked foundresses obtained during two consecutive seasons in southern Spain. Foundresses that hibernated in the same aggregation were more likely to start a nest together, but all of the foundresses at a nest were seldom from a single aggregation. Changes in group composition were frequent throughout the preworker period, mainly because some foundresses disappeared and other wasps joined established groups. Within-group relatedness, however, was not affected by the late arrival of wasps. Our results suggest that waiting to join an established group is a common nesting strategy in *P. dominulus*. Only 16% of marked wasps used more than one nest. Foundresses that moved between groups tended to move to groups in which genetic relatedness among the resident foundresses was higher, but not necessarily relatedness to the moving wasp herself. Overall, nestmate choices were not associated with a single factor. High failure rates, particularly of single-foundress nests, however, suggest that ecological constraints (e.g. risk of predation, lack of resources) may have a stronger effect on individual nesting choices than previously considered.

Groups of cooperating individuals are observed from large marine mammals to unicellular amoebae (Strassmann et al. 2000; Mesnick et al. 2003). In most species, individuals form only temporary associations, frequently when breeding or foraging (Wilson 1975; Early & Dugatkin 2010). However, extreme forms of cooperation, in which group members partially or entirely forfeit their reproduction and never leave their natal groups, exist in at least two very distinct groups: vertebrates and insects (Wilson 1971; Reeve 1992; Clutton-Brock 2002). In the aculeate Hymenoptera, in particular, this radical type of cooperation, that is, eusociality, has evolved several times (Wilson 1971; Bourke & Franks 1995). Explaining why individuals sacrifice their own offspring production to assist in the reproduction of others has long puzzled evolutionary biologists (Hamilton 1964; Grafen 1991; Bourke 2011).

Kin selection has provided the major framework for understanding how altruistic behaviours have evolved (Hamilton 1964;

Frank 1998). Models based on this theory have made clear predictions about the range of conditions in which cooperative associations should be formed (reviewed in Foster et al. 2006; Lehmann & Keller 2006). Although empirical support for these predictions has been found in many taxa, the focus on within-group reproductive partitioning and relatedness has diverted attention from the process of group formation per se, especially under natural conditions (but see Aron et al. 2009).

Paper wasps of the common temperate species *Polistes dominulus* have a long period of nest foundation (ca. 2 months). Hence, they provide a valuable opportunity to scrutinize the early stages of group formation under natural field conditions. Mated foundresses emerging from their winter diapause refuges in early spring can pursue at least three nesting strategies: nest alone (monogyny), associate with other females forming multiple-foundress nests (polygyny) or remain on their winter refuges and 'sit and wait' to adopt orphaned nests later in the season (Reeve 1991; Starks 2001). Furthermore, before the emergence of workers at the beginning of summer, foundresses may switch groups or usurp established nests, that is, forcibly take the place of others in a group (Reeve 1991).

Potentially, foundresses make crucial behavioural (reproductive) decisions during the preworker period, that is, before dominance is established and group composition is stable. At the beginning and

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end of winter, when temperatures permit, foundresses frequently interact at their winter aggregation sites (Pardi 1942). Interactions range from simple antennation to trophallaxis (exchange of regurgitated food) and dominance interactions (Dapporto et al. 2005). Nevertheless, it remains to be clarified whether wasps that hibernate in the same aggregation are more likely to nest together later.

Once nests are initiated, foundresses are likely to meet exclusively at nests when they attempt to establish new groups or join established ones. Earlier studies indicate that as in other paper wasps, *P. dominulus* foundresses frequently move between nests before worker emergence (reviewed in Reeve 1991; Nonacs & Reeve 1995; Seppa et al. 2002). In seminatural conditions, Pratte (1979) reported that up to 75% of the foundresses switched from their original nest during the first 12 days of the nesting period, visiting on average three nests before settling permanently in a group. It has been suggested that nest-switching foundresses may be assessing the relative reproductive payoffs associated with the available nesting choices (Nonacs & Reeve 1995). Chemical profiles (epicuticular hydrocarbons) can potentially be used to discriminate dominant from subordinate wasps within recently established groups of *P. dominulus* (Sledge et al. 2004). However, foundresses that hibernate in the same winter aggregation have very similar chemical profiles (Dapporto et al. 2004). Thus, additional cues are likely to be used to select individual cofoundresses. Individual variations in body size and colour patterns exist and could potentially be used to select nestmates. Clypeal colour patterns are used in individual recognition by *Polistes fuscatus* females and in the establishment of dominance in *P. dominulus* foundress associations, that is, foundresses with larger and more disrupted clypeal marks tend to be dominants (Tibbetts 2002; Tibbetts & Dale 2004; but see Cervo et al. 2008; Green & Field 2011). However, there is little detailed information on the frequency and magnitude of foundress movements for most paper wasp species (e.g. Seppa et al. 2002; Sumner et al. 2007), so that the generality of these hypotheses remains to be tested.

We analysed the group formation process by examining the nesting histories of individually marked foundresses, and their movement patterns between different groups. We first investigated whether females that hibernate in the same winter aggregations later preferentially found nests together. We then tested the hypothesis that fluctuations in group composition caused by the late arrival of wasps determine intragroup genetic relatedness. Finally, we examined whether foundresses that visit different groups are choosing to join a group according to the kinship structure of the group and within-group variability in body size and facial patterns.

METHODS

Field Data Collection

We carried out field observations and collections at two semi-rural sites in southwestern Spain (Conil de la Frontera, Province of Cadiz; Site 1: 36°17′11N, 06°04′28W; Site 2: 36°17′11N, 06°03′57W). The habitat at both sites consisted of hedges of prickly pear cactus (invasive *Opuntia* sp., Barbera et al. 1992) surrounded by pasture and crop fields. Hedges were 1.5–3 m high, and 2–21 m wide. Five and four transects, adding up to a total of 500 and 180 m of hedge, were used in Sites 1 and 2, respectively.

Starting on 18 February 2004 and 11 February 2005, we monitored each site every other day (between 1000 and 1400 hours) to locate winter aggregations and newly founded nests. All groups detected were numbered and their locations mapped. On a subsequent day, before wasps were active (0700–0800 hours), females in winter aggregations were marked on the thorax with a large dot of enamel paint, with a unique colour for each aggregation. Wasps were marked directly in the hibernaculum with a long thin brush, since a pilot

study showed that when removed from it they did not usually return ($N = 10$ aggregations, 207 wasps marked, three returned). The number of wasps marked in each aggregation depended on its location and size.

All wasps found on new nests were gently collected with long forceps, placed into plastic bags and stored temporarily at 4 °C. Within 4 h of collection, wasps were individually marked (2004: four enamel paint dots; 2005: numbered tags from a honeybee queen marking kit: Thorne, Market Rasen, U.K.) and subsequently released onto their original nests to minimize any possible effect of removal. The proportion of marked wasps that were observed only once (at their original nest) was significantly higher in the second year (0.32 in 2004 and 0.52 in 2005; $\chi^2_1 = 28.963, P < 0.0001$), indicating that the tag marking used in 2005 was more disruptive for the wasps. Marked wasps observed only once at their original marking were not included in our group composition and wasp movement analyses. Wasps marked with numbered tags occasionally lost their tags, but could be identified by the presence of residual glue on the thorax and subsequently re-marked.

Every other day, we inspected all nests in each site early in the morning to detect changes in group composition. All wasps were identified, and newly arrived unmarked wasps were collected, marked and released on the same day.

Wasps that changed nests were placed into three categories: (1) movement with replacement: foundresses that left their initial nest up to 2 days after other foundresses (potential usurpers) arrived; (2) movement without replacement: foundresses that left their initial nest without the arrival of new wasps; and (3) nest-switching foundresses that moved two to three times between the same pair of nests.

Before the first workers started to emerge (May), all remaining marked foundresses and their nests were collected and stored at −80 °C.

Morphological Data Collection

Wings were carefully removed from frozen wasps, unfolded, mounted between glass slides and measured under a 16× Leica binocular microscope. The internal length of the longitudinal cell (Discoidal I) of the right wing was used as a measure of size. Wing length is highly correlated with overall body size (Sullivan & Strassmann 1984).

Wasps' heads were mounted on a glass slide and measured using a 30× Zeiss monocular microscope and the software NIH Image version 1.55 (http://rsbweb.nih.gov/nih-image/). The contour of the black clypeal marks was traced, and the area of the resulting polygon used as an estimate of clypeal mark size.

Genotyping

Total DNA was extracted from the anterior section of the thorax using 300 µl of grinding buffer (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 ultrafiltered distilled water. DNA was extracted from between two and 11 wasps per nest (mean ± SD = 4 ± 2), representing 87% of all foundresses present at collection (mean ± SD = 94 ± 42%).

Multiplex polymerase chain reactions (PCR) were performed using five fluorescently labelled, previously described primer pairs (Pdom 7, Pdom 20, Pdom 127b, Pdom 139, Pdom 140; Henshaw 2000). PCR was carried out using a Peltier Thermal Cycler using 10 µl reactions with 2 µl of DNA sample, 2 µl of reaction buffer ((NH$_4$)$_2$ SO$_2$), 0.6 µl of MgCl$_2$, 0.2 µl of each DNTP, 0.8 µl of each primer and 0.05 µl of Taq polymerase. 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 (Applied Biosystems, Inc., Foster City, CA, U.S.A.). Genotypes that appeared inconsistent with those of nestmates 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 one base pair or less were considered to be the same. Moreover, 27 samples previously genotyped by Cant et al. (2006) for four of the five loci were regenotyped. Scores differed for only one allele in each of two samples (error rate $= 2/(27 \times 2 \times 4) = 0.009$).

Relatedness Estimation

Genetic relatedness was estimated using the program RELATEDNESS 5.08, which calculates regression relatedness values using population allele frequency data based on the formula described by Queller & Goodnight (1989). Groups were weighted equally, and confidence intervals for relatedness estimates were obtained by jackknifing over loci.

Pairs of foundresses were assigned to the categories of full sister or nonsisters (i.e. cousins or unrelated, since *P. dominulus* are single mated) using a likelihood-based method implemented by the program Kinship (Goodnight & Queller 1999). With $\alpha = 0.05$, the power to detect full-sister pairs was 99% (based on 2000 simulations).

Statistical Analysis

We used generalized linear models (GLM), assuming normal error distributions, to test whether within-group relatedness was affected by variation in individual arrival times, summarized for each group by the standard deviation of the arrival dates of the wasps in the group. The proportion of wasps that disappeared with replacement and the proportion of wasps that disappeared without replacement were also tested as potential explanatory variables. In addition, group size, intragroup variation in body and clypeal mark size (SD of wing sizes and clypeal mark area, respectively), proportion of wasps with clypeal marks per group, site and year were used as explanatory variables. The effect of year and site is not reported in the Results unless significant.

We conducted two kinds of analysis to examine whether foundresses that moved between different nests could be choosing between potential groups according to their composition. First, we used paired *t* tests to compare pairs of nests visited by the same wasp in terms of each of six within-group traits summarizing group composition: within-group relatedness (including the moving wasp), mean body size, mean clypeal mark size, variation in body size (within-group SD), variation in clypeal mark size and the number of foundresses (group size). When assumptions of normality were not met, Wilcoxon signed-ranks tests were used instead. All morphological variables were calculated excluding the moving wasp herself. The proportions of full sisters of focal wasps that moved between two nests were also compared using a paired Student's *t* test.

In a second set of analyses, we used GLMs to test whether differences between nests in the same within-group traits depended on the type of foundress movement, that is, (1) movement with replacement; (2) movement without replacement; and (3) nest switching. For each within-group trait (e.g. relatedness), differences between nests visited by the same wasp were used as a response variable. Foundress movement type was tested as an explanatory factor and between-group differences in the remaining nest traits (e.g. group size and clypeal mark size variation) were tested as additional explanatory variables, along with the date when foundresses moved and the distance between successive nests. Differences between nests used by the same wasp were always calculated as the value of the trait in the original nest minus the value of the trait in the new nest.

We used GLMs, assuming a normal error distribution, to compare the wing size of wasps that moved and a random sample of wasps that did not move between different nests (using only one wasp per nest). Wing size was used as a response variable and wasp type (moved or did not move) as a factor. A chi-square test was used to compare the frequency of clypeal marks in these two types of wasps.

For all GLMs, all explanatory variables were initially fitted. A minimally adequate model was found by the subsequent removal of explanatory terms (Faraway 2004). Starting with the two-way 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 significance of each term (or two-way interactions) is reported when they were added last to the minimally adequate model. Interactions were not included in the Results unless significant.

Relatedness estimates and proportion of full-sister pairs per group were arcsine transformed to improve the fit of residuals to assumptions of normality (Crawley 2005). For all analyses we used the statistical package R versions 2.6.1 and 2.10.1 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org).

RESULTS

Group Formation, Group Size and Nesting Success

Overall, 58 winter aggregations were detected. A total of 492 foundresses were marked in 23 of these winter aggregations of 7–100 or more wasps each. In 18 of these aggregations, all wasps were marked. In the remaining five large aggregations (50 or more wasps), formed by layers of wasps, approximately half of all wasps could be marked. In total, 110 females marked in the winter aggregations were later found at a nest. Most of these foundresses (89 wasps) were observed nesting with at least one other wasp from the same aggregation (22 nests). In 13 of these nests, at least 50% or more of the foundresses ($N = 68$) came from the same winter aggregation. In contrast, 21 wasps marked at their winter aggregation were later found nesting exclusively with unmarked wasps, that is, wasps of unknown origin. The proportion of marked wasps that nested with at least one foundress known to be from the same winter aggregation was significantly bigger than expected by chance, that is, if wasps formed groups at random with respect to their winter aggregation ($\chi^2_1 = 21.891$, $P < 0.0001$). The distance between a wasp's winter aggregation and the nest it subsequently used ranged from 0.1 to 105.6 m (mean $\pm$ SD $= 13.57 \pm 13.57$; Fig. 1).

Overall, 2227 foundresses on 622 nests were individually marked and 58% of these wasps were subsequently observed more than once
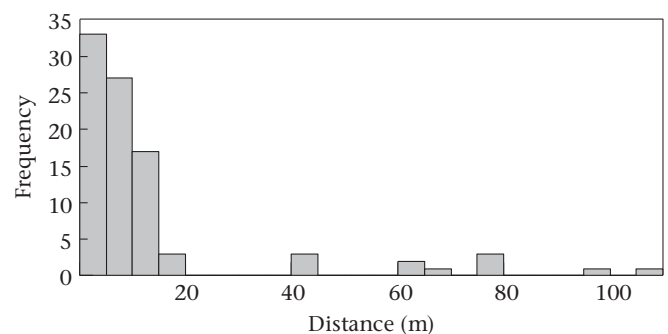
Figure 1. Distribution of distances between winter aggregations and nests used by the same individually marked wasps ($N = 91$ individuals).

at their original group, that is, they were not affected by marking. Most nests ($N = 317$) had three or fewer wasps when detected for the first time (143 nests were initiated by single foundresses). New nests were detected until May (late spring), but the vast majority of nests were founded during early and mid-spring (28% and 63%, respectively).

Nesting attempts by single wasps were less likely to succeed than nests started by two or more foundresses: 63% and 50% of new nests failed, respectively, within the first 10 days after discovery ($\chi^2_1 = 7.609$, $P = 0.005$). Success rates for nests started by two, three, four and five foundresses were not significantly different from each other ($\chi^2_3 = 2.701$, $P = 0.441$).

Group sizes ranged from two to 23 foundresses per nest. Groups of two and three foundresses were the most frequent (Fig. 2). Of 134 nests present at the end of the foundation season, before the first brood emerged, only nine had a single foundress (0.8% of all 1059 remaining foundresses).

Vacant nests could be found throughout the nest foundation period in 2004 and 2005 (111 and 54 nests in total, respectively). Most (101 of 165) had 15 or fewer cells containing only eggs. Vacant nests were adopted by new foundresses on only five occasions. All adopted nests were less than 2 weeks old.

Genetic Relatedness and Fluctuations in Group Composition

Complete historical (from the time of foundation), genetic and morphological data were obtained for 68 of 134 nests collected at the end of the foundation season. During the foundation season, at least one female disappeared from 60 of 68 nests. In 34 cases, foundresses disappeared without being replaced, that is, no other wasp arrived in the group during the previous 2 days. In six nests all disappearances of foundresses occurred with replacement, and in the remaining 20 nests both forms of group composition change occurred. Nine of the 196 wasps that disappeared from these nests were subsequently found in a different group.

Within-group relatedness was not affected by the arrival of new wasps at established groups (Table 1). Group size and the proportion of wasps that disappeared from nests also had no significant effect on relatedness (Table 1). In addition, the date groups were
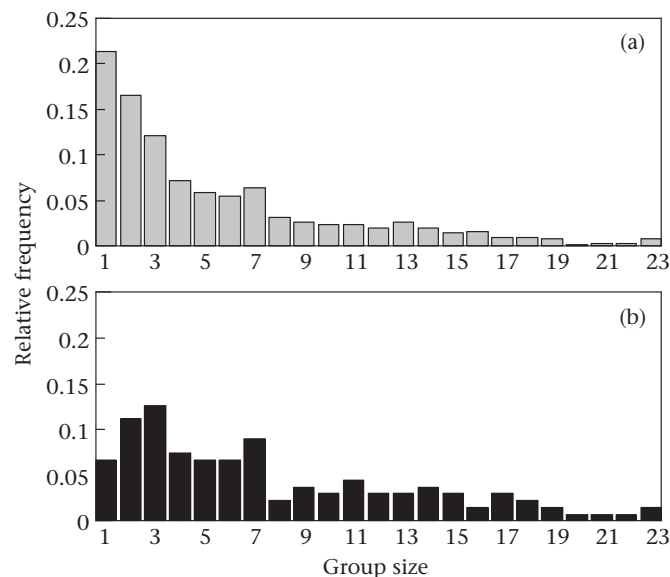
Table 1
Results of GLM testing whether within-group relatedness was affected by variation in group composition

Variables summarizing group composition	F	P
Within-group variation in arrival date (SD of individual arrivals)	1.926	0.171
Total number of foundresses in the group	0.397	0.531
Proportion of wasps that disappeared with replacement	0.286	0.594
Proportion of wasps that disappeared without replacement	0.519	0.473
Date group was founded	1.811	0.183
Within-group variation in body size (SD of wing size)	0.001	0.997
Within-group variation in clypeal mark size (SD of mark area)	2.741	0.102
Proportion of wasps with clypeal marks	5.978*	0.017*

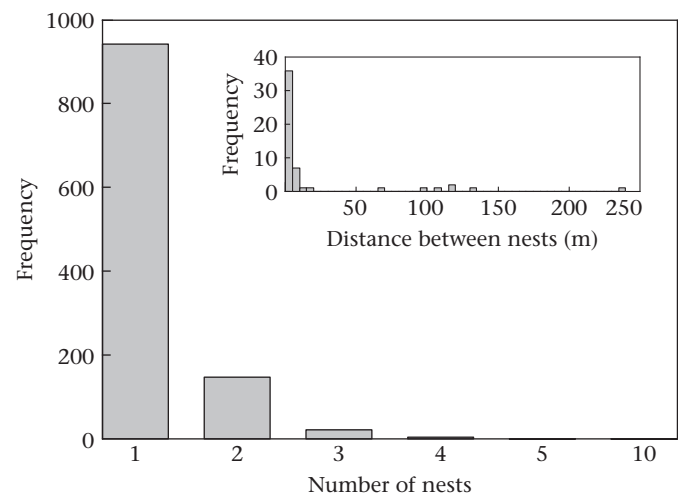F and P values for adding each explanatory variable to the minimal adequate model are shown.
* After the removal of two nests in which all wasps had marks, values changed to $F = 1.461$ and $P = 0.231$.

founded and within-group variation in either body size or clypeal mark size also had no significant effect (Table 1). The only variable with a significant effect on relatedness was the proportion of wasps that had clypeal marks (Table 1): within-group relatedness was lower in groups in which more foundresses had marks. However, this result is dependent on two nests with two unrelated foundresses each, in which all wasps had clypeal marks. When these nests are excluded from the analysis, nest foundation date is the only variable with a marginally significant effect ($F = 4.208$, $P = 0.044$). Nests that were initiated at the end of the foundation period (April) tended to have lower within-group relatedness. This effect was particularly important in the second year, when a larger number of nests were founded during this period.

Movements between Nests

Wasp movement between nests was relatively infrequent: only 16% of all successfully marked foundresses were observed at two or more nests (Fig. 3). Wasps that visited more than one nest were not different in size from wasps that were always in the same group (GLM: $F = 1.345$, $P = 0.247$). Black clypeal marks were equally common on foundresses that moved and foundresses that did not ($\chi^2_1 = 0.111$, $P = 0.739$).

The distance between pairs of nests used by the same wasp ranged from 0.1 to 235 m, but most pairs were less than 3 m apart

Figure 2. (a) Overall number of nests detected throughout the foundation period ($N = 597$) and (b) number of nests still active at the end of the foundation period, just before the emergence of the first workers ($N = 134$).

Figure 3. Number of nests used by individually marked wasps during the 4 months of the preworker period. Inset: distribution of distances between nest pairs where the same wasp was observed ($N = 178$ individuals).

(Fig. 3). Three foundresses that were nesting alone moved to multiple-foundress nests, and no wasp did the reverse.

Genetic and morphological data were obtained for all the nests visited by 59 foundresses that used more than one group. Wasps performed one of three types of movement: (1) movement with replacement: 15 wasps left their initial nest when one or more foundresses arrived (up to 2 days before their departure; in total, 19 wasps arrived); (2) movement without replacement: 32 wasps left their initial nest but no other foundress arrived (up to 2 days before their departure); and (3) nest switching: 12 foundresses moved two to three times between the same pair of nests, and on no occasion did their movements coincide with the arrival of other wasps.

In the 15 cases of movement with replacement, seven of the newly arrived wasps were cousins or unrelated to the wasps that left the group (later collected at other nests), three were sisters of one or more of the departed wasps and nonsisters of others, while in five cases, kinship could not be established because the newly arrived wasps disappeared before genotyping. Only one of the 19 newly arrived wasps had a black clypeal mark (not significantly different from the overall population, 94 of 654: Fisher's exact test: odds ratio = 0.33, P = 0.49).

No significant differences were found between the number or size of foundresses and the mean size of clypeal marks in the first and the second nest visited by a given wasp (Table 2). However, clypeal marks tended to be more variable in size on the last nest visited than on the initial nest (Table 2). Foundresses that used more than one nest moved to groups with higher within-group relatedness than the groups they left (first nest: mean ± SE = 0.113 ± 0.017; second nest: 0.227 ± 0.018; Table 2). This trend was more accentuated for wasps that moved at the end of the nest foundation period (Table 3). No other covariates were significant in this analysis (Table 3). In 50% of the cases in which a wasp moved to a nest with higher relatedness than the one it had left (N = 31), two or more full sisters not related to the wasp moving were present at the second nest. When we considered only the full sisters of the wasp moving (proportion in each nest), no significant difference was found between the two nests (Table 2). In short, although relatedness was higher at the second nest, in 50% of the cases this was a property of the other wasps in the group: wasps that moved were not necessarily moving to join closer relatives.

DISCUSSION

Group Formation, Group Size and Nesting Success

Our results provide evidence that foundresses that hibernate in the same aggregation tend to initiate nests together. In contrast, earlier experimental studies indicated that foundresses from the same winter group do not initiate nests more frequently than foundresses from different aggregations (Pratte 1982). However, the experimental design used by Pratte (1982) severely restricted the

Table 2
Summary of paired comparisons (t test and Wilcoxon V test) between nests visited by the same wasp

Nest traits	1st nest–2nd nest		
	Student's t	df	P
Group size (no. of foundresses)	0.177	54	0.861
Mean wasp body size per nest	0.961	52	0.342
Body size variation (SD)	1.419	47	0.162
Mean clypeal mark size (area) per nest	161*	35	0.117
Clypeal mark size variation (SD)	2.38	33	0.023
Within-group genetic relatedness	−3.961	53	<0.001
Proportion of full sisters of the focal moving wasp	−0.447	53	0.656

* Wilcoxon V.

Table 3
Summary of GLM results testing the effects of foundress movement categories and associated changes in group composition on within-group relatedness differences between nests used by the same foundresses

Explanatory variables	F	P
Foundress movement category	0.117	0.889
Distance between nests	0.314	0.579
Date movement occurred	−2.426	0.021
Difference in within-group clypeal mark variation (SD of area of marks)*	−0.202	0.841
Difference in within-group size variation (SD of wing length)*	0.223	0.825
Difference in group size*	2.511	0.122

F and P values for adding each explanatory variable to the minimal adequate model are shown.
Foundress movement categories are with replacement, without replacement and nest switching.
* Values in original nest minus values in new nest.

interactions between foundresses in winter aggregations, and could have introduced bias in his results.

Dapporto et al. (2004) have provided experimental evidence that foundresses that share the same winter refuge, independently of their natal nest site, will found nests together the following spring. Our results support their findings under natural field conditions in which foundress nesting options are unrestricted. Our findings indicate that hibernating in the same aggregation increases the probability that two or more females will found a nest together. However, in the present study only a small fraction of groups were formed exclusively by wasps known to have hibernated together. Therefore, it is unlikely that hibernating together is the sole determinant of group composition.

Multiple-foundress nests clearly had a higher chance of surviving until the emergence of workers than single-foundress nests. High failure rates for single-foundress nests have been observed in other populations of P. dominulus, and in other polistines (Hughes & Strassmann 1988; Seppa et al. 2002; Tibbetts & Reeve 2003). However, the small proportion of single-foundress nests (9/134) compared to multiple-female nests (125/134) that achieved worker production suggests that monogyny may be even more constrained in our population than others.

Although it was not possible to confirm directly whether wasps that disappeared had died or moved to an undetected group, disappearance is likely to be associated with death. Most wasps (63%) that used more than one nest moved less than 3 m (Fig. 3), so that undetected groups would usually have had to be within 3 m of known nests. At this distance, we would have detected most of these nests during our intensive searches. Nevertheless, the possibility that foundresses move to undetected nests cannot be completely excluded since we have recorded wasps moving between nests up to 235 m apart (Fig. 3). In addition, the average flight range (distance covered by wasps foraging) of polistine wasps is over 130 m (averaged across nine species; Ugolini 1983; Prezoto & Gobbi 2005).

In the present study, multiple-foundress associations of different sizes had similar failure rates. This agrees with a previous experimental study on the same population of P. dominulus, which again found that nest survival was not correlated with group size (Shreeves et al. 2003). Overall, this suggests that although foundresses in polygynic nests may benefit from increased survivorship in relation to single foundresses, these benefits are not directly correlated with the number of foundresses in the group. Hence, survivorship insurance benefits (sensu Nonacs & Reeve 1995) alone cannot explain why foundresses form large groups.

Nest adoption was very uncommon in our population. Although vacant nests were present throughout the foundation period, most of them were small and contained only eggs, so that they were probably unattractive to potential adopters (Starks 1998). A low frequency of

nest adoption has also been reported for the same population of *P. dominulus*, in which only two of 15 experimentally vacated single-foundress nests were reoccupied (Shreeves et al. 2003). Previous evidence of nest adoption by foundresses that were 'waiting' is restricted to studies under seminatural conditions in which adopted nests were relatively large and mature (with more developed larvae) and from which all foundresses had been experimentally removed (Nonacs & Reeve 1993; Starks 2001). The availability of large and mature nests under more natural conditions may be low since these nests are less likely to be completely abandoned by resident foundresses (Strassmann 1981; Tibbetts & Reeve 2003). Hence, the importance of nest adoption, especially as part of the 'sit and wait' alternative nesting strategy, may have been overestimated.

Since the order of arrival at a nest is correlated with rank for wasps that are towards the front of the dominance queue (first and second in the queue; Zanette & Field 2009), late joiners, unless they are usurpers, may seldom be dominants. This implies that an individual adopting a 'sit and wait' strategy may initially have limited reproductive success, since in *P. dominulus* reproductive skew is almost complete and cofoundresses are frequently unrelated (Queller et al. 2000; Liebert & Starks 2006; Zanette & Field 2008). Our results show that the late arrival of wasps had no effect on within-group relatedness. This suggests that the 'sit and wait' strategy is independent of indirect fitness benefits. Alternatively, late joiners may benefit through having a better chance of surviving until they can inherit the dominant position. During the waiting period, foundresses are likely to have a reduced mortality rate since they seldom leave their refuges (L. Zanette, personal observation). In addition, the large number of foundresses that disappeared from established nests indicates that the overall mortality of foundresses is high in the early stages of the nesting cycle. Hence, late joiners may have a longer life span than foundresses that started nesting early in the season. In *P. dominulus,* subordinates with higher ranks improve their chance of inheritance by spending less time in dangerous off-the-nest activities (Cant & Field 2001). Consequently, 'sit and wait' before joining can be a viable frequency-dependent strategy if the turnover of dominants is sufficiently frequent. Future studies could explore these hypotheses by testing whether late joiners live longer and inherit the dominant position more frequently than other wasps in their groups.

Genetic Relatedness and Fluctuations in Group Composition

Groups that were founded later in the season had lower within-group relatedness, particularly in 2005. This may indicate that close to the end of the nest foundation period, foundresses are constrained to nest with any available cofoundresses. The late arrival of wasps (potential usurpers), however, did not lead to low within-group relatedness. This suggests that usurpers, which are also common in several other species of *Polistes*, do not preferentially usurp unrelated wasps (Hughes & Strassmann 1988; Reeve 1991). Note that the assumption made here that all late joiners are potential usurpers may overestimate the frequency of nest usurpation. Usurpers are defined as wasps that forcibly take the place of others in a group. Therefore, direct observation of wasps arriving at nests and their subsequent interactions with nest residents would be necessary to identify usurpers unambiguously. However, even if we assume that usurpation occurred only when females left groups after others joined (i.e. movements with replacement), our results are unchanged: no significant effects of number and date of arrival of joiners on relatedness were detected.

Movements between Nests

A relatively small proportion of foundresses (16%) visited more than one nest. This suggests that foundresses may typically not

evaluate potential cofoundresses by visiting different established groups. Our findings appear to contrast with an earlier study, which found that foundresses nesting in an experimental enclosure visited three nests on average (Pratte 1979). Although possible effects of marking were accounted for (37% of the movements occurred after marking), it is unclear whether the number of nests visited could have been artificially increased by Pratte's (1979) experimental design. In our study, movements were recorded every other day. Consequently, foundress movements between nests were probably underestimated. Continuous behavioural observations of nests and individually marked wasps (through filming or electronic tags) would be necessary to examine more accurately the movement patterns of *P. dominulus* foundresses under field conditions (e.g. Sumner et al. 2007). Most importantly, filming would be essential to analyse the interactions between resident wasps and joiners.

Foundresses that did visit two or more nests tended to move to nests with significantly higher within-group relatedness than in the groups they left, especially at the end of the foundation period, when within-group relatedness is often low. However, the number of full sisters (of the focal moving wasp) did not differ between the two nests. This apparent contradiction is explained by the fact that on half of the occasions on which a wasp moved to a nest with higher relatedness, a different sib-ship was present at the second nest, unrelated to the focal wasp itself. These findings agree with previous studies showing that foundresses of *P. dominulus* cannot discriminate different levels of relatedness (reviewed in Gamboa 2004; but see Queller et al. 2000). They suggest instead that groups containing more closely related individuals (e.g. a pair of full sisters) are either more susceptible, or more attractive, to foundresses leaving other groups.

Conclusions

Foundresses that hibernated in the same winter aggregation were more likely to initiate a nest together, but groups formed exclusively by wasps from the same aggregation were rare. Hence, interactions at the winter aggregations are unlikely to be the only factor determining nestmate selection by *P. dominulus* foundresses.

Changes in group composition were frequent throughout the nest foundation period. Foundress disappearances (probably deaths) and the arrival of joiners at established groups were the main causes. Single-foundress nests were uncommon in the population, suggesting that this strategy has low viability. Polygynic nest foundation and 'sit and wait' were the most common nesting strategies observed. The latter, however, was not associated with the adoption of abandoned nests but was dependent on the frequency of established groups. Within-group relatedness was not affected by the late arrival of wasps, suggesting that indirect fitness benefits alone do not explain their nesting choices. By sitting and waiting, late joiners may be increasing their life span so that their chances of gaining direct fitness benefits through nest inheritance increase.

There was no single factor associated with movements of foundresses between nests. Movements were not generally associated with the arrival of new wasps (potential usurpers) at nests and were also not influenced by group size, or by the number of close relatives present in each group. But foundresses frequenntly moved to nests with higher within-group relatedness than in their original group. Overall, the observed high failure rates of both single- and multiple-foundress nests suggest that ecological constraints (e.g. risk of predation, lack of resources, parasitism) during the foundation period may have a stronger effect on individual nesting choices than previously assumed.

Acknowledgments

References

Aron, S., Steinhauer, N. & Fournier, D. 2009. Influence of queen phenotype, investment and maternity apportionment on the outcome of fights in cooperative foundations of the ant *Lasius niger*. *Animal Behaviour*, **77**, 1067–1074.

Barbera, G., Carimi, F. & Inglese, P. 1992. Past and present role of the Indian-fig prickly-pear *Opuntia ficus-indica* (L. Miller, Cactaceae) in the agriculture of Sicily. *Economic Botany*, **46**, 10–20.

Bourke, A. F. G. 2011. *Principles of Social Evolution*. Oxford: Oxford University Press.

Bourke, A. F. G. & Franks, N. R. 1995. *Social Evolution in Ants*. Princeton, New Jersey: Princeton University Press.

Cant, M. A. & Field, J. 2001. Helping effort and future fitness in cooperative animal societies. *Proceedings of the Royal Society B*, **268**, 1959–1964.

Cant, M. A., English, S., Reeve, H. K. & Field, J. 2006. Escalated conflict in a social hierarchy. *Proceedings of the Royal Society B*, **273**, 2977–2984.

Cervo, R., Dapporto, L., Beani, L., Strassmann, J. E. & Turillazzi, S. 2008. On status badges and quality signals in the paper wasp *Polistes dominulus*: body size, facial colour patterns and hierarchical rank. *Proceedings of the Royal Society B*, **275**, 1189–1196.

Crawley, M. J. 2005. *Statistics: An Introduction using R*. Chichester: J. Wiley.

Clutton-Brock, T. 2002. Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, **296**, 69–72.

Dapporto, L., Pansolli, C. & Turillazzi, S. 2004. Hibernation clustering and its consequences for associative nest foundation in *Polistes dominulus* (Hymenoptera Vespidae). *Behavioral Ecology and Sociobiology*, **56**, 315–321.

Dapporto, L., Palagi, E. & Turillazzi, S. 2005. Sociality outside the nest: helpers in prehibernating clusters of *Polistes dominulus*. *Annales Zoologici Fennici*, **42**, 135–139.

Early, R. & Dugatkin, L. A. 2010. Behavior in groups. In: *Evolutionary Behavioral Ecology* (Ed. by D. Westneat & C. Fox), pp. 285–307. Oxford: Oxford University Press.

Faraway, J. 2004. *Linear Models with R*. Boca Raton, Florida: Chapman & Hall/CRC.

Foster, K. R., Wenseleers, T. & Ratnieks, F. L. W. 2006. Kin selection is the key to altruism. *Trends in Ecology & Evolution*, **21**, 57–60.

Frank, S. A. 1998. *Foundations of Social Evolution*. Princeton, New Jersey: Princeton University Press.

Gamboa, G. J. 2004. Kin recognition in eusocial wasps. *Annales Zoologici Fennici*, **41**, 789–808.

Goodnight, K. F. & Queller, D. C. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, **8**, 1231–1234.

Grafen, A. 1991. Modelling in behavioural ecology. In: *Behavioural Ecology: an Evolutionary Approach* (Ed. by J. R. Krebs & N. B. Davies), pp. 5–31. 3rd edn. Oxford: Blackwell Scientific.

Green, J. P. & Field, J. 2011. Interpopulation variation in status signalling in the paper wasp *Polistes dominulus*. *Animal Behaviour*, **81**, 205–209.

Hamilton, W. D. 1964. The genetical evolution of social behaviour, I and II. *Journal of Theoretical Biology*, **7**, 1–52.

Henshaw, M. T. 2000. Microsatellite loci for the social wasp *Polistes dominulus* and their application in other polistine wasps. *Molecular Ecology*, **9**, 2155–2157.

Hughes, C. R. & Strassmann, J. E. 1988. Foundress mortality after worker emergence in social wasps (*Polistes*). *Ethology*, **79**, 265–280.

Lehmann, L. & Keller, L. 2006. The evolution of cooperation and altruism: a general framework and a classification of models. *Journal of Evolutionary Biology*, **19**, 1365–1376.

Liebert, A. E. & Starks, P. T. 2006. Taming of the skew: transactional models fail to predict reproductive partitioning in the paper wasp *Polistes dominulus*. *Animal Behaviour*, **71**, 913–923.

Mesnick, S., Evans, K., Taylor, B. L., Hyde, J., Escorza-Treviño, S. & Dizon, A. E. 2003. Sperm whale social structure: why it takes a village to raise a child. In: *Animal Social Complexity: Intelligence, Culture and Individualized Societies* (Ed. by F. B. M. de Waal & P. L. Tyack), pp. 444–464. Cambridge, Massachusetts: Harvard University Press.

Nonacs, P. & Reeve, H. K. 1993. Opportunistic adoption of orphaned nests in paper wasps as an alternative reproductive strategy. *Behavioural Processes*, **30**, 47–60.

Nonacs, P. & Reeve, H. K. 1995. The ecology of cooperation in wasps: causes and consequences of alternative reproductive decisions. *Ecology*, **76**, 953–967.

Pardi, L. 1942. Ricerche sui polistine. V. La poliginia iniziale in *Polistes gallicus* (L.). *Bollettino Dell'istituto Di Entomologia Della Università di Bologna*, **14**, 1–106.

Pratte, M. 1979. Mobilité des fondatrices de *Polistes gallicus*. L. In: *Ecologie des Insectes Sociaux* (Ed. by D. Cherix), pp. 133–140. Lausanne: Musée de Zoologie.

Pratte, M. 1982. Former relations and foundress association in *Polistes gallicus* L. *Insectes Sociaux*, **29**, 352–357.

Prezoto, F. & Gobbi, N. 2005. Flight range extension in *Polistes simillimus* Zakin, 1951 (Hymenoptera, Vespidae). *Brazilian Archives of Biology and Technology*, **48**, 947–950.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Queller, D. C., Zacchi, F., Cervo, R., Turillazzi, S., Henshaw, M. T., Santorelli, L. A. & Strassmann, J. E. 2000. Unrelated helpers in a social insect. *Nature*, **405**, 784–787.

Reeve, H. K. 1991. Polistes. In: *The Social Biology of Wasps* (Ed. by K. G. Ross & R. W. Matthews), pp. 99–148. Ithaca, New York: Cornell University Press.

Reeve, H. K. 1992. Queen activation of lazy workers in colonies of the eusocial naked mole-rat. *Nature*, **358**, 147–149.

Seppa, P., Queller, D. C. & Strassmann, J. E. 2002. Reproduction in foundress associations of the social wasp, *Polistes carolina*: conventions, competition, and skew. *Behavioral Ecology*, **13**, 531–542.

Shreeves, G., Cant, M. A., Bolton, A. & Field, J. 2003. Insurance-based advantages for subordinate co-foundresses in a temperate paper wasp. *Proceedings of the Royal Society B*, **270**, 1617–1622.

Sledge, M. F., Trinca, I., Massolo, A., Boscaro, F. & Turillazzi, S. 2004. Variation in cuticular hydrocarbon signatures, hormonal correlates and establishment of reproductive dominance in a polistine wasp. *Journal of Insect Physiology*, **50**, 73–83.

Starks, P. T. 1998. A novel 'sit and wait' reproductive strategy in social wasps. *Proceedings of the Royal Society B*, **265**, 1407–1410.

Starks, P. T. 2001. Alternative reproductive tactics in the paper wasp *Polistes dominulus* with specific focus on the sit-and-wait tactic. *Annales Zoologici Fennici*, **38**, 189–199.

Strassmann, J. E. 1981. Parasitoids, predators, and group-size in the paper wasp, *Polistes exclamans*. *Ecology*, **62**, 1225–1233.

Strassmann, J. E., Solis, C. R., Peters, J. M. & Queller, D. C. 1996. Strategies for finding and using highly polymorphic DNA microsatellite loci for studies of genetic relatedness and pedigrees. In: *Molecular Zoology Advances, Strategies, and Protocols* (Ed. by J. D. Ferraris & S. R. Palumbi), pp. 528–549. New York: J. Wiley/A.R. Liss.

Strassmann, J. E., Zhu, Y. & Queller, D. C. 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature*, **408**, 965–967.

Sullivan, J. D. & Strassmann, J. E. 1984. Physical variability among nest foundresses in the polygynous social wasp, *Polistes annularis*. *Behavioral Ecology and Sociobiology*, **15**, 249–256.

Sumner, S., Lucas, E., Barker, J. & Isaac, N. 2007. Radio-tagging technology reveals extreme nest-drifting behavior in a eusocial insect. *Current Biology*, **17**, 140–145.

Tibbetts, E. A. 2002. Visual signals of individual identity in the wasp *Polistes fuscatus*. *Proceedings of the Royal Society B*, **269**, 1423–1428.

Tibbetts, E. A. & Dale, J. 2004. A socially enforced signal of quality in a paper wasp. *Nature*, **432**, 218–222.

Tibbetts, E. A. & Reeve, H. K. 2003. Benefits of foundress associations in the paper wasp *Polistes dominulus*: increased productivity and survival, but no assurance of fitness returns. *Behavioral Ecology*, **14**, 510–514.

Ugolini, A. 1983. Homing in the wasp *Polistes gallicus* (L): information gained enroute and initial orientation. *Monitore Zoologico Italiano*, **17**, 212–213.

Wilson, E. O. 1971. *The Insect Societies*. Cambridge, Massachusetts: Harvard University Press.

Wilson, E. O. 1975. *Sociobiology: the New Synthesis*. Cambridge, Massachusetts: Harvard University Press.

Zanette, L. & Field, J. 2008. Genetic relatedness in early associations of *Polistes dominulus*: from related to unrelated helpers. *Molecular Ecology*, **17**, 2590–2597.

Zanette, L. & Field, J. 2009. Cues, concessions, and inheritance: dominance hierarchies in the paper wasp *Polistes dominulus*. *Behavioral Ecology*, **20**, 773–780.