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Group formation in a social wasp

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Submitted for Examination of PhD Degree

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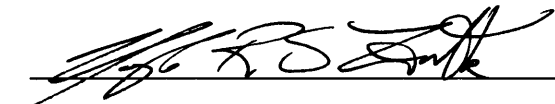
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Abstract

Group formation is a fundamental step in the evolution of cooperation, yet there are few models of this process in social animals, and even fewer empirical tests. The prolonged nest initiation phase in temperate paper wasps provides a valuable opportunity to study group formation. Data were obtained for 180 groups of females collected from a large population of *Polistes dominulus* in two sites in south-western Spain.

Foundresses on early nests were more closely related then females in winter aggregations or in stable groups (just before workers emerged). Most stable groups ($\geq$ 85 %) had one or more females that were unrelated (or distantly related) to the remaining members of the group. Mean wasp size did not markedly change during the different stages of the nesting cycle. Only 15 % of all foundresses had one or more clypeal marks. Wasps with marks were more common at the end of the nest foundation period than at early nests suggesting that these wasps may be adopting a "sit and wait" strategy.

Foundresses that hibernated in the same aggregation were more likely to start a nest together. Changes in group composition were likely to be caused by the disappearance of foundresses (death) and the frequent arrival of joiners to established groups. Within-group relatedness was not affected by the arrival of late joiners, but was negatively correlated with the date that nests were initiated. Only 16 % of all successfully marked wasps visited more than one nest. These wasps tended to move to nests with higher within-group relatedeness and less clypeal marks variability than their original group, but not necessarily a higher number of their own relatives.

Dominance hierarchies were experimentally inferred for 53 nests. Rank was negatively correlated with the number of full-sisters foundresses an individual had in its group. Highly-ranked wasps (rank 1 and 2) were less likely to share a nest with their full-sisters than wasps of lower rank, but an individual's rank was not affected by whether its full-sisters had a higher or lower position than it in the dominance hierarchy. Rank was also correlated with the size of clypeal marks, but only one foundress had a mark on 15 nests out of the 20 nests where wasp with marks were present (out of 53).

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CHAPTER 1:   General Introduction

1.1 Group formation

Groups of cooperating individuals are observed in organisms ranging from large marine mammals to unicellular amoebae (Strassman et al., 2000; Mesnick et al., 2003; Eichinger et al., 2005). The level of cooperation between group members and the social structure of cooperative groups varies greatly. In most species, individuals form only temporary associations, frequently when breeding or foraging (Wilson, 1975). However, extreme forms of cooperation, where group members partially or entirely forfeit their reproduction and never leave their groups exist in at least two very distinct groups: mammals and insects (Wilson, 1971; Reeve, 1992; Clutton-Brock , 2002). In the aculeate Hymenoptera, in particular, this radical type of sociality has evolved several times (Wilson, 1971; Bourke and Franks, 1995). Explaining why individuals may sacrifice their own offspring production to assist in the reproduction of others has long puzzled evolutionary biologists (Grafen, 1991; Bourke, 1997).

Kin selection theory has provided the major framework for understanding how reproductive sociality evolves (Hamilton, 1964; Bourke 1997; Frank, 1998; Queller, 2000). The numerous models constructed upon this theoretical basis have allowed clear predictions about the range of conditions where cooperation is favoured (reviewed in: Foster et al., 2006; Lehman and Keller, 2006). More specifically, transactional skew models, extending the basic premises of kin selection, predict that reproductive partitioning within groups is a function of not only the genetic relatedness between group members and the constraints on solitary living (versus group

living) but also the power asymmetries between group members (Emlen, 1997; Johnstone, 2000; Reeve and Keller, 2001).

Despite their generality and potential to predict when helping should be observed, the specific predictions made by transactional models regarding variation in reproductive skew have not found consistent empirical support (Reeve et al., 2000; Nonacs et al., 2004; Liebert and Starks, 2006; Nonacs et al., 2006). In eusocial wasps, in particular, the observed levels of skew are frequently high, i.e. dominants usually monopolize reproduction, regardless of the social structure of the groups or within-group relatedness (Queller et al., 2000; Nonacs et al., 2006). Outside the transactional framework, however, few modelling attempts have been made to explain the lack of variation in reproductive skew (Nonacs, 2002; Nonacs, 2006). The importance of this question is amplified by the fact that wasps in the same cooperative group are not necessarily close relatives, hence indirect fitness benefits cannot be obtained (Queller et al., 2000).

One possibility that has not received much attention is that social structures and the associated reproductive skew may be set at the very early stages of group formation, i.e. when cooperative associations start (Nonacs, 2006). Paradoxically, the focus on within-group reproductive partitioning has diverted attention from the process of group formation per se, i.e how individuals form a group under natural, unconstrained conditions. Early group formation studies have focused primarily on the trade-offs associated with changes in group size, but they have typically assumed that within-group variation is negligible (Pullian and Caraco, 1984; Higashi and Yamamura, 1993). However, the validity of this assumption for most social insects is limited, in particular, when dominance hierarchies exist (Reeve and Emlen,

2000). Social structure often reflects asymmetries between group members; hence these are likely to have an effect on group formation and cannot be neglected (Cant and English, 2006). Nevertheless, models and empirical tests of the importance of within-group variation in the formation of groups remain scarce (e.g. Seppa et al., 2002; Cant and English, 2006).

Temperate paper wasps of the genus *Polistes* start to found nests in late winter, soon after the first foundresses have left their winter refuges. The nest foundation period ends inl late spring, when all winter refuges are completely empty and the first workers are about to emerge (Reeve, 1991). During this prolonged nest foundation period (ca. 2 months) groups are started by one or a few foundresses but constantly change composition with the arrival and departure of individual foundresses. Hence, they provide a valuable opportunity to scrutinize the early stages of group formation under natural field conditions, to reveal which traits may determine group composition and the formation of social structures in eusocial wasps.

1.2 *Polistes* systematics and distribution

Polistes is one of the 29 genera in the vespid subfamily Polistinae (Carpenter, 1991). Paper wasps are the most diverse group of social wasps, both in species richness (943 species) and morphological and behavioural diversity (Carpenter, 1991; Arévalo et al., 2004). Polistinae can be divided in two major behavioural groups, based on their type of colony foundation and social structure (Jeanne, 1991). Independent founding is characterized by small, simply constructed nests without a protective paper envelope, founded

by a single (monogyny) or few mated females (polygyny), without the assistance of workers (Gadagkar, 1991). In contrast, swarm founding species have large colonies (usually over 50 adult wasps), often with a protective envelope, initiated by swarms formed by several mated females and a large number of workers (Jeanne, 1991). *Polistes,* a monophyletic group with 204 recognized species, is one of the five paper wasp genera where independent-founding occurs (the other four are: *Mischocyttarus, Belonogaster, Parapolybia* and *Ropalidia;* Arévalo et al., 2004, Pickett et al., 2006). *Polistes* is distributed throughout the world, although it is concentrated in the tropics, particularly in the New World (Carpenter, 1991).

1.3 *Polistes* as model organisms

There are several reason why *Polistes* are the most extensively studied group of social wasps. They are considerably common, their nests are seldom in cavities, and are usually small and not covered by a paper envelope (Pardi 1942; Reeve, 1991). Their life cycle is simple and they are relatively tolerant to manipulation in the field or laboratory, in particular before the emergence of workers (Pardi, 1996; Burian, 1996). Most important, however, is the primitive form of social organization in *Polistes,* without morphologically differentiated castes (Reeve, 1991; Röseler, 1991; but see Hunt, 2006). This is particularly evident in the early stages of the nesting cycle when only foundresses are present on the nests. Independently of their rank status, all foundresses retain their reproductive totipotency (Röseler, 1991; Sledge et al., 2004). Hence, foundresses are not morphologically constrained to adopt any particular

nesting strategy (Reeve, 1991; Pardi, 1996). A subordinate individual can potentially abandon its initial group and start its own nest, or usurp an established nest and became dominant e.g. (Makino and Sayama, 1991; Cervo and Lorenzi, 1996). Moreover, in most species foundresses can also switch groups, and in some cases adopt abandoned nests (Reeve, 1991; Starks, 2001). Such behavioural flexibility creates ideal opportunities for comparative studies on the evolution of cooperative reproductive strategies and group formation (Burian, 1996; Choe and Crespi, 1997; Starks and Fefferman, 2006).

1.4 *Polistes dominulus* (Christ, 1791)

1.4.1 Distribution

Polistes dominulus is one of the most abundant *Polistes* species in the Old World. Its distribution includes most of central and southern Europe, although it is absent from the coldest parts of northern Europe (Pekkarinen and Gustafsson, 1999; Cervo et al., 2000). It is particularly abundant in countries of the Mediterranean basin such as Spain, France, Italy, Greece and North Africa (Cervo et al., 2000). *P. dominulus* also occurs in the Middle East, including the countries around the Caspian Sea, and across Russia and China (Rusina et al., 2006).

In the past 30 years *P. dominulus* has successfully invaded North America, with its range expanding across two thirds of the United States (Johnson and Starks, 2004). It has been suggested that multiple independent

P. dominulus invasions occurred in the northeaster United States, nevertheless the exact locality of these invasions remains to be established (Johnson and Starks, 2004). *P. dominulus* has recently (2006) been observed in Hawaii (http://www.hawaii.gov/hdoa/pi/ppc/2006-annual-report/new-pest-detections- Hawaii Department of Agriculture, 2006).

1.4.2 Nesting cycle

Polistes dominulus exhibits an annual colony cycle, typical of polistine wasps in temperate regions (Reeve, 1991). Mated females (gynes) overwinter in aggregations ranging from a few to more than a hundred individuals. Winter diapause refuges, or hibernacula, are often re-used by females in different years and although their location varies considerably, they are commonly observed behind large nests of the previous year (Pardi, 1942; Dapporto et al., 2004; Turillazzi et al., 2006). In late winter/early spring, foundresses leave their winter refuges and start to found new nests, either singly (monogyny) or jointly with auxiliary foundresses (polygyny) (Pardi, 1942; Reeve, 1991). On polygynic nests, one individual becomes 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; Röseler, 1991; Reeve 1991). As in other polistines, nests are built with a mixture of woody fibres, water and proteinaceous glandular secretions (Wenzel, 1991; Downing, 1991; Curtis et al., 2005). Foundresses provision their nest cells with masticated insect larvae, primarily of Lepidoptera (Richter, 2000).

Towards the middle of the founding period (early spring), 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. Usurpers usually destroy the previous foundress's eggs and early instar larvae (Nonacs and Reeve, 1995; Starks, 2001). Nest foundation ceases and changes in group composition become less frequent in early summer, just before the first brood (mainly workers) starts to emerge (Shreeves et al., 2003). In mid summer, workers stop emerging and the first reproductives start to appear. This reproductive phase lasts until early-mid fall when colonies dissolve. Reproductives (males and females) do not participate in the activities of their natal nest and generally mate away from it (Pardi, 1942). As in other paper-wasps, males patrol and defend unique mating territories surrounding perching points (e.g. large trees), which are landmarks used by females as orientation cues (Turillazzi and Beani, 1985; Beani and Turillazzi, 1988; Reeve, 1991; Beani et al., 1992). Males die shortly after mating, and mated gynes disperse to winter diapause refuges (Pardi, 1942; Reeve, 1991).

1.5 Thesis structure and objectives

In the four main chapter of this thesis I analyse group formation in *P. dominulus*, primarily considering the importance of two factors: genetic relatedness and morphological differences between foundresses. In chapters (2) and (3), I focus on group composition changes across successive stages of the nest foundation period. In chapter (4), I focus on individual movement patterns and choices of nesting strategy. In chapter (5) I focus on the formation of social structures (dominance hierarchies) in foundresses associations. All four chapters are presented as independent manuscripts to be published separately, and their specific objectives are as follows.

Chapter 2

To 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. In particular, to test whether genetic relatedness increases as group formation progresses, as expected if females preferentially choose close relatives as nest-mates.

Chapter 3

To test the hypothesis that foundresses of *P. dominulus* preferentially form groups with morphologically dissimilar wasps, and thus, maximize within-group stability. This is expected if conflicts are less likely to occur between individuals with clear morphological differences, directly correlated to their fighting ability. I analyse the changes in within-group body size and clypeal mark size at successive stages of the nesting cycle, before group composition becomes stable and workers start to emerge. In particular, I test whether within-group morphological differences are more evident in stable groups at the end of the founding period than in early unstable groups at the beginning of the foundation period.

Chapter 4

The general goal of this chapter is to examine the basis of movements by individually marked foundresses in order to reconstruct their nesting history and examine the potential success of different nesting strategies. The specific objectives are:

a. To test the hypothesis that foundresses that hibernate in the same winter aggregations later preferentially found nests together, as expected if interactions between foundresses at their winter aggregations determine nest-mate choices;

b. To test the hypothesis that the arrival of late joiners decreases within -group genetic relatedness, as expected if late joiners are unrelated nest

usurpers;

c. To test the hypothesis that foundresses that switch groups are selecting which group to join according to the composition of the groups. In particular, to test whether nests used by the same foundress differ in their kinship structure and within-group morphological variability.

Chapter 5

The central objective of this chapter is to examine the basis of dominance queuing in foundress associations. The specific objectives are:

a. To test the hypothesis that dominance status in foundresses associations of *P. dominulus* is determined by a convention: the order of arrival at newly founded spring nests;

b. To test whether rank status is associated with two potential indicators of general fitness, body size and the size of clypeal marks;

c. To test the hypothesis that unrelated subordinates obtain higher positions in the dominance queue, thus providing a direct fitness incentive for them to remain in the group. I test whether unrelated subordinates are more likely to be at the front of dominance hierarchies, and whether an individual's status is correlated with the proportion of its nest-mates that are full sisters.

CHAPTER 2: Genetic relatedness in early associations of *Polistes dominulus*

Abstract

Indirect benefits obtained through the reproduction of relatives are fundamental in the formation and maintenance of groups. Here I examine the hypothesis that females of the common temperate paper wasp *Polistes dominulus* preferentially form groups with close relatives. Genetic relatedness data were obtained for 199 groups of females collected at the early stages of the nesting cycle of a large population of *P. dominulus* in two sites in south-western Spain. Average within-group relatedness values ranged from 0.139 to 0.491. Foundresses on early nests were significantly more closely related than females in winter aggregations and on nests (just before workers emerged). Within-group relatedness was independent of group size. The vast majority of worker-destined females (85 %) had one or more females that were unrelated or distantly related to the remaining members of the group. These results suggest that kin benefits alone are unlikely to explain why *P. dominulus* foundresses form cooperative associations.

Abstract

Indirect benefits obtained through the reproduction of relatives are fundamental in the formation and maintenance of groups. Here, I examine the hypothesis that females of the common 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 south-western Spain. Average within-group relatedness values ranged from 0.189 to 0.491. Foundresses on early nests were significantly more closely related then 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 (ca 85 %) had one or more females that were unrelated (or distantly related) to the remaining members of the group. These results suggest that indirect fitness benefits alone are unlikely to explain why *P. dominulus* foundresses form cooperative associations.

2.1 Introduction

Kin selection theory has provided a fundamental framework for the study of cooperative behaviour and altruism (Grafen, 1984; Frank, 1998; Griffin and 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 and 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; Turillazzi 2003). Such behavioural flexibility has provided a rare opportunity to accurately measure the costs and benefits associated with cooperative and non cooperative reproductive strategies within the same population (Queller, 1996).

Central to kin selection theory is the idea that individuals can obtain indirect fitness benefits through 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 relatedness studies based on allozyme data indicated that nest mates of at least 8 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 sub-tropical species: *P. bellicosus* (Field et al., 1998); *P. fuscatus* (Reeve et al., 2000); *P. carolina* (Seppa et al., 2002).

In contrast, unexpected low levels of genetic relatedness have been found in an Italian population of *P. dominulus*, where 35 % of nest mate foundresses were non relatives (Queller et al., 2000). Cooperation between unrelated individuals occurs in other communal (Kukuk and Sage, 1994) and eusocial Hymenoptera (Bourke and Franks 1995, Bernasconi and Strassmann, 1999). However, in *P. dominulus* associations the dominant female produces most or all the eggs (Liebert and Starks, 2006; Queller et al., 2000). This almost complete reproductive skew in groups of unrelated individuals is uncommon in social insects (Queller *et al.,* 2000).

Interestingly, 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 foundresses associations are composed mainly of full-sisters has been found for another European *P. dominulus* population (South of Spain; Cant et al., 2006b). Here, I 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.

2.2 Methods

2.2.1 Natural history of *Polistes dominulus*

Polistes dominulus has a colony cycle typical of polistine wasps in temperate regions (Reeve, 1991). Mated females (gynes) overwinter in aggregations ranging from a few to more than a hundred individuals. Winter diapause refuges, or 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 be found 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). New nests are founded either singly (monogyny) or jointly with auxiliary foundresses (polygyny; 2 to 23 females in my studied population) (Pardi, 1942). In Southern Spain, the founding period is relatively long, usually starting in the end of February and 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 (Chapter 4, but see Queller et al., 2000). Usurpers usually destroy the previous foundress's eggs and early instar larvae (Nonacs and Reeve, 1995; Starks, 2001). In the same period, at my study site, *P. dominulus* is subject to attack by a social parasite, *P. 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 become less frequent (i.e. stable groups). In mid summer, new workers stop emerging and the first reproductives start to emerge. These gynes 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, going to winter diapause refuges (Pardi, 1942; Pardi, 1996).

2.2.2 Specimen Collection

Females of *P. dominulus* were collected from two nearby sites (720 m apart) in south-western Spain (Conil de la Frontera, Cadiz, 36°15' N, 06°10'; Figure 1). 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

30
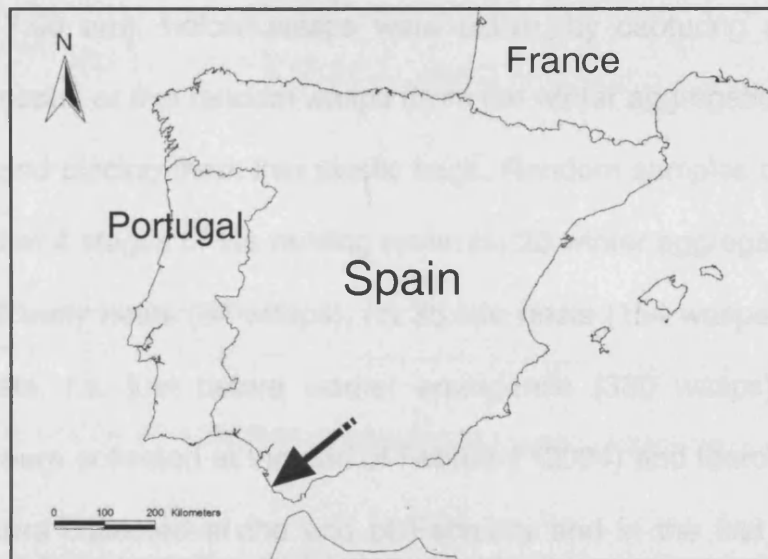
Figure 1 - A Location of the study sites on the Iberian Peninsula. Both sites were in a semi-rural area of the town of Conil de la Frontera (arrow) in the province of Cadiz. **B** Initial section of Site 1.

morning (ca. 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 4 stages of the nesting cycle: (a) 23 winter aggregations (99 wasps), (b) 27 early nests (84 wasps), (c) 35 late nests (154 wasps) and (d) 95 stable nests, i.e. just before worker emergence (380 wasps). Winter aggregations were collected at the end of February (2004) and March (2005). Early nests were collected at the end of February and in the first week of March (in both years). Late nests were collected during 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 Ecología Aquàtica", University of Cadiz, Spain). Samples represented 15% of all detected nests at stages (b) and (c), and 40 % and 35% at stage (a) and (d) respectively.

2.2.3 DNA extraction, amplification and visualization

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

Multiplex polymerase chain reactions (PCR) were performed using 5 previously described primers, fluorescently labelled (Table 1; Henshaw,

2000). PCR was carried out on a Peltier Thermal Cycler using 10µl 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. 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 (v. 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 base pair or less were considered to be equal. Moreover, 27 random samples previously genotyped for 4 of the same loci (Cant et al., 2006a), were re-genotyped, scores were not confirmed only for one allele in two samples (error rate = 2/[27X2X4] = 0.009).

2.2.4 Relatedness estimation

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

Pairs of foundresses were also assigned to sib categories using a

likelihood-based method implemented by the program Kinship (Goodnight and Queller, 1999) (http://www.gsoftnet.us/GSoft.html). 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 versus 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 and 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 and Queller, 1999), and therefore was not investigated here.

2.2.5 Statistical Analysis

Generalized linear models (GLMs), assuming normally distributed errors, were used to compare estimates of within-group relatedness between

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the 4 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 pair-wise 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 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.

Chi-squared tests were used to compare the total number of pairs, out of all possible pairs, assigned to the category of full-sisters (r = 0.75) at different stages of the nesting cycle. Independent comparisons (no. 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; http://www.r-project.org).

A sub-set 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 and Avise, 2004).

Heterozygote deficiency, expected if null alleles are present, was tested for each locus and sub-set of samples using the score test (U-test) (Rousset and Raymond, 1995). Fisher's exact test was used on pair-wise comparisons of these subsets to test for population differentiation across loci, i.e. differences between sites 1 and 2 (Raymond and Rousset, 1995a). Exact P values for both analyses were estimated using a Markov chain method (Guo and Thompson, 1992), with 10000 dememorization steps, 500 batches and 10000 iterations per batch, using the software GENEPOP 3.4 (Raymond and Rousset, 1995b) (http://wbiomed.curtin.edu.au/genepop/). Average heterozygosity for each locus was calculated following Nei (1987).

2.3 Results

2.3.1 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.

Genetic differentiation was 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 ($P = 0.030$ and $P = 0.013$, respectively). Because of these minor differences between the sites, relatedness values and likelihoods for pairwise relationship categories were estimated using the allele frequencies obtained for each site (not the overall frequencies).

2.3.2 Genetic relatedness between foundresses

Overall relatedness values for each site were relatively low and not significantly different ($r_{site\ 1} = 0.\ 293 \pm 0.032$ S.E. and $r_{site\ 2} = 0.358 \pm 0.021$ S.E; $t = 1.664$, $P = 0.098$). 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 (Figure 2). The distribution of group relatedness

Table 1 - Maximum number of alleles and average heterozygosity (observed and expected) for each microsatellite loci

Locus	Repeat*	Primers (5' – 3')	No. of alleles	H(obs.)	H(exp.)
Pdom 127	$(AAT)_{13}...(AAT)_6AA$	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.

Table 2 - Probability (*U-test*) of heterozygote deficiency for each of the 5 microsatellite loci, and respective standard errors (across loci)

Site -Year	Locus									
	Pdom 127		Pdom 139		Pdom 140		Pdom 20		Pdom 7	
	P	±S.E.	P	±S.E.	P	±S.E.	P	±S.E.	P	±S.E.
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

Table 3 - Pairwise comparisons between site 1 and 2 (2004 and 2005) for genetic differentiation across all 5 microsatellite locus

Site (Year) pairs	X^2_{10}	P
Site 1(04) X Site 2(04)	23.275	0.009
Site 1(04) X Site 1(05)	9.573	0.478
Site 1(04) X Site 2(05)	13.652	0.189
Site 2(04) X Site 1(05)	21.918	0.015
Site 2(04) X Site 2(05)	14.695	0.143
Site 1(05) X Site 2(05)	8.748	0.556

estimates (r) for each period shows three distinctive peaks: close to zero, at intermediate values of relatedness (0.2 ≤ r ≤ 0.5), and near the haplodiploid full sister value of three-quarters (Figure 3). 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 at the extremes of the relatedness distribution (Figure 3). Initially, at the winter aggregations stage, groups of non relatives 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 ≥ 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, indicated the prevalence of groups of non relatives. Just before worker emergence, at the stable nest stages very low relatedness values (r ≤ 0.1) were found for 37 % of the groups.

The results of the GLM analysis confirmed the trends observed in the relatedness distributions. Stage of the nesting cycle had a significant effect on within-group relatedness (F = 3.018, df = 3, P = 0.031). Independent contrasts between the early nest 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 among nests and to more than 100 wasps in winter aggregations, it had no significant effect on within-group relatedness (F= 0.7870, df = 1, P = 0.377). The interaction term between group size
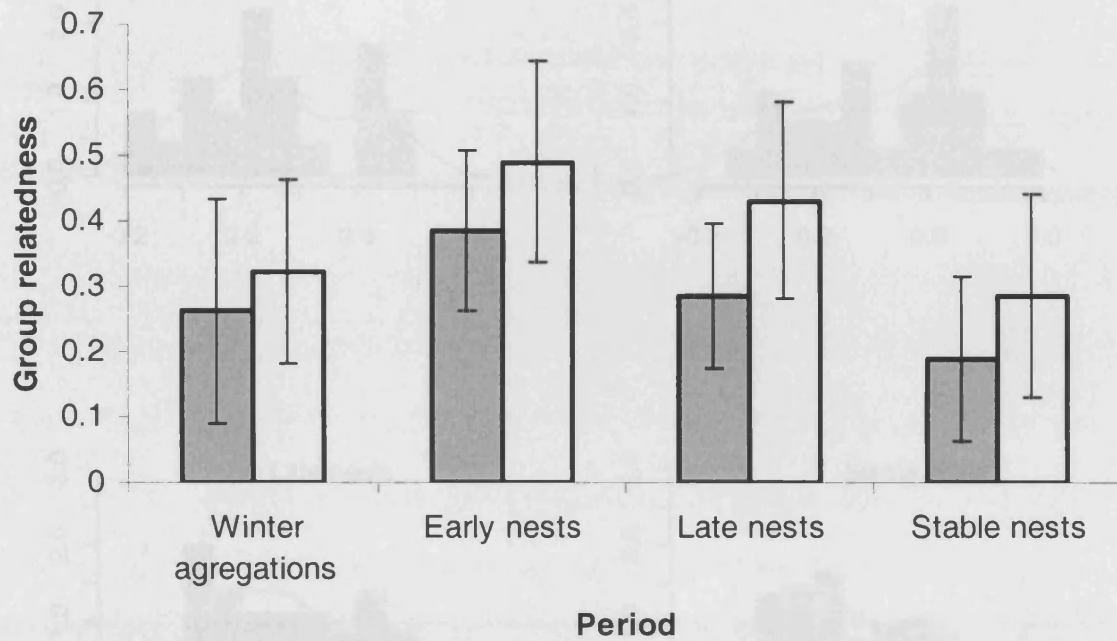
Figure 2 - Group genetic relatedness estimates for the four stages of the nesting cycle at both sites (Grey bars= Site1; White bars = Site 2; whiskers indicate 95 % C.I.). Significant differences were found between early nests and winter aggregations (t = - 2.035, *P* = 0.045) and between early nests and pre-worker emergence stable nests (t = - 2.989, *P* = 0.003).
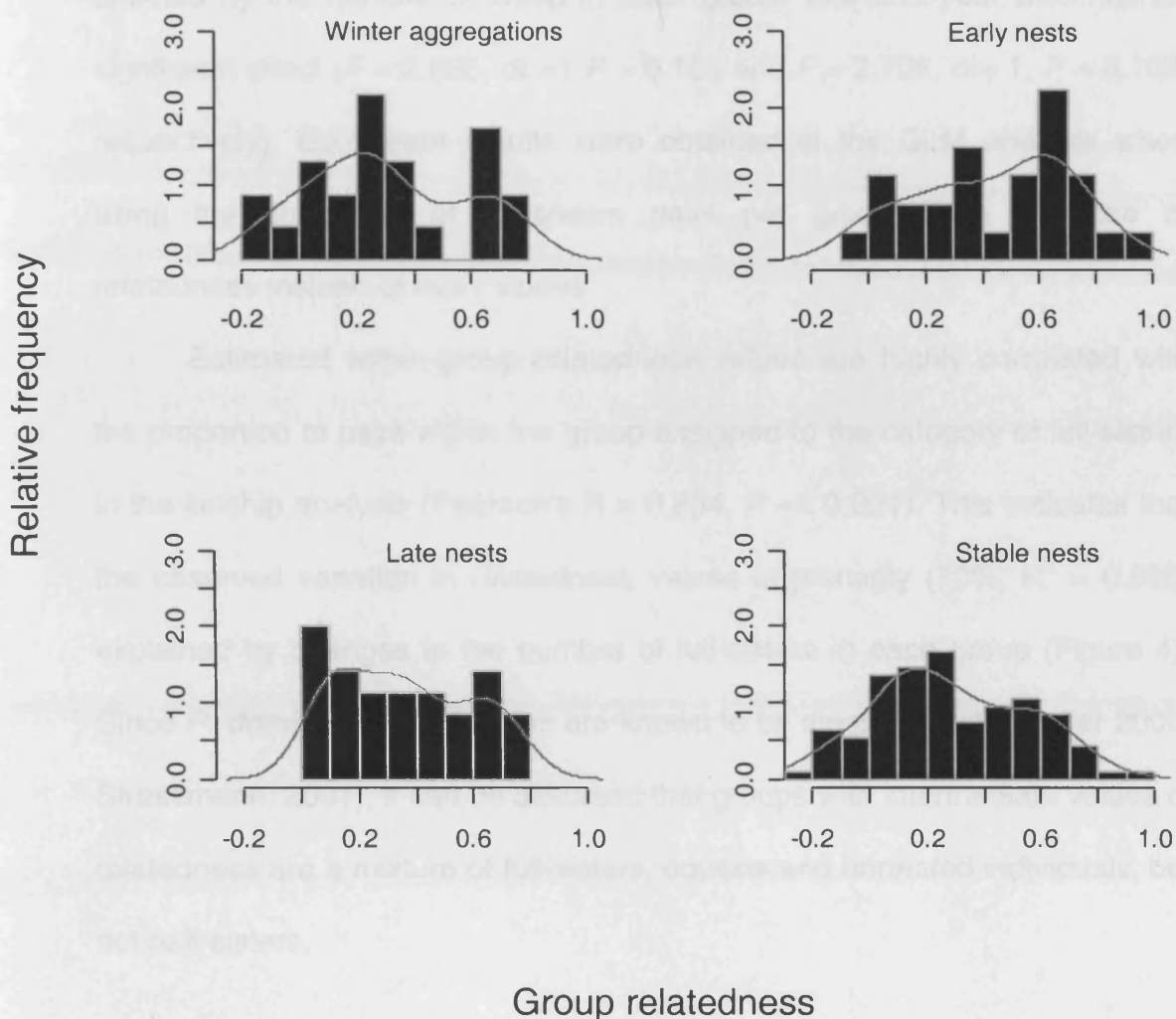
Group relatedness

Figure 3 - Distribution of raw relatedness estimates for the different periods of the nesting cycle, and respective kernel density plots. 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).

and stage of the nesting cycle was also not significant ($F = 0.872$, df = 3, $P = 0.459$), indicating that the differences in relatedness between stages were not affected by the number of wasp in each group. Site and year also had no significant effect ($F = 2.685$, df =1 $P = 0.105$ and $F = 2.726$, df= 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 (Figure 4). Since *P. dominulus* foundresses are known to be singly mated (Queller 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.

2.3.3 Overall proportion of full-sisters

Considering all possible different pairs of foundresses that could be formed with all the wasps collected at each stage of the nesting cycle, the proportion of wasp pairs assigned to the haplodiploid full-sister category differed considerably between stages. At the beginning of the nesting cycle (early nests stage) 0.066 of all pairs were assigned to the full-sister category; prior to worker emergence (stable nest stage) this proportion drops to 0.036

(Figure 5). The number of pairs assigned to the category of full-sisters was significantly larger at the early nest stage than at the winter aggregation and stable nest stages (X^2 = 6.754, P = 0.009; X^2 = 25.745, P < 0.001, respectively).
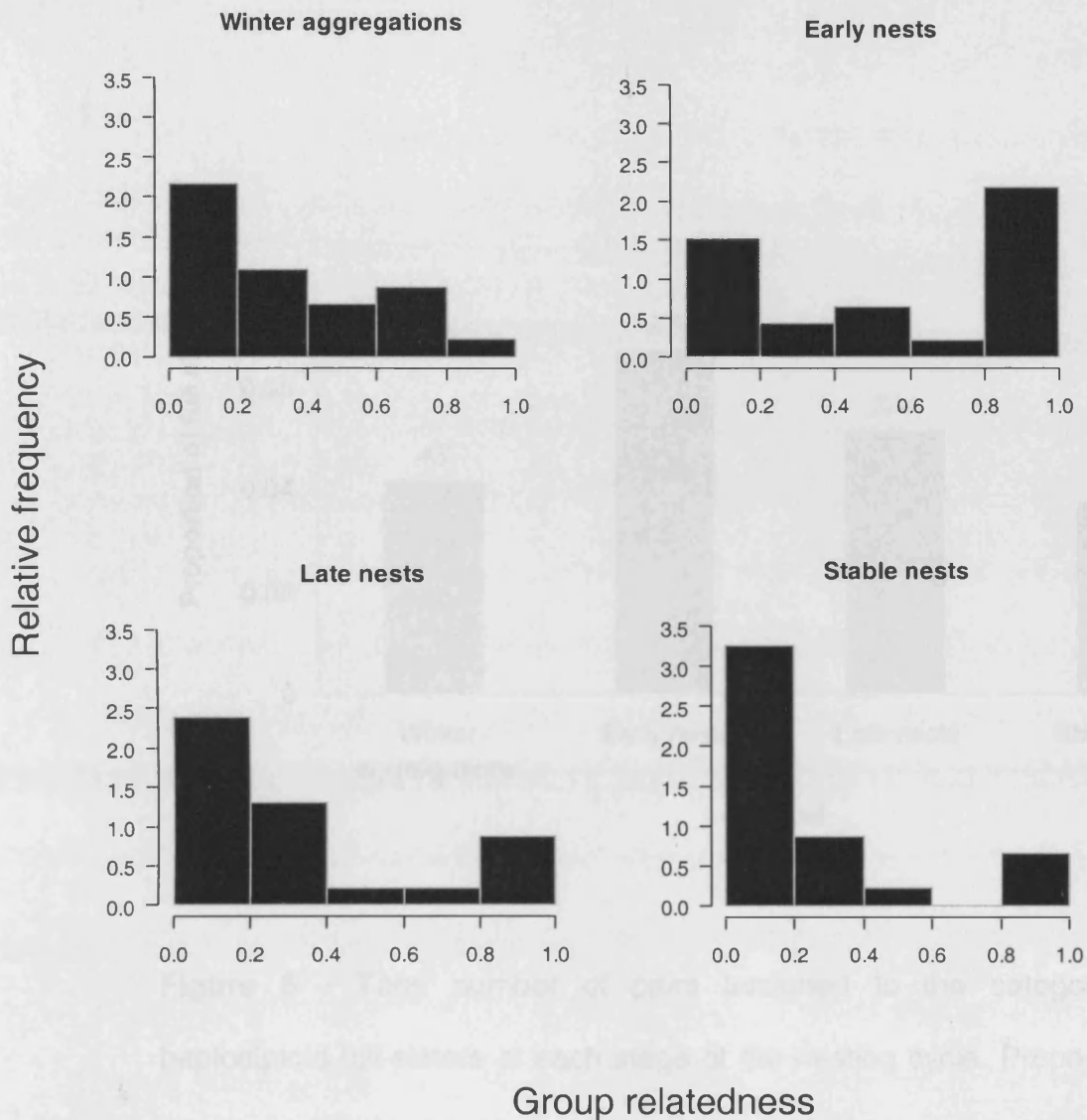
Figure 4 – Distribution of groups with different fractions of pairs assigned to the category of full sisters in the kinship analyses. Overall number of groups considered: 23 winter aggregations (99 wasps), 27 early nests (84 wasps), 35 late nests (154 wasps) and 95 stable nests (350 wasps).
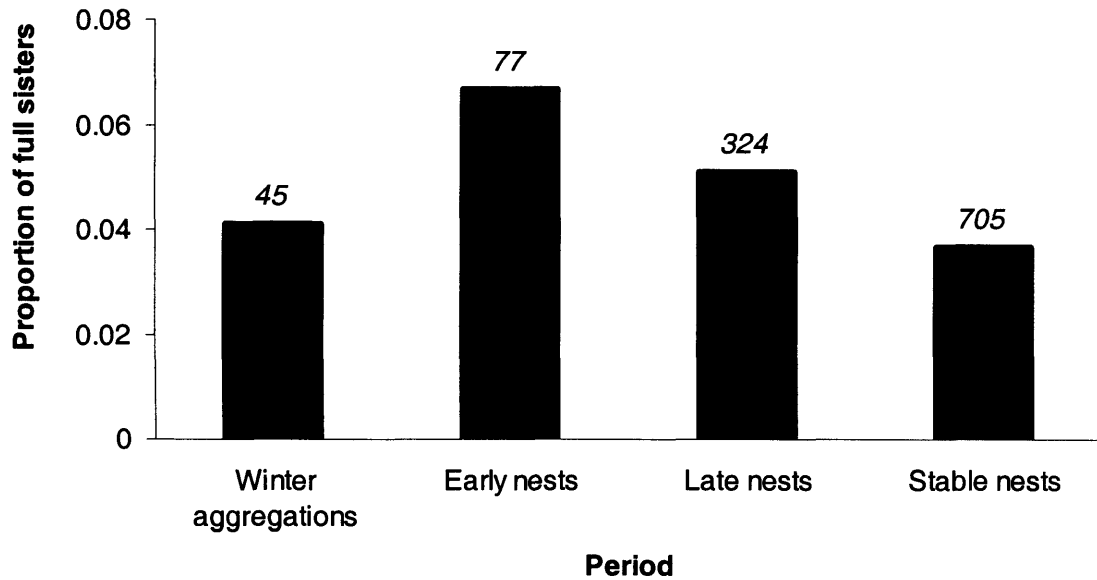
Figure 5 - Total number of pairs assigned to the category of haplodiploid full-sisters at each stage of the nesting cycle. Proportions were calculated using all possible pairs at each stage, independently of their group, i.e. the nest or hibernacula were the pair was collected. Number above bars are totals of full sister pairs.

2.4 Discussion

The results presented here show a surprising level of variation in within-group relatedness in foundresses associations of *P. dominulus*. Although full-sister groups could be found throughout the nest foundation period, the vast majority (ca 80 %) of worker-producing groups, i.e. stable nests, had one or more females which were unrelated (or distantly related) to the remaining members of the group. Associations between unrelated foundresses have been observed previously in other *P.dominulus* populations (Liebert and Starks, 2006; Queller et al., 2000). However, the high frequency of within-group genetic asymmetries, i.e. different sibships in the same group, observed in my Spanish population has not been reported before.

The levels of 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 also indicated that winter aggregations are usually formed by gynes which emerge from different nests during the previous year (Pratte, 1982; Starks, 2003). *P. dominulus* foundresses have also been observed over-wintering with other wasp species (Pardi, 1942, L. Dapporto pers. comun.). 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 why unrelated foundresses end up nesting together (Dapporto et al., 2004; Gamboa, 2004). However, since co-foundresses do not necessarily come from the same winter aggregation (Chapter 4), the effect of this winter mixing of cues on the formation of groups of unrelated wasps is uncertain. In addition, it has been experimentally shown that foundresses of *P. fuscatus,* preferentially associate with full-sisters whether they hibernate with them or not (Post and Jeanne, 1982). Despite it not being clear whether all 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 (Figure 1) 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; see Chapter 2). In addition, at the early nests stage the overall proportion of number of pairs in the whole population assigned to the full sister category was significantly larger than at any other stage (Figure 4). Hence, by chance alone foundresses were more likely to encounter their close kin at the beginning of the nest founding period. Although this would be a small effect, coupled with the tendency of females to return to their natal locality

(philopatry) it could explain why higher within-group relatedness values were more common on early nests (David, 1994; Jeanne and Morgan, 1992; Klahn, 1979; 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.

Apart from seasonal changes, the main differences between early nests and the subsequent two stages (late and stable nests) is the number of active foundresses present in the sites. Therefore, the significant decrease in relatedness observed in the stable nests could be a consequence of the arrival of unrelated joiners (potential usurpers) in already established groups (but see Chapter 4). The within-group relatedness values observed in stable nests also 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., 1998a; Reeve et al., 2000; Seppa et al., 2002). My 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 and co-workers (J. Field, pers. comun.).

Overall, the observed relatedness distribution (of stable nests) is similar to the pattern described by Queller and co-workers for *P. dominulus* in Tuscany (Italy), with a large peak of unrelated foundresses and a large peak of full-sisters. However, direct comparisons with their results are limited by their use of pair-relatedness values instead of mean within-group values. Interestingly, my results show that a relatively small fraction (ca 10 %, Figure

4) of the stable nests, i.e. 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 co-foundresses. 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*. Anecdotal evidences suggest that subordinate foundresses are evicted from worker-producing nests of *P. dominulus* (Pardi, 1942), but this needs to be confirmed. Furthermore, data on dominance turnover in the nests are scarce for most Polistinae (Field et al., 1998b; Queller et al., 2000). Such information is crucial to examine the importance of any form of direct benefits through nest inheritance.

The very low frequency of monogynic nests observed in my study population (0.06% of all foundresses; Chapter5) 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 possibility for subordinate foundresses. Nevertheless, low levels of relatedness between nest mates have also been found in *P. dominulus* populations where single foundress nests are more frequent and successful (Queller et al., 2000; Liebert and Starks, 2006). These contrasting results suggest that the assumption that monogyny is a successful strategy in *P. dominulus* may not hold for all populations. Future tests of predictions derived from kin-selection based models, i.e. transactional skew models, should consider this possibility.

In conclusion, the results present here show that within-group genetic asymmetries can be very common in foundresses associations of *P. dominulus*. The prediction that foundresses of *P. dominulus* preferentially nest with their close relatives could not be confirmed. Local constraints on single nesting could explain why distantly related wasps associate, but a considerable fraction of co-foundresses are completely unrelated (Figure 4). Hence, indirect fitness benefits are unlikely to be the sole explanation for the occurrence of cooperative behaviour in these groups.

CHAPTER 3: Morphological cues and nest-mate choices in foundresses associations of the paper wasp *Polistes dominulus*
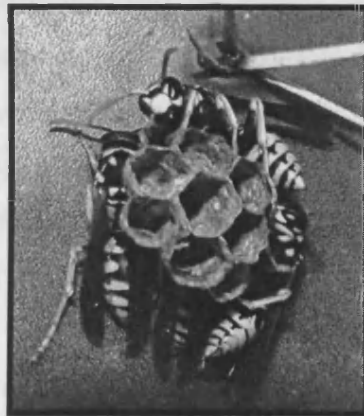
Abstract

In most social organisms group composition is a fundamental determinant of group stability and functionality, consequently also of individual survival. Hence, it may be expected that reliable strategies to select group members would evolve. Here, I examine the hypothesis that foundresses of the common temperate paper wasp *Polistes dominulus* use body size and facial colour patterns to select their co-foundresses. Morphological 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 south-western Spain. Overall, foundresses body size (Discoidal I cell length) ranged from 6.1 to 8.72 mm (mean = 7.56 ± 0.405 S.D., mode = 7.6 mm). Mean wasp size did not markedly change during the different stages of the nesting cycle, but foundresses on early nests were significantly more similar in size than females on winter aggregations and on stable groups (just before workers emerged). Clypeal marks were highly varible in size and shape, but foundresses with marks were relatively rare in the studied population (ca 15 % of 704 wasps), and within-groups (63 % of nests had a single wasp with a clypeal mark). Wasps with marks were significantly more frequent at the end of the nest foundation period than at early nests, suggesting that these wasps may be adopting a "sit and wait" nesting strategy. Overall, these results suggest that body size and clypeal colour patterns are unlikely to be of major importance as visual cues for nest-mate selection by *P. dominulus* foundresses.

3.1 Introduction

The absence of morphologically differentiated castes is one of the defining traits of facultatively eusocial organisms (Crespi and Yanega, 1995). This is particularly intriguing in species where the dominant individual monopolises reproduction (e.g. Liebert and Starks, 2006). Conflicts to achieve such rewarding positions are likely to be accentuated between individuals of similar quality, i.e. similar strength (Maynard-Smith and Parker, 1976). Determining how within-group conflicts are resolved and the resulting reproductive skew achieved has been a topic of considerable theoretical and empirical work (Bourke, 2005).

Temperate paper wasps of the genus *Polistes* have been used extensively as a model to examine these questions (Reeve, 1991; Turillazzi and West Eberhard, 1996). In most species, mated females frequently found nests jointly during early spring, after emerging from hibernation (Reeve, 1991). Groups are relatively small, with on average less than 10 individuals, and co-foundresses are of similar age and are usually full-sisters, so that within-group morphological variation is not usually marked (e.g. Field et al., 1998; Seppa et al., 2002). Changes in group composition and within-group conflicts are common at the early stages of nest foundation season (Cant et al., 2006a; Chapter 4). It has been suggested that during this period foundresses may be assessing the potential of other foundresses as future nest-mates (Reeve, 1991; Nonacs and Reeve, 1995). Although morphological differences are not extreme, individual variation in body size and colour pattern exists, and could potentially be used as cues to select nest-mates.

Nonacs and Reeve (1995) observed that foundresses of *P. dominulus* that switched nests at the beginning of the nesting season tended to be smaller than their nest-mates on the nest they moved to, suggesting that foundresses could be using body size as a cue to select which group to join. Furthermore, clypeal colour patterns have recently been shown to serve as visual cues in nest-mate recognition in *P. fuscatus* (Tibbetts, 2002). They also appear to represent signals of quality used in the establishment of dominance hierarchies in *P. dominulus* foundresses associations (Tibbetts and Dale, 2004). Visual cues may be particularly important since wasp chemical signatures (cuticular hydrocarbons) are not variable enough at the beginning of the nesting season to allow individual nest-mate discrimination (Sledge et al., 2004)

A fundamental feature of *Polistes* foundress associations is that stable groups are characterized by a linear dominance hierarchy, directly correlated with the amount of risky foraging that each female carries out (Shreeves et al., 2003). The establishment and especially the stability of these hierarchies is essential for group functionality and hence for individual group members to obtain the benefits derived from group living (e.g. increased protection, productivity; Cant et al., 2006a). Therefore, foundresses on more stable groups, or groups that achieve stability more rapidly, are likely to have an advantage over foundresses on unstable groups. Thus, actively selecting dissimilar nest-mates (less likely to fight) could be an advantageous nesting strategy for foundresses of *P. dominulus*. As a result, it may be expected that within-group morphological differences may be more evident within stable groups at the end of the founding period (before the emergence of workers) than in early, unstable foundresses associations at the beginning of the

foundation season. In this chapter, I test these hypotheses by analysing the changes in within-group morphological variability at successive stages of the nesting cycle, before group composition becomes completely stable and workers start to emerge. I consider two basic traits; body size and clypeal colour patterns.

3.2 Methods

3.2.1 Natural history of *Polistes dominulus*

Mated females of *Polistes dominulus*(gynes), as other polistine wasps in temperate regions, overwinter in aggregations ranging from a few to more than a hundred individuals (Reeve, 1991). In late winter/early spring (February-March, in Spain), foundresses leave their winter diapause refuges and start to found new nests, either singly (monogyny) or jointly with auxiliary foundresses (polygyny; 2 to 23 females in my study population) (Pardi, 1942). In Southern Spain, the nest founding period is relatively long, usually starting at the end of February and ending in mid May.

All foundresses can potentially reproduce (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 (Chapter 4, but see Queller et al., 2000). Usurpers usually destroy the previous foundress's eggs and early instar larvae (Nonacs and Reeve, 1995; Starks, 2001). During the same period at the study site, *P. dominulus* is subject to attack by a social parasite, *P. 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, when changes in group composition become less frequent (i.e. groups are stable). In mid-summer, new workers stop emerging and the first reproductives start to emerge. These gynes do not participate in the activities of the nest and generally mate away from their natal nest (Pardi, 1942). The reproductive phase lasts until the early fall when the colonies dissolve and gynes disperse, going to winter diapause refuges (Pardi, 1942).

3.2.2 Specimen Collection

Females of *P. dominulus* were collected from two nearby sites (720 m apart) in south-western Spain (Conil de la Frontera, Cadiz, 36°15' N, 06°10'). Seven hundred and four wasps from 180 groups were collected between February and May 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 locations recorded. Nests are easy to spot on these plants, so that few groups remained undetected. Collections were made in the early morning (ca 7:00 am), before wasps were active, by capturing all group members (at the nests) or 5 random wasps (from the winter aggregations) with long forceps and placing them into plastic bags. A set of random samples of groups was collected at 4 stages of the nesting cycle: (a) 23 winter aggregations (99 wasps), (b) 27 early nests (84 wasps), (c) 35 late nests (154 wasps) and (d) 95 stable nests, i.e. just before worker emergence (380 wasps). Winter

aggregations were collected at the end of February (2004) and March (2005). Early nests were collected at the end of February and at the first week of March (in both years). Late nests were collected during 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 Ecología Aquàtica", University of Cadiz, Spain). Samples represented 15% of all detected nests at stages (b) and (c), and 40 % and 35% at stage (a) and (d) respectively.

3.2.3 Morphological data collection

In the laboratory (Woflson House - University College London), wasps were divided into four parts: head, anterior thorax, posterior thorax and abdomen. Wings were carefully removed, unfolded, mounted between glass slides and measured under a 16 x Zeiss 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 know to be highly correlated with overall body size (Sullivan and Strassmann, 1984).

Wasp heads were mounted on a ruler and photographed with a 10 x Macro Fuji digital camera. The resulting images were used to measure the size and darkness of the clypeal marks. The contour of the marks was traced, and the area of the resulting polygon used as an estimate of size. The average number of grey pixels inside these polygons was used as measure of darkness. Image analyses were performed with the software Image/J (version 1.33u: http://rsb.info.nih.gov/ij/). These simplified measurements of clypeal

patterns were preferred over more complex measurements (e.g. "broken-

ness"; sensu Tibbets and Dale 2004) since they could also be obtained from

relatively low definition images of live specimens in the field. In addition,

Tibbets (2006) has shown that clypeal marks can be simply categorized by

their size and disruption (in 1-13 scale) and that these categories are highly

significantly correlated to more elaborate measurements. The accuracy of my

photography-based measurements was assessed by directly measuring the

clypeal marks using a 30 x monocular microscope and the software NIH

Image (version 1.55, http://rsb.info.nih.gov/nih-image). The correlation

between measurements was highly significant (R = 0.903, df = 73, p <<

0.001).

3.2.4 Statistical Analysis

Generalized linear models (GLM) were used to compare body size

(wing length), within-group size variability (S.D. of wing size), and the

occurrence of wasps with clypeal marks in the four different stages of the

nesting cycle. The limited number of groups where at least two wasps had

clypeal marks in the first three stages of the nesting cycle (see Results)

comparisons of within-group mark size and variability between stages. Raw

wing size measurements were log 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 stages of

the nesting cycle (Faraway, 2004). A binomial error distribution was assumed

for the GLM with presence/absence of wasps with clypeal mark(s) as the

response variable (*y* variable).

For all GLMs, stage of the nesting cycle was used as an explanatory variable (factors). In addition, within-group genetic relatedness, group size, site and year were used as explanatory variables. In the GLM with presence/absence of wasp with clypeal marks as the *y* variable, mean wasp body size (per group) was also initially considered as a potential explanatory co-variable. In all GLMs, all potential explanatory variables and their pair-wise interactions were initially fitted. A minimally adequate model was found by the subsequent removal of non-significant terms (Faraway, 2004). Starting with the interactions, terms were dropped until further removal led to significant (P < 0.05) increase in deviance, assessed from tabulated values of F and Chi square (when using normal and binomial error distributions, respectively; Crawley, 2005). The significance of each term (or two-way interaction) is reported when adding them to the minimally adequate model. Two-way interactions were not included in the results unless significant. The effects of site and year are also not reported unless significant. Estimates of within-group relatedness were obtained from a previous study on the same the same population (Chapter 1).

A Paired t-test was used to compare the size of wasps with or without clypeal black marks in the 65 groups that contained both wasps with marks and without. All analyses were carried out using the statistical package R (version 1.9.1; http://www.r-project.org).

3.3 Results

3.3.1 Body size

Overall, foundress body size (Discoidal I cell length) ranged from 6.1 to 8.72 mm (mean = 7.56 ± 0.405 S.D., mode = 7.6 mm). Mean wasp size did not change markedly during the different stages of the nesting cycle (Figure 1). In the GLM, stage of the nesting cycle had no significant effect on wasp body size (F = 1.231, df = 3, P = 0.299). There was no significant association between mean wasp body size and the number of wasps in the group (F = 0.675, df = 1, P = 0.412). Year had a significant effect on wasp body size: smaller foundresses tended to be more common on 2005 (GLM; F = 6.2922, df = 1, P = 0.0129).

Co-foundresses on early nests tended to be more similar in size than wasps on winter aggregations or late/stable nests (Figure 2). GLM results show that stage of the nesting cycle had no significant effect on within-group size variation (F = 2.127, df = 3, P = 0.098). The number of foundresses in each group also had no significant effect on within-group size variation (F = 0.965, df = 1, P = 0.327). Overall, within-group genetic relatedness also had no significant effect on wasp size (F = 0.229, df = 1, P = 0.632). However, wasp size tended to be less variable in early nests founded by more closely related wasps than nest early founded by unrelated or distantly related wasps. Nevertheless, this correlation was not significant (R = - 0.374, t = - 1.755, P = 0.095).
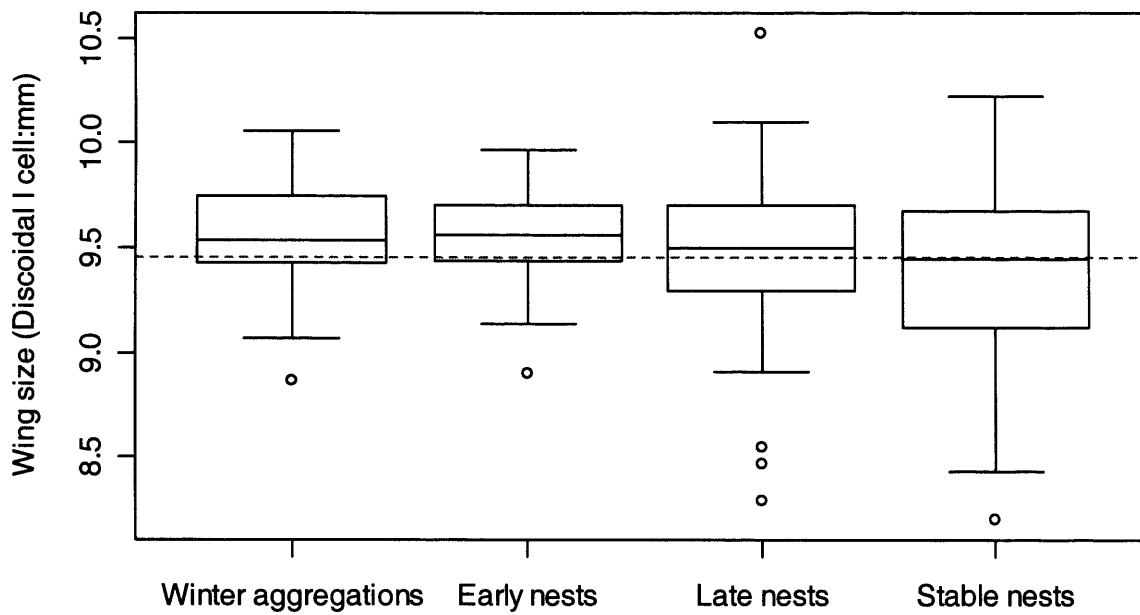
Figure 1 - Box-plot of wasp size data for the four stages of the nesting cycle.

No significant differences were found between stages. Dashed line indicates

overall mean wasp size (mean = 7.56 ± 0.405 S.D.).
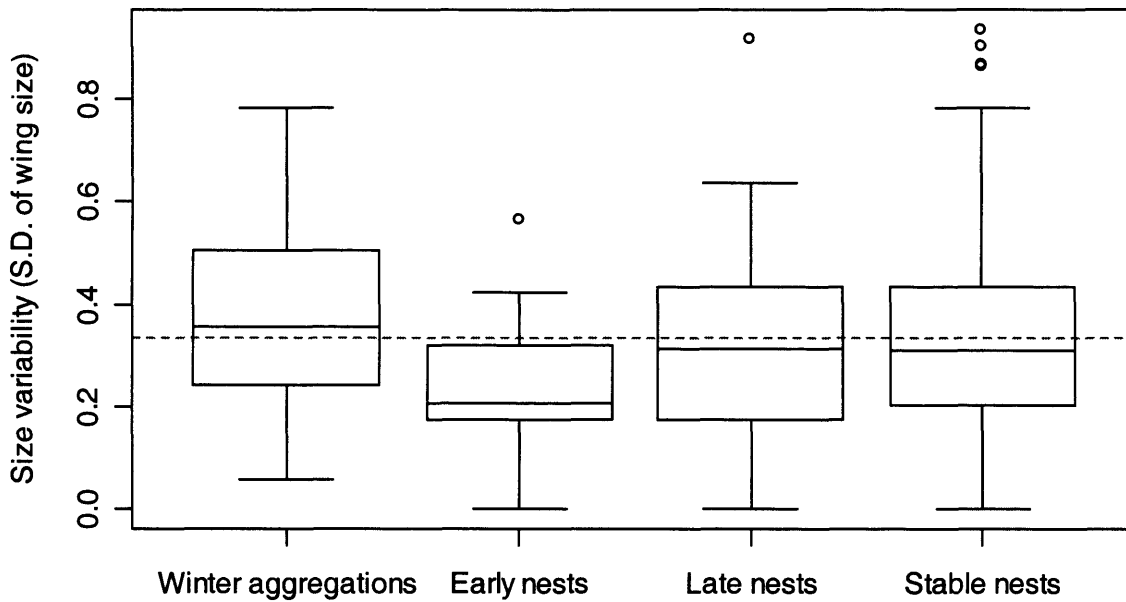
Figure 2 - Box-plot of within-group size variation for the four stages of the nesting cycle. Foundresses on early nests were significantly more similar in size than wasps collected during the three remaining stages. The dashed line indicates overall mean S.D of wasp size per group (mean of S.D. = 0.335 ± 0.195 S.D.).

3.3.2 Clypeal marks

Overall, 110 wasps had distinguishable black clypeal marks (15% of all wasps photographed). Seven foundresses had three marks, 29 had two marks and 74 had one continuous black mark (Figure 3). Marks usually had an irregular border, varying considerably in shape and darkness (mean no. of grey pixels per mark = 202.201 ± 11.113 S.D.). The total area of marks ranged from 0.002 to 0.283 mm^2, but the majority of marks were relatively small (area ≤ 0.05 mm^2; Figure 4). Foundresses with black clypeal marks were found in 12 winter aggregations and in 52 nests (out of 23 and 157 groups collected, respectively). In 33 of the nests only one wasp had a clypeal mark (Figure 5). Overall, clypeal mark size was not significantly correlated with wasp size (Pearson's R = 0.089, df = 105, P = 0.367). Foundresses with clypeal marks were not different in size from foundresses without marks in the same nest (t = 0.136, df = 64, P = 0.891), or in the overall population (t = 0.901, df = 64, P =0.366).

The proportion of foundresses with clypeal marks varied considerably across the foundation period. Wasps with marks were significantly more frequent at winter aggregations and stable nests (just before worker emergence) than at early and late nests (X^2 = 4.475, df = 3, P = 0.034; Figure 6). GLM results show stage of the nesting cycle had a significant effect on the number of groups where wasps with clypeal marks were present (Early nest; Z = - 2.379, P = 0.017, Late nests; Z= - 2.061, P = 0.039). In contrast, group size and within-group relatedness had no significant effect (Z = - 0.589, P = 0.557, Z= - 1.246, P = 0.216, respectively). Wasp body size and within-group

size variation had no marked effect on the occurrence of wasps with clypeal marks (GLM; body size, Z= - 1.475, $P = 0.142$; S.D. of body size, Z = 1.239, P = 0.215). The same results was obtaining when removing the foundresses with marks from the analysis. Overall wasps with clypeal marks were more common in Site 2 (n= 66, 19 % of all wasps) than in Site 1 (n = 28, 8% all of wasps). Site had a marginally significant effect on the occurrence of wasps with clypeal marks (GLM; Z = 1.239, $P = 0.051$).
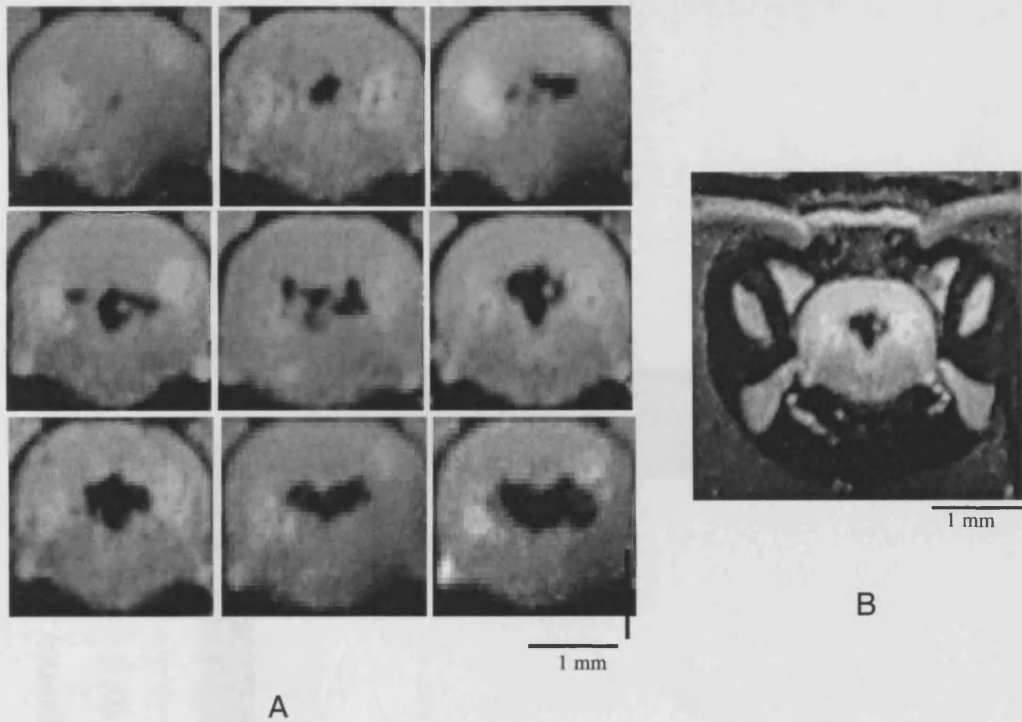
Figure 3 - (A) Observed variation in size and shape of clypeal marks found on foundresses of *Polistes dominulus*. A large fraction (ca 67%) of all foundresses with clypeal marks had only one distinguishable mark. (B) Standard digital image of *P. dominulus* head used to measure the size and darkness of clypeal marks.
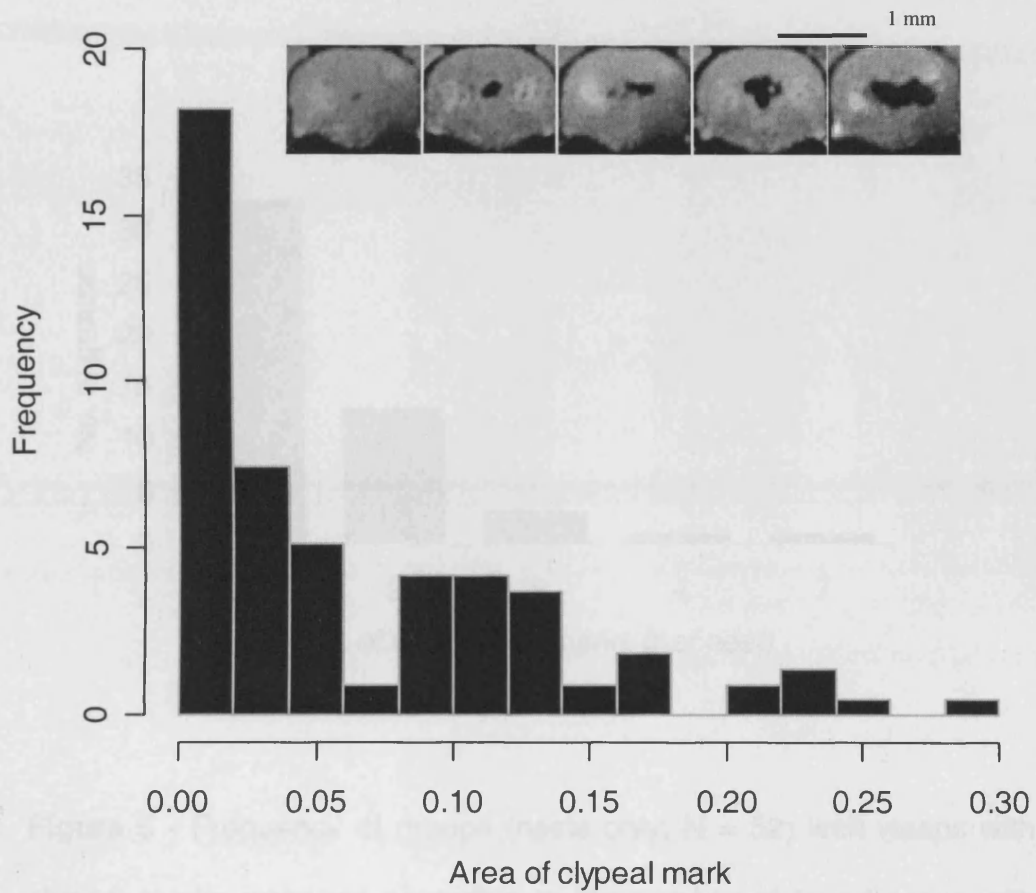
Figure 4 - Frequency of different clypeal mark sizes (area in mm^2) observed in 110 foundresses collected during 2004 and 2005. Images (inset) show an example of clypeal marks for the first five 0.05 mm^2 size intervals.
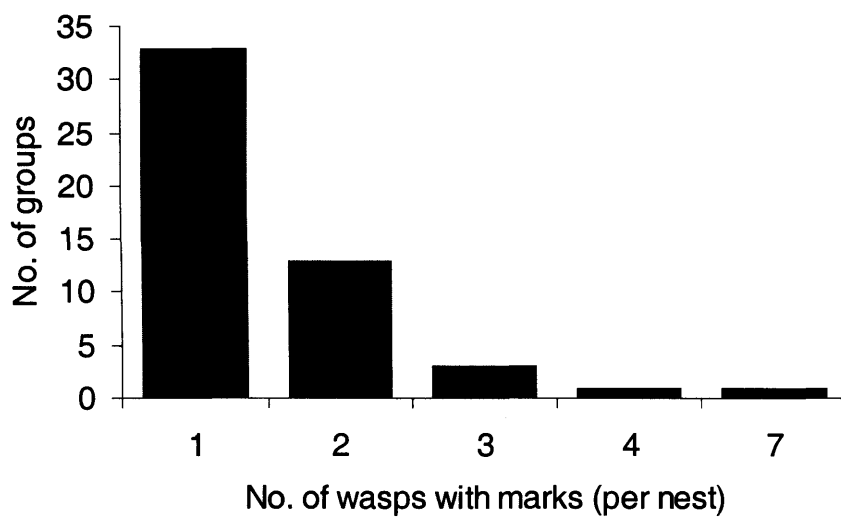
Figure 5 - Frequency of groups (nests only; N = 52) with wasps with clypeal marks arranged according to the number of foundresses with marks in each group.
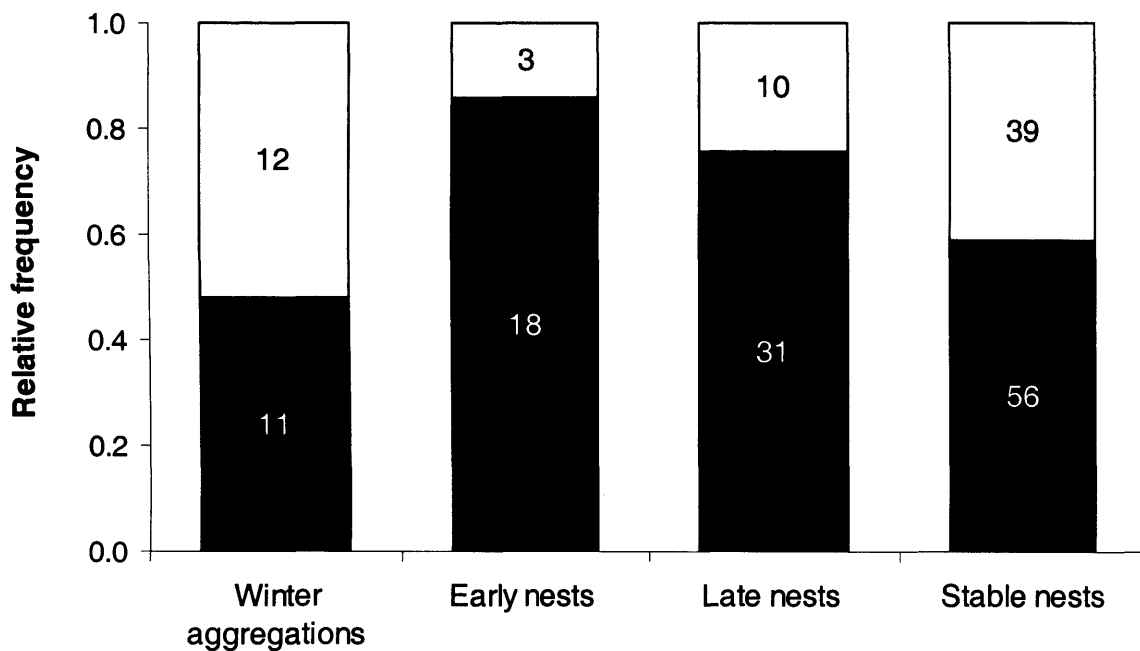
Figure 6 - Frequency of groups where at least one foundress had a black clypeal mark (white bars) and where all foundresses had and entirely yellow clypeus (black bars). Females with marks were significantly more frequent at stable nests groups than at early and late nests (X^2 = 4.475, df = 3, P = 0.034). Numbers inside bars represent sample sizes.

3.4   Discussion

The results presented here show that differences in body size tended to be more evident between foundresses in stable groups than foundresses in recently established, unstable associations. This conforms to the hypothesis that strategies that lead to the formation of morphologically variable groups may be favoured in cooperative associations of paper wasps.

At this stage, however, is not possible to determine whether the observed increase in within-group size variability is a direct result of foundresses actively choosing to nest with dissimilar wasps, or whether it is a by-product of foundresses co-founding nests with distantly related females. The negative correlation between within-group relatedness and size variation found in early nests suggests that the latter may be the case. Overall, morphophysiological differences are known to be more accentuated between unrelated individuals than closely related ones in *P. dominulus* (Dani et al., 2004). Furthermore, the differences in within-group variability between the four stages of the nesting cycle were small, suggesting that body size is unlikely to be a trait used by *Polistes* foundresses to select nest-mates.

Overall, consistent evidence that body size is important in the group formation process is scarce for most polistine wasps (e.g. Nonacs and Reeve, 1995). Although body size can vary considerably among reproductive females, direct correlations between size and reproductive potential (e.g. number of offspring produced or dominance rank) have seldom been found, suggesting that body size may not be a reliable cue for nest-mate choice in paper wasps (Tsuchida, 1991; Field et al., 1998; Seppa et al., 2002; Kudo,

2005). However, behavioural cues, e.g. egg-laying, are known to be used by workers of *P. dominulus* to assess the reproductive potential of dominant foundresses (Liebig et al., 2005). Thus, the possibility that behavioural cues are used by other foundresses to choose potential nest-mates should be considered in the future. Further research could also explore the importance of within-group size variability by measuring the reproductive success of foundresses (i.e. number of gynes produced) in nests where wasps of known size have been removed to either increase or decrease within-group size variation. Nonacs and Reeve (1995) , although not controlling for within-group relatedness, have presented evidence that groups of *P. dominulus* where dominant and subordinates foundresses are more dissimilar grow faster (in terms of no. of cells in the nest) and produce more workers.

The low frequency, both within groups and overall of wasps with clypeal marks suggests that the importance of these marks as visual cues during group formation may be limited. It is consistent with marks being costly to produce and thus an uncommon trait (Maynard-Smith and Harper, 2003). However, foundresses with clypeal marks were considerably more common in American populations of *P. dominulus*, where at least 65.8% of all wasps had marks (Tibbetts and Dale, 2004). These contrasting results suggest that the frequency of foundresses with clypeal marks may not be only associated with their potential physiological costs, but also with environmental factors (Griffith et al., 1999). This could explain why the frequency of these marks varies considerably between populations, even between very close ones, as in the present study.

The lack of correlation between wasp body size and clypeal mark size contrasts with a previous study on *P. dominulus* foundresses showing a

positive correlation between wasp size and mark quality (i.e. "brokenness"; Tibbetts and Dale, 2004). However, the biological significance of the correlation reported by these authors is questionable since only a very small fraction of the variation in clypeal marks was explained by wasp body size (R^2 = 0.028; page 219 in Tibbetts and Dale, 2004).

Interestingly, the number and type of clypeal mark(s) were also uncorrelated with several measures of foundresses condition, such as body weight and rank, in *Polistes fuscatus* (Tibbetts, 2002). Nevertheless, the possibility that clypeal marks are reliable quality signals in *Polistes* cannot be completely disregarded. Although it is still unclear which fitness advantages foundresses with clypeal marks may have, the association between these marks and higher dominance rank has been confirmed in independent studies of distinct populations of *P. dominulus* (Tibbetts and Dale, 2004; this study: Chapter 5). In addition, it has been shown that clypeal marks may be socially costly. Foundresses with experimentally enhanced marks, i.e. fake high quality badges, receive more aggression in paired contests then wasps with honest high quality marks (Tibbetts and Dale, 2004). Hence, it can be assumed that clypeal marks are functional signals. Moreover, although body size is frequently a good indicator of individual quality (Blanckenhorn, 2000), its effect on the overall fitness of social and solitary Aculeata is variable, and frequently small (Alcock et al., 2006) and references therein). Hence, future studies need to examine the potential relation between clypeal marks and other traits associated with individual fitness (e.g. parasite load) to determine whether clypeal marks are reliable quality signals or not.

The late appearance of wasps with clypeal marks, which were much more frequent at stable nests than at earlier nests, suggests that foundresses

with marks may be adopting a "sit and wait" nesting strategy (Starks, 2001; Chapter 3). Foundresses that "sit and wait" may have advantage over females that found their own nest early in the season when the mortality rate at the nests is higher (Chapter 4). Although "sitting and waiting" is not adopted exclusively by wasps with clypeal marks, they may have an advantage over other foundresses (without marks) adopting the same strategy, since their chances of obtaining a dominant position are higher, even when they arrive late in a group (Chapter 5). The correlation between clypeal marks and "sit and wait" strategies needs to be examined further to test this hypothesis. If marks confer an advantage to foundresses trying to join a group, it may be expected that these foundresses would encounter less resistance from nest residents than foundresses without marks, and that resistance would change according to the quality implied by the mark.

Overall, within-group variations in body size and clypeal mark are small throughout the nest foundation period of *P. dominulus*. Hence, it may be that these morphological traits are not of major importance as visual cues in nest-mate choice. The observed increase in within-group size variation at end of the nest founding period is likely to be a consequence of the large number of groups formed by unrelated individuals. Clypeal marks, although varible in size and shape, are a relatively rare trait in the studied population. Thus it is unlikely that foundresses of *P.dominulus* rely on them to select nest-mates. However, wasps with clyepeal marks may preferentially adopt a "sit and wait" nesting strategy, since their late arrival does not prevent them from achieving a higher ranked position a reproducing.

CHAPTER 4: Founders versus joiners: early fluctuations in group composition in the paper wasp *Polistes dominulus*
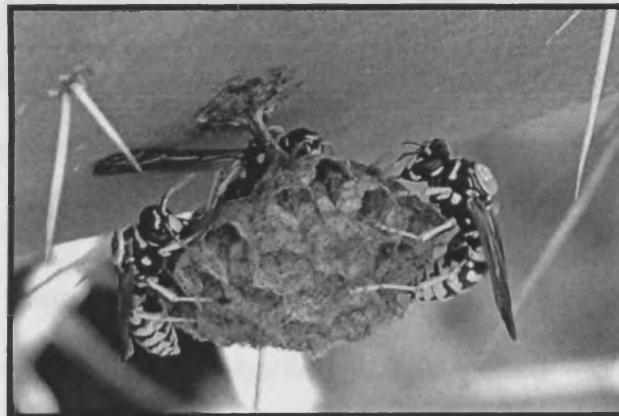
Abstract

The diversity of reproductive strategies presented by paper wasps of the genus *Polistes* has long puzzled social behaviour researchers. Here I investigate whether such variability is affected by interactions between females prior to the foundation of nests and at newly founded nests. Data on the nesting history of individually marked foundresses were obtained in two sites in southern Spain during the nest foundation seasons of 2004 and 2005. Foundresses that hibernated in the same aggregation were more likely to start a nest together, but all members of a group were seldom from a single aggregation. Changes in group composition were frequent throughout the pre-worker period, and were likely to be caused by the disappearance of foundresses (possible death) and the frequent arrival of joiners to established groups. This suggests that "sit, wait and join" is a common nesting strategy in *P. dominulus*. Within-group relatedness was not affected by the arrival of late joiners, but was negatively correlated with the date that nests were initiated. Only 16 % of all successfully marked wasps visited more than one nest. These wasps tended to move to groups where the resident foundresses had smaller and less variable clypeal marks than the wasps in their original nests, suggesting that these marks are visual cues used to select nest mates. In contrast, wasp body size, the proportion of full-sisters, and group size did not differ significantly between the nests left and joined by foundresses that moved between groups.

4.1 Introduction

The behavioural flexibility presented by the paper wasps of the genus *Polistes* has long attracted the attention of naturalists (Pardi, 1942). It has provided a fertile ground for comparative studies of alternative reproductive strategies and is one of the main reasons why this group of wasps has been used extensively as a model to study the evolution of social behaviour (West - Eberhard and Turillazzi, 1991).

The co-occurrence of different nesting strategies is particularly evident in the common temperate species *Polistes dominulus*. 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, i.e. forcibly take the place of others in a group (Reeve, 1991). This considerable number of possible strategies implies that foundresses make crucial behavioural (reproductive) decisions during the pre-worker period. Consequently, it may be that interactions between foundresses occurring at this particular stage may be associated with those decisions.

Before winter starts, foundresses frequently interact at their winter aggregation sites. Interactions range from simple antennation to trophallaxis (exchange of regurgitated food between future foundresses), and dominant-subordinate interactions (Dapporto et al., 2005a). Interactions also occur at

the end of winter when temperatures start to rise (Pardi, 1942). Nevertheless, it remains to be clarified whether wasps that hibernate in the same aggregation are more likely to later nest together.

Once nests are initiated, foundresses are likely to meet exclusively at nests or at their close surroundings, when they attempt to establish new groups or join established ones. Earlier studies indicate that as in other paper wasps, *P. dominulus* foundresses move frequently between nests before worker emergence (reviewed in Reeve, 1991; Nonacs and Reeve, 1995; Seppä et al., 2002). In semi-natural 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 and Reeve, 1995). Chemical profiles (epicuticular hydrocarbons) are not variable enough within recently established groups of foundresses of *P.dominulus* to allow individual recognition (Sledge et al., 2004). Moreover, foundresses that hibernate in the same winter aggregation have very similar chemical profiles (Dapporto et al., 2004). Thus, chemical signatures alone are unlikely to be used as cues to select co-foundresses. In contrast, individual variations in body size and colour patterns exist and could potentially be used to select nest-mates. Clypeal colour patterns have recently been shown to be used in individual recognition by *P. fuscatus* females and in the establishment of dominance in *P. dominulus* foundress associations, i.e. foundresses with larger and more disrupted clypeal marks tend to be dominants (Tibbetts, 2002; Tibbetts and Dale, 2004). However, detailed information on the frequency and magnitude

of foundress movements remain still scarce for most paper wasp species (e.g. Seppä et al., 2002), so that the validity of these hypotheses remains to be tested.

At the very beginning of the nest foundation season (February-March) within-group relatedness is on average higher than when groups are about to produce the first workers (Queller 2000, Chapter 1). This decrease in within-group relatedness may be associated to the arrival of unrelated late joiners, foundresses that "sit and wait" and join established groups, occasionally replacing resident wasps (Starks, 2001). Although resident foundresses could abandon their nests when new wasps arrive (E. Almond, unpublished), they may be constrained to stay in their original nest if joiners (potentially usurpers) arrive too late in the season (just before worker emergence), so that starting a new nest is no longer feasible.

Here, I first investigate whether females that hibernate in the same winter aggregations later preferentially found nests together. I then test the hypothesis that the arrival of late joiners and the associated group composition changes decrease intra-group genetic relatedness. Finally, I examine 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 wasp body size and facial patterns. In summary, this study investigates the basis of movements by individually marked foundresses in order to reconstruct their nesting history and examine the potential success of different nesting strategies.

4.2 Methods

4.2.1 Study sites

Field observations and collections were carried out at two adjacent sites (720 m apart) in south-western Spain (Conil de la Frontera, Province of Cadiz; Site 1 - 36°17'11 N, 06°04'28 W and Site2 - 36°17'11 N, 06°03'57 W). The habitat at both sites consisted of hedges of prickly pear cactus (*Opuntia*) surrounded by pasture and crop fields. Hedges were 1.5 to 3 meters high, and 2 to 21 meters wide. Five and four transects, adding up to a total of 500 and 180 meters of hedge were used in Site 1 and 2, respectively.

4.2.2 Field data collection

Starting on 18/02/2004 and 11/02/2005, each site was monitored every other day (between 10.00 - 14.00 h) 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 (07.00 - 08.00 h), females in winter aggregations were marked on the thorax with a large dot of enamel paint (Revell), using a unique colour for each aggregation. Wasps were marked directly in the hibernacula with a long thin brush, since a pilot study showed that when removed from it they did not return (n = 10 aggregations, 207 wasps marked, 3 returned). The number of wasps marked in each aggregation depended on its location and size. Large aggregations (≥

50 wasps) were usually formed by layers of wasps, so that not all wasps could be reached when the aggregations were behind old nests or in small cavities.

Wasps found on new nests were gently collected with long forceps, placed into plastic bags and stored at 4 °C. In 2004, each individual was marked on the thorax with a unique combination of 4 enamel paint dots and subsequently released onto its original nest to minimize any possible effect of removal. In 2005, wasps were individually marked using numbered tags glued to the thorax (queen marking kit: Thorne, UK). The proportion of marked wasps that were observed only once in their original nest was significantly higher in the second year (X^2 = 107.001, df = 1, $P <<$ 0.001), indicating that the tag marking used in 2005 was more disruptive for the wasps.

Every second day after their discovery, all nests were inspected 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 categorized in three groups: (a) movement with replacement: foundresses that left their initial nest up to 2 days after other foundresses (potential usurpers) arrived; (b) movement without replacement: foundresses that left their initial nest without the arrival of new wasps and (c) nest switching foundresses that moved two to three times between the same pair of nests. Foundresses were considered to be in a group only if observed at leasttwice in that particular group.

Before the first workers started to emerge (May), all marked foundresses and their nests were collected and stored at -80 °C (at the Laboratorio de Ecología Aquàtica, University of Cadiz, Spain).

4.2.3 Morphological data collection

In the lab (UCL), wasps were divided into four parts: head, anterior thorax, posterior thorax and abdomen. Wings were carefully removed, unfolded, mounted between glass slides and measured under a 16 x Zeiss 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 known to be highly correlated with overall body size (Sullivan and Strassmann, 1984).

Wasps heads were mounted on a ruler and photographed with a 10 x Macro Fuji digital camera. Images were used to measure the size and darkness of the clypeal marks. The contour of the marks was traced, and the area of the resulting polygon used as an estimate of size. The average number of grey pixels inside these polygons was used as measure of darkness. Image analyses were performed with the software Image/J (version 1.33u: http://rsb.info.nih.gov/ij/). The accuracy of measurements was assessed by measuring the heads using a 30 x monocular microscope and the software NIH Image (version 1.55, http://rsb.info.nih.gov/nih-image). The correlation between measurements was strong and highly significant ($R = 0.903$, $df = 73$, $p << 0.001$).

4.2.4 DNA extraction, amplification and visualization

Total DNA was extracted from the anterior section of the thorax using 300 μl of grinding solution (0.1M NaCl; 0.1M Tris-HCl, h = 8.0, 0.05M EDTA; 0.05% SDS), following Strassmann (1996) with minor modifications. DNA

extractions were diluted 1:10 with ultra-filtered distilled water. From each winter aggregation or nest collected, DNA was extracted from up to 4 wasps.

Multiplex polymerase chain reactions (PCR) were performed using 5 fluorescently labelled primer pairs (Pdom 7, Pdom 20, Pdom 127b, Pdom 139, Pdom 140 (Henshaw, 2000). PCR was carried out using a Peltier Thermal Cycler using 10µl 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 (v. 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 base pair or less were considered to be equal. Moreover, 27 samples previously genotyped by Cant et al. (2006a) for four of the five loci used in my study were re-genotyped. Scores were not confirmed only for one allele in two samples (error rate = 2/[27X2X4] = 0.009).

4.2.5 Relatedness estimation

Genetic relatedness was estimated using the program RELATEDNESS 5.08 (http://www.gsoftnet.us/GSoft.html), which calculates regression relatedness values using population allele frequency data based on the formula described by Queller and Goodnight (1989). Groups were weighted

equally, to account for differences in sample size between them. Confidence intervals for relatedness estimates were obtained by jacknifing over loci.

Pairs of foundresses were assigned to the categories of full-sister or non –sisters (i.e. cousins or unrelated, since *P. dominulus* are single mated) using a likelihood-based method implemented by the program Kinship (Goodnight and Queller, 1999; see details of methods in Chapter 1) (http://www.gsoftnet.us/GSoft.html). With $\alpha = 0.05$, the power to detect full-sister pairs was 99% (based on 2000 simulations).

4.2.6 Statistical Analysis

4.2.6.1 Relatedness and arrival

Generalized linear models (GLM), assuming normal error distributions, were used to test whether group relatedness was affected by variation in individual arrival times, summarized for each group by the standard deviation (S.D.) of the arrival dates of the wasps in the group. The proportion of wasps that disappeared within 2 days of the arrival of other wasps in the group (replaced wasps, i.e. wasps that left after new ones arrived), and the proportion of wasps that disappeared without being replaced were also considered as potential explanatory variables. In addition, group size, intra-group variation in body and clypeal mark size (S.D. 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 are not reported in the results unless significant.

4.2.6.2 Nest- mate choices

To examine whether foundresses that move between different nests are choosing between potential groups according to relatedness or morphological traits, several features of groups visited by the same wasp were compared using paired Student's t - tests. When assumptions of normality were not met, Wilcoxon matched pairs signed rank tests were used instead. Four within-group traits summarising group composition were considered: relatedness, variation in body size and clypeal mark size (within-group S.D.) and the number of foundresses (group size). GLMs were then used to analyse whether differences between nests (in any of these traits) were associated with the three different types of foundress movements between groups: **(a)** movement with replacement; **(b)** movement without replacement **(c)** nest switching. Differences in the within-group trait between the nests visited by the same wasp (e.g. relatedness) were used as a response (y) variable. Wasp category was tested as an explanatory factor and within-group differences in the remaining nest traits (e.g. body size and clypeal mark size variation) were tested as additional explanatory variables. In addition, when foundresses moved (date), the distance between nests (in meters) and differences in group size between the nests were considered as potential covariates. 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 second nest.

For all GLM, all explanatory variables 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 ($P < 0.05$) increase in deviance, assessed from tabulated values of F (Crawley, 2005). The 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.

Relatedness estimates and proportions of full-sister pairs per group were arcsine transformed to improve the fit of residuals to assumptions of normality (Crawley, 2005). All analyses were carried out using the statistical package R (version 1.9.1; http://www.r-project.org).

4.3 Results

4.3.1 From winter aggregations to nests

Overall, 680 groups were detected during the two years (58 winter aggregations and 622 nests). A total of 143 nests were initiated by single foundresses (23 % of all new nests), and 51 %(n = 317) of all nests had three or fewer wasps when detected for the first time (Figure 1). 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) (Figure 1).

At least 492 wasps were marked in 23 winter aggregations ranging from 7 to ≥ 100 individuals in size. In 18 of these, all wasps were marked. In total, 110 females marked in the winter aggregations were later found nesting. The potential bias in detecting wasps that come from the same winter aggregation caused by incomplete marking (in 5 large aggregations) can be assumed to be relatively small, since only 28 % of all wasps later observed nesting came from these five winter groups.

Most foundresses from known winter aggregations (n = 89 wasps) were observed nesting with at least one other wasp from the same aggregation (no. nests = 22). In 13 of these nests, 50% or more of the foundresses came from the same winter aggregation (Figure 2). In contrast, 21 wasps (marked at their winter aggregation) were found nesting exclusively with unmarked wasps, i.e. wasps of unknown origin. The proportion of marked wasps that nested with at least one wasp from the same winter aggregation was significantly bigger than expected by chance, i.e. if wasps form groups at
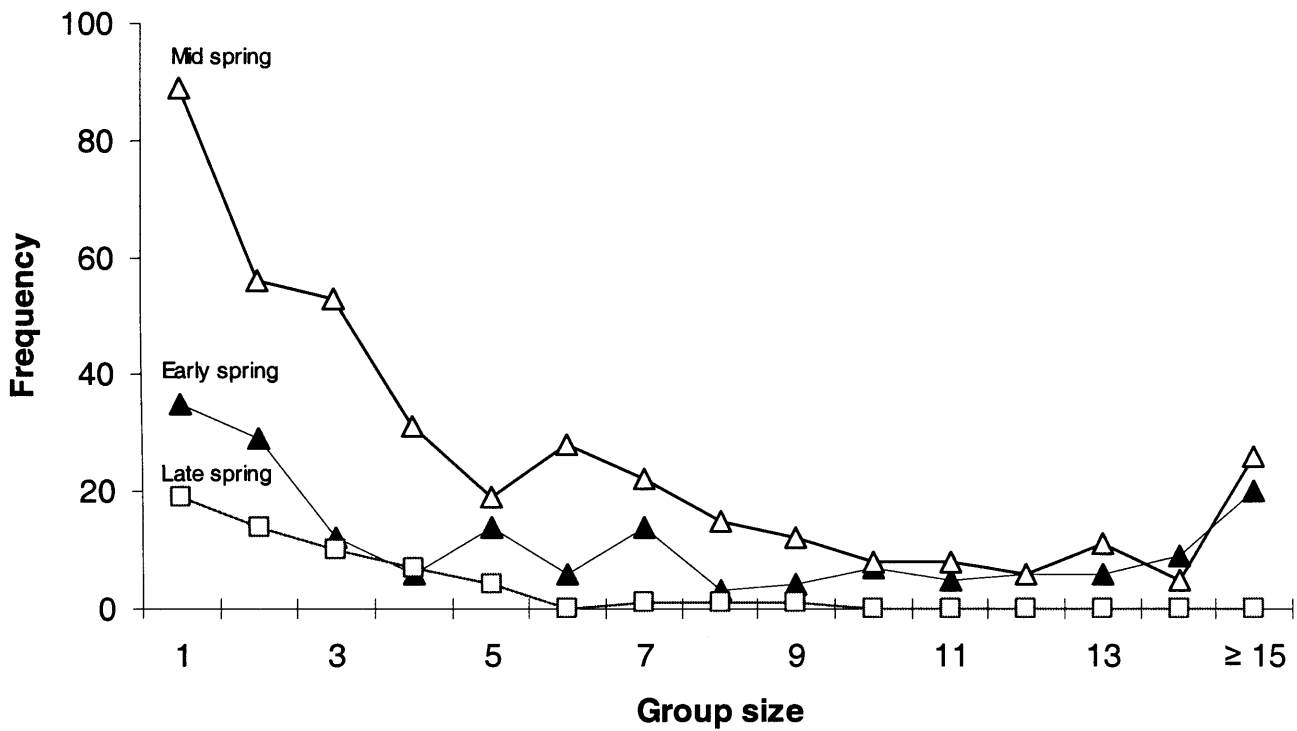
Figure 1 - Overall number of single and multiple foundresses nests discovered at different periods of the nesting season: early spring (black triangles) 11th February - 4th March; mid spring (open triangles) 5th March – 11th April; and late spring (open squares) 12th April – 28th May.
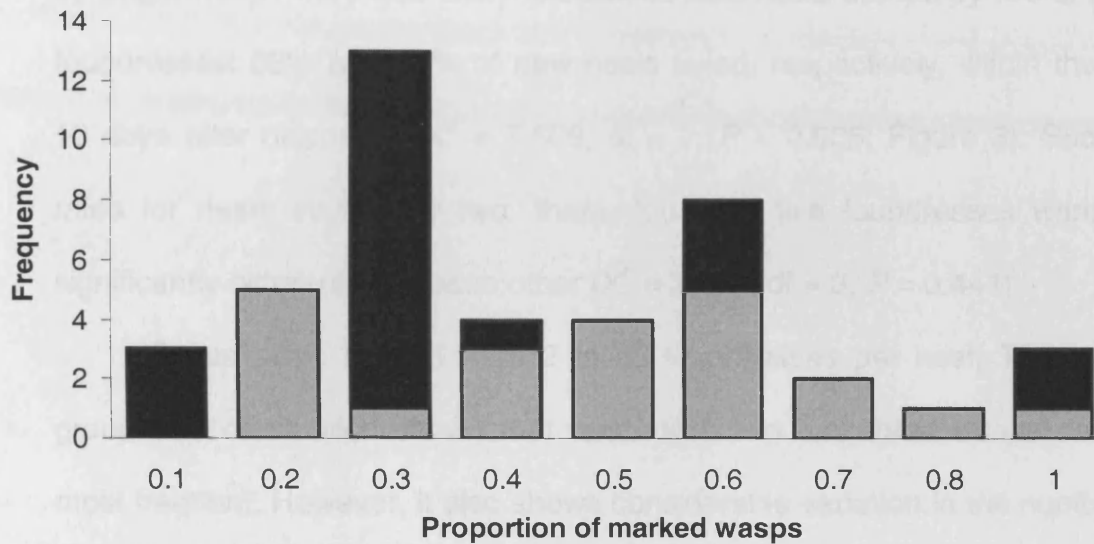
Figure 2 – Proportion of wasps marked at the same winter aggregation observed on newly established nests where one (black bars) or more (grey bars) foundresses marked at their winter aggregation were present (total no. nests = 43). 17 % of 492 foundresses marked in 23 aggregations were re-encountered at the nests.

random with respect to their winter aggregation (X^2 = 21.891, df = 1, p << 0.001).

In total, 2227 wasps were individually marked on the nests, and 58% of these were observed more than once at their original group. 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 (X^2 = 7.609, df = 1, P = 0.005; Figure 3). Success rates for nests started by two, three, four and five foundresses were not significantly different from each other (X^2 = 2.701, df = 3, P = 0.441).

Group sizes ranged from 2 to 23 foundresses per nest. The overall group size distribution shows that nests with two and three wasps are the most frequent. However, it also shows considerable variation in the number of foundresses per nest, with groups of very different sizes being equally frequent, (e.g. 4 to 7 and 8 to 18 wasps; Figure 4). Nests with only one wasp represented only 6 % of all nests present at the end of the foundation period, before the first brood emerged (n $_{total}$ = 134). Thus, single foundresses represented only 0.8 % of all foundresses that survived until that stage.

Empty nests could be found throughout the nest foundation period in 2004 and 2005 (111 and 54 nests in total, respectively). Most of them (101 out of 165) had 15 or fewer cells containing only eggs. Empty nests were adopted by new foundresses in 5 occasions only (two in 2004 and three in 2005), and all adopted nests were less then 2 weeks old.
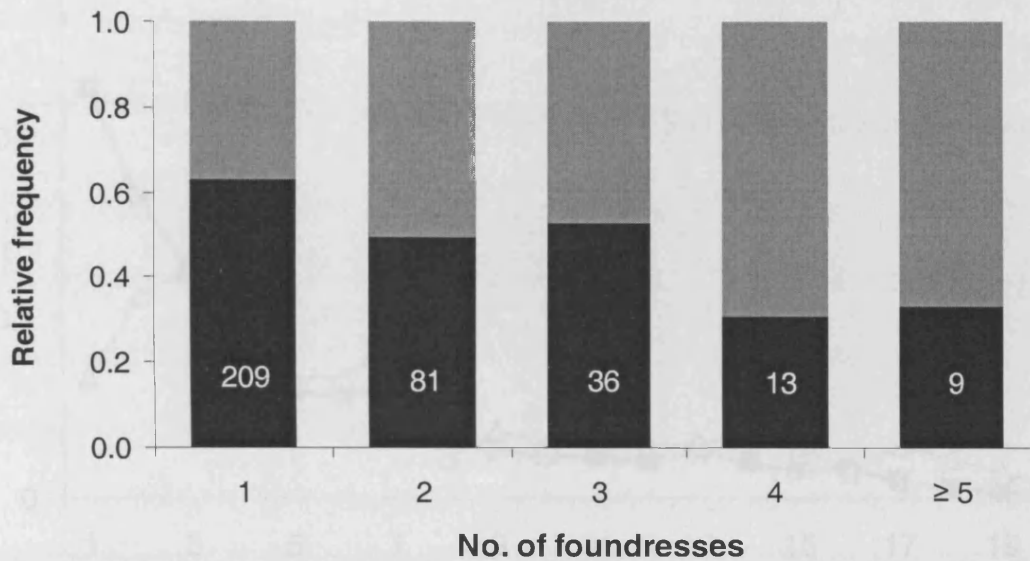
Figure 3 - Proportion of nests that survived until the end of the foundation period, just before worker emergence (grey bars) and nests that were completely abandoned within 10 days of being discovered (black bars), divided according to the number of foundresses present at the nest when it was first seen. Numbers at the bottom are sample sizes (no. of nests).
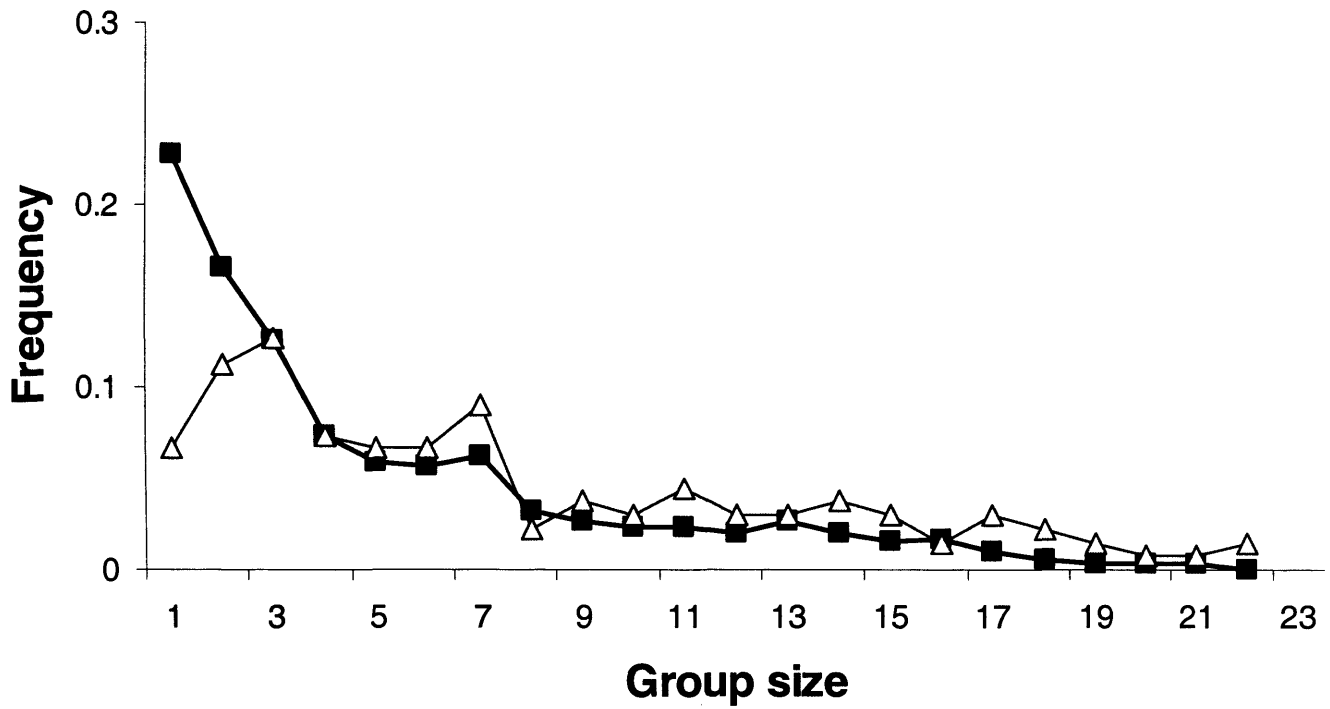
Figure 4 - Overall number of nests detected throughout the foundation period (black squares) (n $_{total}$ = 597), and number of nests still active at the end if the foundation period, just before the emergence of the first workers (empty triangles) (n $_{total}$ = 131).

4.3.2 Fluctuations in group composition and relatedness

At least one female disappeared from 60 out of 68 nests for which complete historical, genetic, and morphological data were obtained. In 34 cases, foundresses disappeared without being replaced, i.e. no other wasp arrived in the group within two days. In six nests all foundresses disappearances occurred with replacement, and in the remaining 20 nests both forms of group composition change occurred. The number of wasps that disappeared from each group ranged from 1 to 9. Only 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 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 where more foundresses had marks. However, this result is dependent on three nests where all wasps had clypeal marks. When these nests are excluded from the analysis, the date when the nest were started is the only variable with a marginally significant effect ($F = 4.208$, $P = 0.044$). Overall, nests that were initiated at the end of the foundation period (April) tended to have lower within-group relatedness. This effect was particularly

Table 1 - Effect of group composition (and changes) on within-group relatedness. F values and the respective probabilities associated (P) are reported for when each explanatory variable was added to a minimal adequate model (GLM).

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

* After the removal of three nests where all wasps had marks values changed to: $F = 1.461$ and $P = 0.231$

2004
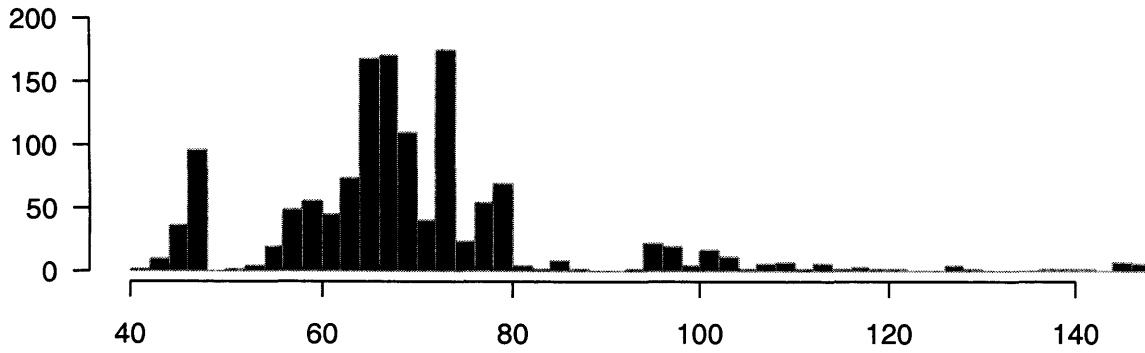
2005
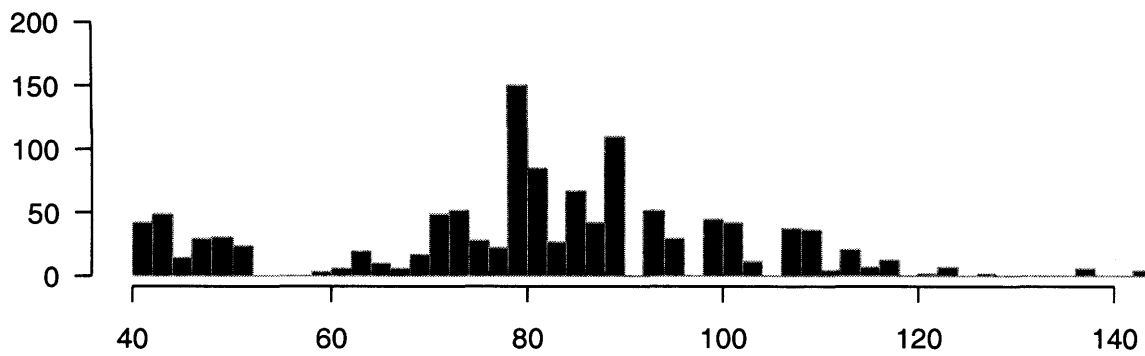
Days since 1st of January

Figure 5 - Frequency distribution of dates of nest foundation .The nesting season in 2005 was longer due possibly to a prolonged period of cold weather (≤ 15 °C) in the beginning of spring (March).

important in the second year, when a larger number of nests were founded during this period (Figure 5).

4.3.3 Wasp movements between nests

Wasp movement between nests was relatively infrequent: only 16 % of all successfully marked foundresses were observed at two or more nests (Figure 6). Wasps that visited more than one nest were not different in size from wasps that were always in the same group (GLM, $F = 0.591$, $P = 0.621$), and black clypeal marks were equally common on foundresses that moved and foundresses that did not ($X^2 = 2.734$, df = 3, $P = 0.434$).

The distance between pairs of nests used by the same wasp ranged from 0.1 to 235 meters, but most pairs were less than three meters apart (Figure 7). Three foundresses that were nesting alone moved to multiple foundress nests, and no wasp did the reverse. In 2004, movements between nests were more frequent during early spring (second half of March). In the following year, movements were relatively frequent throughout spring (Figure 8).

Genetic and morphological data was obtained for all the nests visited by 59 foundresses that used more than one group. Overall, 29 wasps left a group that dissolved within a week of their departure. The remaining 30 foundresses moved between groups that remained active throughout the study. 55 of these wasps could be classified in three categories of movement: (a) movement with replacement: 15 wasps left their initial nest when other foundresses arrived (up to 2 days before their departure); (b) movement

without replacement: 28 wasps left their initial nest independently of the arrival of new wasps and (c) nest switching: the remaining 12 foundresses moved two to three times between the same pair of nests, and on no occasion did their movements coincided with the arrival of other wasps.

For the 15 cases of movement with replacement (a), the newly arrived wasps were cousins or unrelated to the wasp that left the group in seven occasions, in three cases they were a mixture of sisters and non-sisters and in the remaining five occasions the kinship relation could not be established since the newly arrived wasps disappeared before genotyping. Only one of the 19 newly arrived wasps had a black clypeal mark.

The composition of groups visited by the same wasp was considerably similar. No significant differences were found between the number and size of foundresses in the first and the second nest (Table 2). Wasps also had similar clypeal colour patterns in both sets of nests (Table 2). Nevertheless, clypeal marks tended to be more variable (in size) on the last nest visited than on the initial nest. Interestingly, foundresses that used more than one nest moved to groups with higher within-group relatedness than the groups they left. (mean $_{1st. nest}$ = 0.113 ± 0.017 S.E., mean $_{2nd. nest}$ = 0.227 ± 0.018 S.E., Table 2). This trend was more accentuated for wasps that moved at the end of the nest foundation period (Table 3, Figure 9). In contrast, the type of foundress movement (a, b or c) had no significant effect on this trend (Table 3). Differences in group composition (group size and within-group morphological variation) also were not correlated to the observed differences in relatedness between nests (Table 3). In 50 % of the cases in which a wasp moved to a nest with higher relatedness then the one it had left (n = 31), two or more full-sisters not related to the wasp moving were present at the second nest.

Considering 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, this was a property of the other wasps in the group, the wasps that moved was not moving to join closer relatives.

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

Nest traits	1st nest X 2nd nest		
	Sudent's t	df	*P*
Group size (total no. of foundresses)	0.177	54	0.861
Wasp body size (average per nest)	0.961	52	0.342
Body size variation (S.D.)	1.419	57	0.162
Clypeal mark size (average per nest)	161*	-	0.117
Mark size variation (S.D.)	171.5*	-	0.053
Genetic relatedness	-3.961	53	<<0.001
Proportion of full-sisters of the wasp moving	-0.447	53	0.656

* Wilcoxon *V.* values

Table 3 – Summary of GLM results, with the effects of distinct foundresses movement types and associated changes in group composition on within-group relatedness differences between nests used by the same foundresses. F values and the respective probabilities associated (P) are reported for when each explanatory variable was added to a minimal adequate model (GLM) .

Variables summarizing foundresses movements	F	P
Type of movement *	0.117	0.889
Distance between nests	0.314	0.57892
Date movement occurred	-2.426	0.021
Differences in within-group clypeal mark variation (S.D. of area of marks) **	-0.202	0.841
Differences in within-group wasp size variation (S.D. of wing length) **	0.223	0.8247
Differences in group size (no. of foundresses in each nest) **	2.511	0.122

* **a**: with replacement; **b**: without replacement and **c**: nest switching

** Value in first nest used minus value in final nest
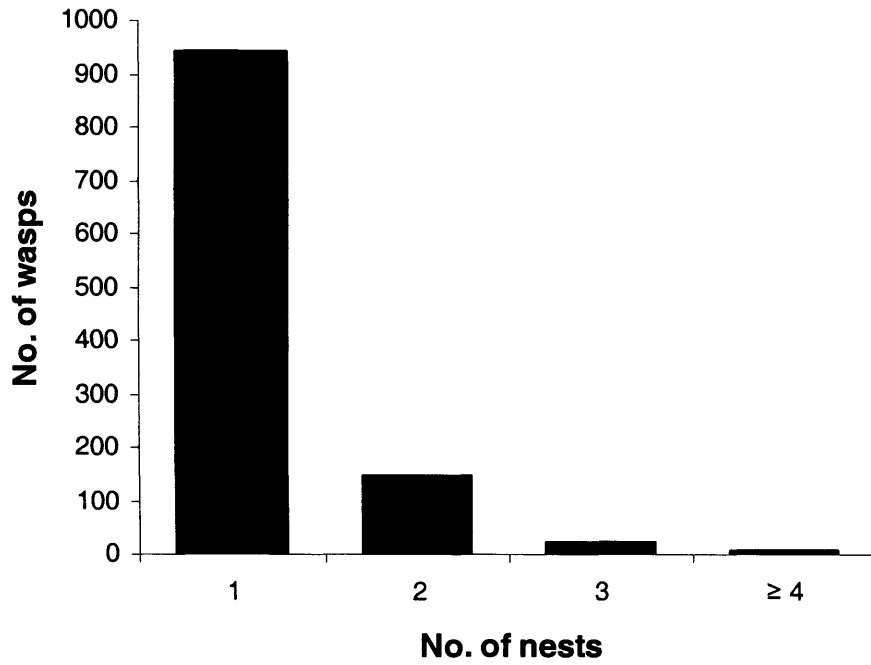
Figure 6 - Overall distribution of the number of nests visited by individually marked wasps throughout the 4 months of the foundation period. Wasps were considered to be visiting a nest if observed in at least 2 census at that nest.
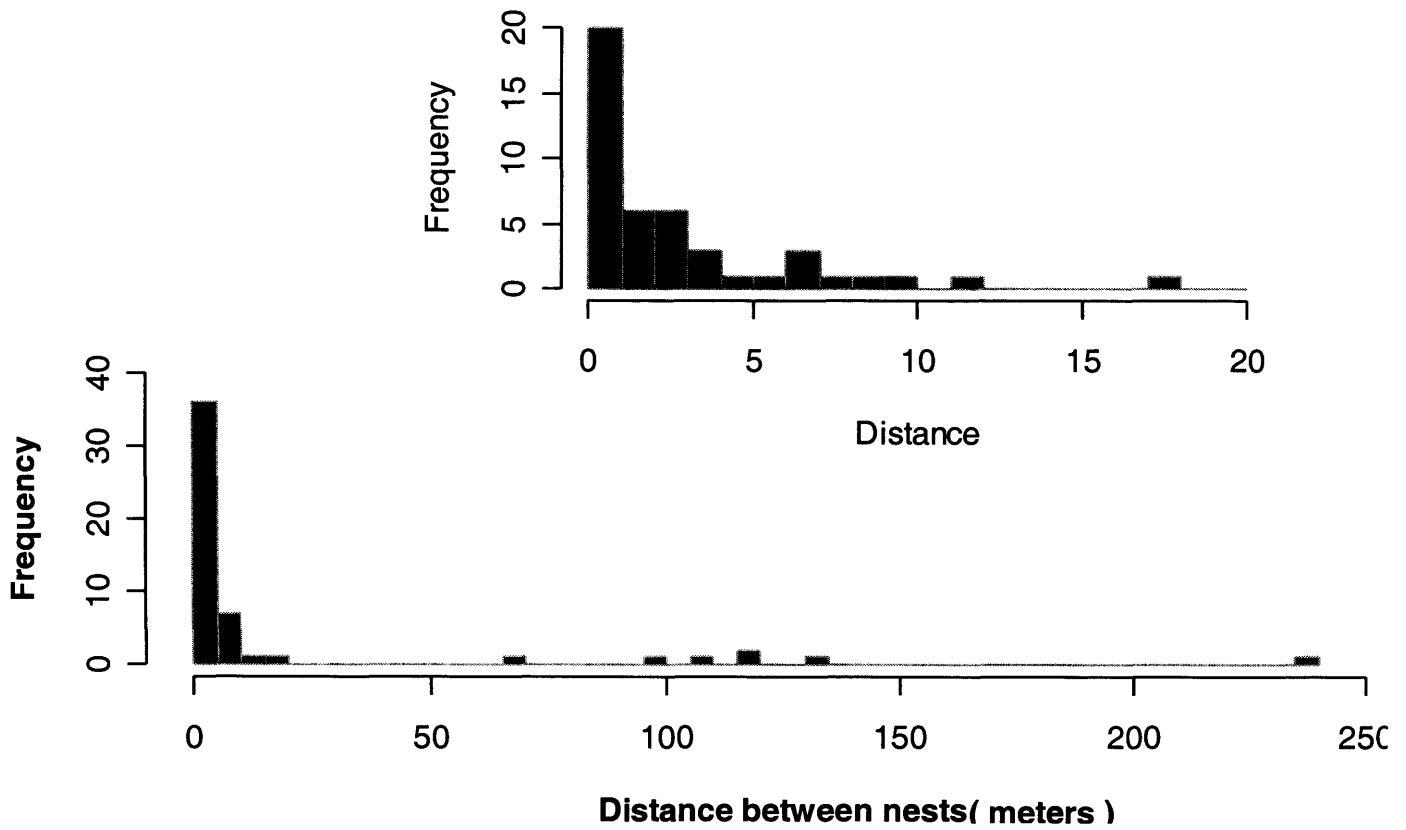
Figure 7 - Distribution of distances between nest pairs visited by the same

wasp (bottom) (n ₜₒₜₐₗ = 178 individuals). Most movers (63%) visited a pair of

nests less than 3 meters apart (inset).
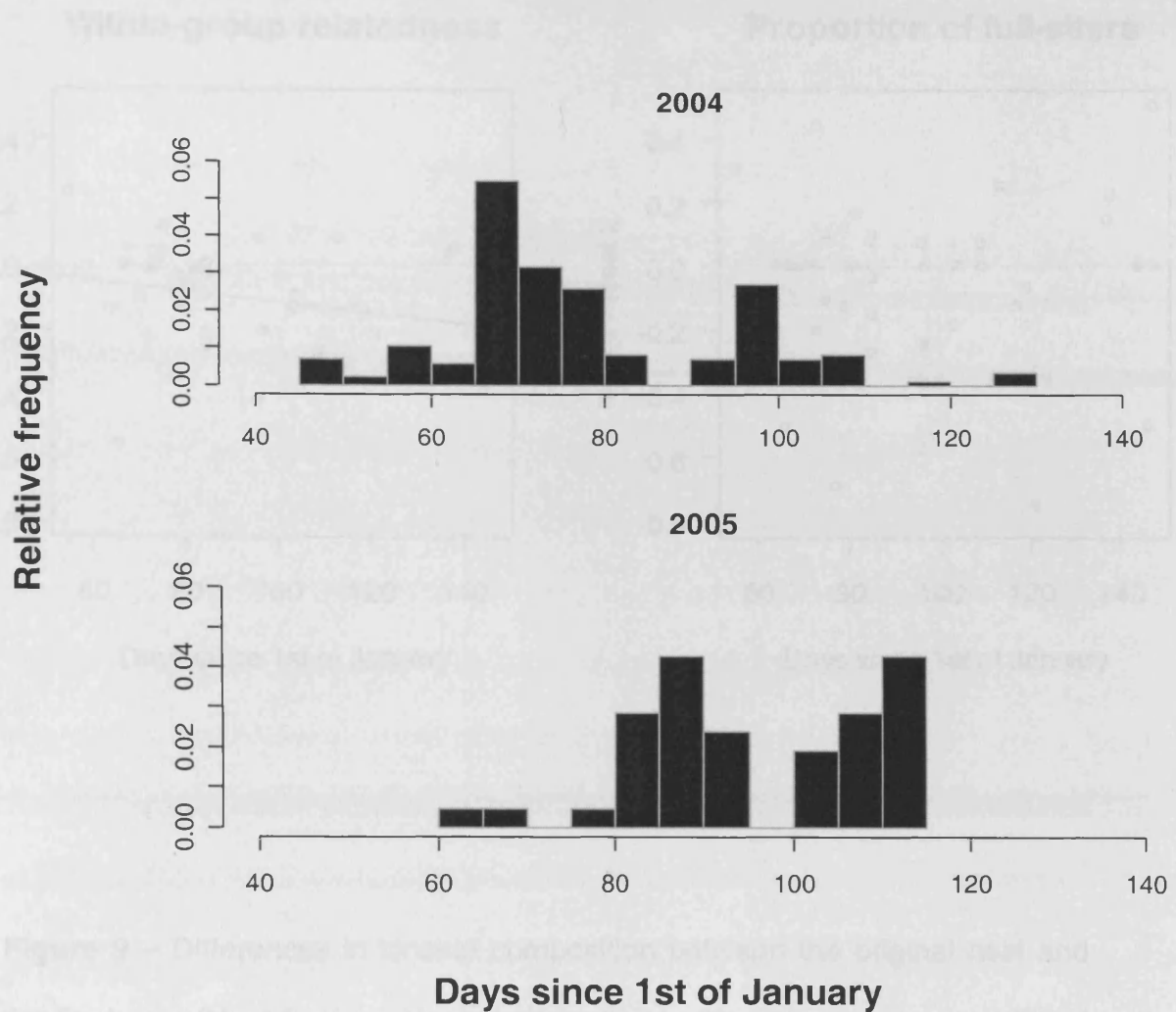
Figure 8 - Dates when foundresses moved between nests. 171 wasps visited more than one nest in 2004, and 46 moved in 2005. Foundresses that moved to a different nest immediately after being marked were not included.
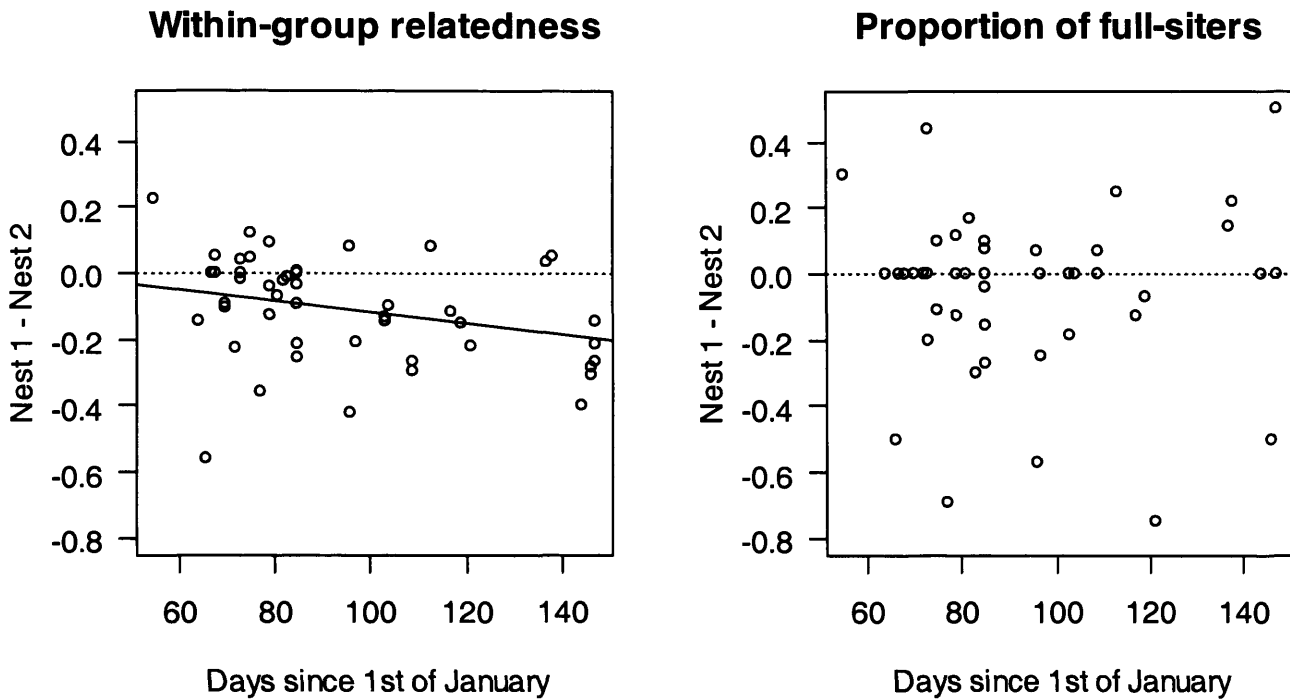
Figure 9 – Differences in kinship composition between the original nest and the final nest (Nest 1 minus Nest 2) in relation to the date of movement (fitted regression line: $y = 0.054 - 0.001x$; $R^2 = 0.085$, df $= 48$, $P = 0.038$). Only full-sisters of the wasp that moved were used to calculate the differences in proportion.

4.4   Discussion

4.4.1   From winter aggregations to nests

The results presented here provide evidence that foundresses that hibernate in the same aggregation tend to initiate nests together. In caontrast, earlier experimental studies indicated that foundresses from the same winter group do not initiate nests more frequently then foundresses from different aggregations (Pratte, 1982). However, the experimental design used by Pratte severely restricted the interactions between foundresses in the winter aggregations, and it could have introduced bias in his results.

Recently, Dapporto and co-workers (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. My results support their findings under natural field conditions where foundress nesting options are unrestricted. My findings indicate that hibernating in the same aggregation may increase the probability of two or more females co-founding a nest. However, in the present study a very small fraction of groups (Figure 2) were formed exclusively by wasps that hibernated together. Therefore, it is unlikely that co-hibernation is the sole determinant of group composition.

4.4.2 Nesting strategies.

4.4.2.1 Monogynic versus polygynic

Multiple foundress nests clearly had a higher chance of surviving until the emergence of workers than single foundress nests. High failure rates for single monogynic nests have been observed in other populations of *P. dominulus*, and in other polistines (Hughes and Strassmann, 1988; Seppa et al., 2002). But the small number of single foundresses nests (n = 9) that achieved worker-production suggests that monogyny may be even more constrained in my population than others. Tibbetts and Reeve (2003) have presented experimental evidence of this marked difference in other *P. dominulus* populations, and argued that it offered support to the survivorship insurance hypothesis (SI), i.e. individual foundresses on multiple-foundress nests are more likely to survive than foundresses who nest alone (Nonacs and Reeve, 1995).

Interestingly, in the present study, multiple foundresses associations of different sizes had similar failure rates. These results agree with a previous experimental study on the same population of *P. dominulus* showing that nest survival was not correlated to 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, SI benefits alone cannot explain why foundresses form groups of different sizes. Alternatively, other forms of insurance-based advantages may be directly correlated to group size. One such advantage, known as Assured Fitness

Returns (AFRs) occurs when offspring of helpers that have only been part-reared can be brought to adulthood by surviving wasps in the group after the helper dies (Gadagkar, 1990). Theoretically, this may favour the permanence of helpers even when helper-offspring relatedness is low (Gadagkar, 1990). AFRs have been elegantly demonstrated to operate in my population of *P. dominulus*, and also in the primitively eusocial hover wasps (Stenogastrinae) *Liostenogaster flavolineata* (Field et al., 2000; Shreeves et al., 2003). It is possible that AFRs are positively correlated with the number of helpers in a group, i.e. in larger groups a larger fraction of the brood of dead foundresses is preserved than in smaller groups. However, there was no evidence of an effect of group size on AFRs in my population (Shreeves et. al., 2003). Moreover, AFRs were not found to be at work in other *P. dominulus* populations (Tibbetts and Reeve, 2003). Thus, the general importance of AFRs in the establishment of foundresses associations remains to be confirmed.

Although it was not possible to directly confirm whether wasps that disappeared had died or moved to an undetected group, disappearance is most likely to be associated with death. Most wasps that used more than one nest moved less then 3 meters (Figure 8), so that undetected groups would usually have had to be within 3 meters of known nests. At this distance, most of these nests would have been detected during intensive searches. Nevertheless, the possibility that foundresses move to undetected nests cannot be completely excluded, since the average flight range (distance covered by wasps foraging) of polistine wasps is over 130 meters (averaged across 9 species; Ugolini, 1983; Prezoto and Gobbi, 2005).

4.4.2.2 "Sit, wait and join"

Nest adoption was very uncommon in my population. Although, empty 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 very low frequency of nest adoption has also been reported for the same population of *P. dominulus* populations in the South of Spain, where only 2 out of 15 experimentally emptied single foundress nests were re-occupied (Shreeves et al., 2003). Previous evidence of nest-adoption by foundresses that were "waiting" is restricted to studies under semi-natural conditions where adopted nests were relatively large and mature (with more developed larvae) and from which all foundresses had been experimentally removed (Nonacs and Reeve, 1993; Starks, 2001). The availability of large and mature nests under more natural conditions may be very low since these nests are less likely to be completely abandoned by its resident foundresses (Strassmann, 1981; Tibbetts and Reeve, 2003). Hence, the importance of nest adoption, especially as part of the "sit and wait" alternative nesting strategy, may have been overestimated.

Nevertheless, "sitting and waiting" is probably a real reproductive strategy in *P. dominulus*. Assuming that the proportion of unmarked foundresses that moved between two or more nests was twice as large as the proportion of marked wasps that did so, 32 % of all unmarked wasps would have come from undetected nests. Hence, the remaining 68 % of unmarked foundresses did not come from another nest, i.e. did not start their own nest. Thus, a large fraction of foundresses was "waiting", possibly at their winter refuges, to later join an established group. These results do support the

existence of a "sit and wait" strategy, which in my studied population is common, but not associated with the adoption of vacant nests.

Considering that the order of arrival at the nest is correlated with rank (Chapter 5), at the initial portion of the dominance queue, late joiners may seldom be dominants (unless they are usurpers). This implies that an individual adopting a "sit and wait" strategy may have limited reproductive success , since in *P. dominulus* reproductive skew is almost complete and co-foundresses are frequently unrelated (Queller et al., 2000; Liebert and Starks, 2006). Interestingly, the arrival of late joiners had no effect on within-group relatedness, suggesting that the "sit, wait and join" strategy is possibly independent of indirect fitness benefits. Alternatively, late joiners may benefit by waiting 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 (pers.obs.). 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. It has been shown that in *P. dominulus,* subordinates with higher ranks improve their chance of inheritance, i.e their survival, by spending less time in dangerous off-the-nest activities (Cant and Field, 2001). Consequently, "sit ,wait and join" can be a viable strategy if dominant turnover is sufficiently frequent. Future studies may explore both hypothesis by testing whether late joiners live longer and inherit the dominant position more frequently than other wasps in their group. Moreover, whether late joiners are physiologically constrained, i.e. not ready to nest, or are facultatively delaying their nesting

attempt is currently unclear and may also be examined in the future (Gadagkar, 1991; Starks, 1998).

4.4.3 Fluctuations in group composition and relatedness

My prediction that the arrival of late joiners (potential usurpers) leads to low within-group relatedness in associations of *P. dominulus* was not confirmed. This suggests that usurpers, which are also common in several other species of *Polistes* do not preferentially usurp unrelated wasps (Reeve 1991; Hughes and Strassmann, 1988). It must be noted 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 have been necessary to clearly identify usurpers. Nevertheless, if it is conservatively assumed that usurpation occurred only when one or more females left the nest after another one joined the group (i.e. movements with replacement), my results are unchanged: no significant effect of number and date of arrival of joiners on relatedness is detected.

Interestingly, the fact that groups that were founded later in the season had lower within-group relatedness, particularly in 2005, is an indication that foundresses may be constrained to nest with any available co-foundresses close to the end of the nest foundation period. At this point, the density of foundresses is at its highest, the likelihood of finding close kin is low (Chapter

1), and the chance of a successful nest foundation decreases since there is less time left in the season.

4.4.4 Wasp movements between nests

The number of foundresses that visited more than one nest was small. This suggests that the idea that foundresses of *P. dominulus* evaluate potential co-foundresses by visiting different established groups may be incorrect. My findings contrast with an early study showing that foundresses nesting in an experimental enclosure visited on average 3 nests, with a similar number of wasps visiting 1 to 3 nests (Pratte, 1979). Although possible effects of marking were accounted for (37 % of the movements occurred after marking), it is unclear whether the number of nest visited could have been artificially increased by Pratte's experimental design. In my present study movements were recorded every other day, and visits to nests were operationally recorded only when a wasp was observed at least twice in a nest. Consequently, foundress movements between nests may have been 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 (Summer 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 frequently moved to nests with significantly higher within-group relatedness than the groups they

departed from, but the number of full-sisters (of the wasp moving) was not different between the nests visited. This apparent contradiction is explained by the fact that on half of the occasions in which a wasp moved to a nest with higher relatedness, a different sib-ship was present at the second nest, i.e. two or more full-sisters which were not related to the joining wasp. Hence, it is unlikely that foundresses of *P. dominulus* in general choose to nest with closely related kin by using kin-related cues to choose which group to join. 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).

Although relatedness may not be directly used to select nest-mates, most foundresses did move to nests with significantly higher within-group relatedness than their original nest. In particular, this trend was more marked at the end of foundation period, when within-group relatedness is frequently low. This suggests that groups where more closely related individuals are present (e.g. a pair of full-sisters) are either more susceptible or attractive to foundresses departing from other groups. Foundresses nesting only with unrelated or distantly related individuals may prevent joiners because they would represent an increase in competition over nest inheritance. Hence, nests with low within-group relatedness may be less attractive to joiners than nests with higher within-group relatedness. However, more detailed information (e.g. nest defence) would be required to test whether groups differ significantly from nests with other types of kin-structure (e.g. only unrelated or only closely-related individuals).

The fact that foundresses usually tended to move away from groups where the size of clypeal marks was more variable suggests that these marks

may be visual cues used in nest mate choice. Foundresses may be actively avoiding individuals with very dissimilar clypeal marks (i.e. much larger). Alternatively, individuals with larger clypeal marks may be more dominant individuals which are able to forcibly evict other wasp from their groups (Tibbetts and Dale, 2004). Currently, it is not possible to determine which explanation is correct. Future experimental studies may address this question by comparing how much aggression foundresses that departed from a group received from the remaining members of their group whether or not their clypeal marks have been experimentally changed.

4.4.5 Summary

Foundresses that hibernate in the same winter aggregation are more likely to initiate a nest together, but groups formed exclusively by wasps coming from the same aggregation are rare. Hence, interactions at the winter aggregations are unlikely to be the only factor determining nest-mate 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 studied population, suggesting that this nesting strategy is locally less viable. Polygynic nest foundation and "sit and wait" were the most common nesting strategies observed. The latter, however, is not associated with the adoption of abandoned nests. Foundresses that "sit and wait" tended to join established groups. Within-group relatedness was not affected by the arrival of these wasps, suggesting that indirect fitness benefits alone do not explain foundress nesting choices. Alternatively, 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.

Movements of foundresses between nests are not generally caused by the arrival of new wasps (potential usurpers) at their original nests. Movements are also not influenced by the overall number of foundresses or the number of close relatives of the wasp moving present in each group. But foundresses frequently moved to nests with higher within-group relatedness than their original group, suggesting that nest-mate choices may be

determined by traits associated with relatedness. In addition, foundresses

tended to move away from groups where the size of clypeal marks was

variable, suggesting that clypeal marks are a visual cue used in the choice of

nest -mates.

CHAPTER 5: Conventions, concessions and inheritances: dominance hierarchies in early associations of *Polistes dominulus*
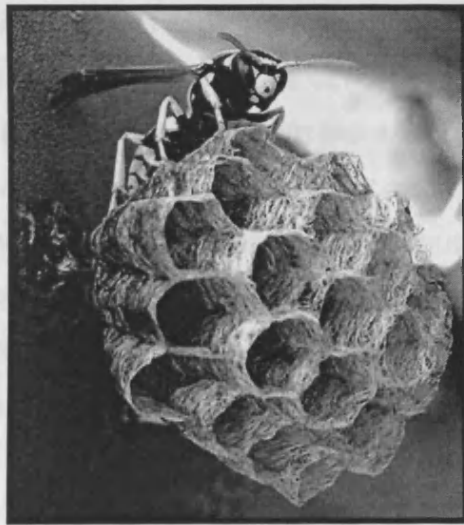
Abstract

Dominance hierarchies constitute the structural base of many social groups. Hence, understanding how they are established is critical. Here I examine how hierarchies are formed in foundresses associations of the common paper wasp *Polistes dominulus*. By comparing field data with computer simulations, I evaluate order of arrival at the nest, body size, facial colour patterns, and within-group kinship structure as determinants of rank. Dominance hierarchies (rank 1 to 5) were experimentally inferred for 53 nests. Overall, the order in which foundresses arrived at the nest and their body size were not significantly correlated with rank. Rank status was negatively correlated with the number of full-sisters foundresses had in their group. Highly-ranked wasps (rank 1 and 2) were less likely to share a nest with their full-sisters than wasps of lower rank, but an individual's rank was not affected by whether their full-sisters had a higher or lower position in the dominance hierarchy in relation to them. Rank was significantly correlated with the size of clypeal marks, but the number of foundresses with clypeal mark(s) in each nest was considerably low. Only one foundress had a mark on 15 nests out of the 20 nests where wasp with marks were present. Overall, my results suggest that within-group kinship is a fundamental factor in the establishment of dominance hierarchies in *P.dominulus* foundresses associations.

5.1   Introduction

Dominance hierarchies are a very common feature of group living, from bumblebees to mandrills (Wilson, 1975). By definition, they reflect the effects of asymmetries between group members on the partitioning of resources and reproductive success (present and future). Since higher ranked individuals obtain a larger share of the benefits of group-living, constant competition to attain dominant status may be expected (Wilson, 1971; Röseler, 1991; Cant et al., 2006a). Conversely, group stability and the synergistic benefits associated with it, i.e. higher group efficiency, depend on the stability of the dominant-subordinate(s) relationship (Bourke and Franks, 1995; Cant and Field, 2001; Cant et al., 2006a; 2006b). Understanding the importance of this trade-off in the evolution of group living has been the subject of considerable theoretical and empirical work (reviewed in Johnstone, 2000; Queller et al. 2000; Camazine et al. 2001; Reeve and Keller, 2001).

Since first being described by Pardi (1942), dominant-subordinate interactions in primitively eusocial wasps have received considerable attention (West, 1967; reviewed in Röseler, 1991; Camazine et al. 2001). In temperate species, before worker emergence, multiple foundresses groups are usually small; all individuals are potentially capable of independent reproduction and are of similar size and age (Reeve, 1991). Moreover, individual chemical profiles are not distinguishable at the beginning of the nesting season (Sledge et al., 2001). This lack of well-defined asymmetries begs the question of what determines dominance status in these groups.

In theory, hierarchies can be established either through direct conflict

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between individuals or through pre-determined conventions based on some arbitrary cue (e.g. order of arrival) which may prevent costly fights between individuals of similar quality (Maynard-Smith and Parker, 1976). Physical contests between foundresses at the early stages of the nesting cycle are common in several polistine species (*P. dominulus* - Pardi, 1942, 1946; *P. metricus* - Gamboa and Dropkin, 1979; *Ropalidia marginata* - Premnath et al., 1996; *P. fuscatus* - Gamboa and Stump, 1996; *Belonogaster juncea juncea* - Tindo and Dejean, 2000). However, the importance of these fights as determinants of the eventual dominance order remains to be confirmed (Cant et al., 2006b). It is not clear whether the aggression between co-foundresses is a cause or consequence of the rank order within the group, does aggression establish the hierarchy or does it represent conflict over status? Evidence that arbitrary cues such as order of arrival and age are used to determine dominance has been found in some primitively eusocial wasps (e.g. order of arrival in *P. carolina*: Seppa et al., 2002; age in *Liostenogaster flavolineata:* Field et al., 1999). However, it is difficult to determine if these cues are arbitrary or direct indicators of general fitness and fighting ability (Strassmann, 1993).

Unlike many vertebrate groups where dominance order can be directly correlated with body size (*in extremis* Buston, 2003), evidence that body size determines rank order in paper wasps is limited (Reeve, 1991). Few studies have found a strong correlation between rank and body size (e.g. Nonacs and Reeve, 1995 in *P. dominulus*). In contrast, it has recently been shown that colour patterns - black marks on the clypeus - affect dominant-subordinate interactions and potentially dominance status (i.e. position in the queue) in *P. dominulus*, and individual recognition in *P. fuscatus* (Tibbetts, 2002; Tibbetts

and Dale, 2004). Although the importance of these visual cues as signals of quality has yet to be confirmed, their relevance in the establishment of dominance hierarchies has to be considered.

Interestingly, within-group genetic relatedness in foundresses associations of *P. dominulus* is not necessarily high, and within-group genetic asymmetries are common (e.g. different sibships; Queller, 2000; Chapter 1). Such asymmetries are likely to generate conflicts between foundresses, since indirect fitness benefits will be unevenly distributed between group members (Keller and Reeve 1996). The possibility of obtaining direct fitness benefits through nest inheritance has been suggested as a possible solution to the conundrum of unrelated foundresses (Queller et al., 2000). More generally, resource inheritance is likely to be a fundamental factor underling the evolution of sociality. In many vertebrate and insect societies, low-ranking individuals can attain breeding status by outliving or supplanting the individuals ahead of them in the group hierarchy (Cant et al., 2006b, and references therein). In this scenario, behavioural strategies that increase the chances of subordinate individuals inheriting the dominant position are expected to evolve. In *P .dominulus,* high-ranked subordinate foundresses spend less time out of the nest and have higher survivorship than low-ranking wasps (Cant and Field, 2001). In addition, within-group conflicts are more frequent between higher ranked subordinates, suggesting that the probability of inheritance is an important factor in the formation of foundresses associations of *P. dominulus* (Cant et al., 2006b). Understanding how dominance hierarchies (i.e. inheritance queues) are established in these paper wasps is therefore critical.

In this chapter, I first examine the hypothesis that dominance status in

foundresses associations of *P.dominulus* is determined by a convention: the order of arrival at the newly founded spring nests. To test this, I examine whether the observed correlation between order of arrival and dominance rank is different from the correlation obtained in simulated populations where rank order was random. I then use the same approach to test whether rank is associated with two potential indicators of general fitness, body size and the size of clypeal marks.

In addition, I examine the potential importance of intra-group relatedness in the establishment of dominance. Concession models from transactional skew theory predict that dominants should concede a larger share of reproduction to unrelated subordinates as an incentive for them to remain in the group (Johnstone, 2000; Reeve and Keller 2001). However, observed levels of subordinate reproduction in *Polistes* do not agree with the prediction of the models (Nonacs et al., 2006). Independent of relatedness, skew is usually very high, i.e. dominant individuals (rank 1) monopolize most or all reproduction (Queller et al., 2000; Liebert and Starks, 2006; but see Cant et al., 2006b). Here, I examine the hypothesis that unrelated subordinates obtain higher positions in the dominance queue, thus providing an alternative incentive for them to remain in the group. I test whether unrelated subordinates are more likely to be at the top of dominance hierarchies, and whether an individual's status is correlated with the proportion of its nest-mates that are full sisters.

5.2 Methods

5.2.1 Natural history of *Polistes dominulus*

Polistes dominulus has a colony cycle typical of polistine wasps in temperate regions (Reeve, 1991). Gynes (mated females) overwinter in aggregations ranging from a few to more than a hundred individuals. Winter diapause refuges 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). In late winter/early spring (February-March, in Spain), foundresses leave their winter diapause refuges and start to found new nests, either singly (monogyny) or jointly with auxiliary foundresses (polygyny; 2 to 23 females in my study population) (Pardi, 1942). In Southern Spain, this founding period is relatively long, usually starting in the end of February and 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 (Chapter 4, but see Queller et al., 2000). Usurpers usually destroy the previous foundress's eggs and early instar larvae (Nonacs and Reeve, 1995; Starks, 2001). In the same

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period, at my study site, *P. dominulus* is subject to attack by a social parasite, *P. 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 became less frequent (i.e. stable groups). In mid summer, new workers stop emerging and the first reproductives start to emerge. Males and gynes do not participate in the activities of the nest and generally mate away from their natal nest (Pardi, 1942). The reproductive phase lasts until the early fall when the colonies dissolve and gynes disperse, going to winter diapause refuges (Pardi, 1942).

5.2.2 Determining the order of arrival

Nests of *P. dominulus* were found on hedges of prickly pear cactus (*Opuntia* sp.) in two nearby rural sites in south-western Spain (Conil de la Frontera, Province of Cadiz; Site 1- 36°17'11 N, 06°04'28 W and Site2 - 36°17'11 N, 06°03'57 W). From February to May in 2004 and 2005, sites were monitored every other day (between 10 - 14 h) to locate newly founded nests. All groups were numbered and their location mapped. The morning after, before they were active (07.00 – 08.00), all wasps were gently collected with long forceps, placed into plastic bags and stored at 4 °C for 15 to 30 min. Wasps were then individually marked using combinations of 4 enamel paint dots (2004) and honey bee tags (2005; queen marking kit: Thorne, UK) and subsequently released on the their original nests to minimize any possible effect of removal. Every other day, all nests were censused early in the

morning to detect changes in group composition. All marked wasps were recorded, and newly arrived unmarked wasps were collected, marked and released on the same day. The order of arrival in the nest was then determined according to the date that foundresses were first recorded in each group (see Figure 1 in results). Wasps that were recorded only once were disregarded.

5.2.3 Identifying dominants and hierarchies

The dominant female on polygynic *P. dominulus* nests can easily be identified from daytime censuses because it leaves the nest less frequently than the remaining females in the group, which spend most of their time foraging (Röseler, 1991; Cant and Field, 2001). During April and May 2004-2005, before the first brood (workers) emerged, daytime censuses were conducted to measure to proportion of time that each foundress spent on the nest. Censuses were carried out only on sunny days ($\geq$ 22 °C) between 10.00 and 15.00, the time when wasps are most active. Two to eight censuses were carried out each day (interval between census: 30 min − 1 h). Following previous studies, wasps were classed as dominants if they were present on the nest for more than 70 % of daytime censuses (mean time in nest of dominants ± S.E. = 88.2 ± 0.017 %) (Cant and Field, 2001; Cant et al., 2006a). This criterion identified a single dominant in 65 nests (out of 67) In the two remaining nests, 2 wasps were present more than 70 % of the time and were considered to be co-dominants.

After dominants had been identified, hierarchies were inferred for 41 experimental nests (2004 only) by removals of successive dominants (Cant and Field, 2001). Starting the day after the removal of the original dominant foundress (rank 1), repeated daytime census were conducted every other day (minimum of 15 census, 2 to 8 census per day), until a new dominant was identified using the criterion above. New dominants were easily identified because only one wasp per nest markedly changed its behaviour after the removal, i.e. spent much less time off the nest. Once identified, the replacement dominant (rank 2) was also removed and the nests censused to identify the next wasp to inherit dominance. The process was repeated until only two wasps were left on the nest (no further removals were necessary) or until workers started to emerge (early May). From this point, new dominants could no longer be identified since more than one foundress was present on the nest during most censuses ($\geq$ 70 %). Overall, 1 to 4 wasps were removed from each experimental nests. In 2005, rank 1 and 2 females were identified in 12 nests also using the criterion above. At the end of the experiment, marked foundresses and their nests were collected and stored at - 80 °C (Laboratorio de Ecología Aquàtica, University of Cadiz, Spain).

5.2.4 Morphological data collection

In the laboratory, wasps were divided into four segments: head, anterior thorax, posterior thorax and abdomen. Wings were carefully removed, unfolded, mounted between glass slides and measured under a 16 x binocular microscope. The internal length of the longitudinal cell (Discoidal I)

of the right wing was used as a size measure, and is known to be correlated with overall body size (Sullivan and Strassmann, 1984).

Wasp heads were mounted on a ruler and photographed with a 10 x Macro Fuji digital camera. Images were used to measure the size and darkness of the clypeal marks. The contour of the clypeal marks was traced, and the area of the resulting polygon used as an estimate of size. The average number of grey pixels inside these polygons was used as measure of darkness. Image analyses were performed with the software Image/J (version 1.33u: http://rsb.info.nih.gov/ij/). The accuracy of these measurements was assessed by measuring the heads using a 22 x monocular microscope and the software NIH Image (version 1.55, http://rsb.info.nih.gov/nih-image). The correlation between measurements was strong and highly significant (R = 0.903, df = 73, p << 0.001).

5.2.5 DNA extraction, amplification and visualization

Total DNA was extracted from the anterior section of the thorax of all 177 ranked wasps using 300 µl of grinding solution (0.1M NaCl; 0.1M Tris-HCl - ph = 8.0, 0.05M EDTA; 0.05% SDS), following Strassmann (1996) with minor modifications. DNA extractions were diluted 1:10 with ultra-filtered distilled water.

Multiplex polymerase chain reactions (PCR) were performed using 5 previously described primers, fluorescently labelled (Pdom 7, Pdom 20, Pdom 127b, Pdom 139, Pdom 140; Henshaw, 2000). PCR was carried out in a Peltier Thermal Cycler using 10µl reactions with: 2.µl of DNA sample, 2 µl of

reaction buffer ((NH$_4$)$_2$SO$_4$), 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 Applied Biosystems 3100 sequencer. Allele sizes were scored against an internal size standard (Applied Biosystems GeneScan ROX 500) using ABI GENESCAN Analysis software (v. 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, 20 random samples were retyped, of which all were re-confirmed.

5.2.6 Relatedness estimation

Pairs of foundresses were assigned to the categories of full-sister or non-sisters (cousins or unrelated) using a likelihood-based method implemented by the program Kinship (see Chapter 1; Goodnight and Queller, 1999; http://www.gsoftnet.us/GSoft.html). 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 versus the likelihood of the same genotypes arising if the females were maternal cousins. Cousins are the next closest possible relationship after sisters, since co-foundresses are of the same generation and multiple mating has not been detected in *P dominulus* so that co-foundresses are very unlikely to be half-sisters (Strassmann, 2001). At α = 0.05, 99% of true sisters should have been correctly assigned to the full-sister category (no. of simulations = 2000, see details in Chapter 1). For each ranked foundress, the proportion of full-sisters

was calculated as the number of wasps assigned to the category of full-sisters of that wasp present in the same group divided by the number of foundresses genotyped in that group.

5.2.7 Statistical analysis

Foundresses in the same group cannot be considered as completely independent data points. Hence, the relationship between rank (dominance status) and order of arrival at nests was evaluated by comparing the observed mean correlation across nests with the mean correlation obtained with simulated groups in which rank was randomly determined. First, Kendall's tau (τ) rank correlation between foundresses arrival order and rank was calculated for each nest, and the overall observed mean correlation across nests obtained. Then, Kendall's tau (τ) was recalculated after rank order was randomly permutated in each nest, and an overall simulated mean correlation obtained. This simulation procedure was repeated 10000 times to obtain null distribution of means (Legendre and Legendre, 1998). The proportion of simulated mean correlation values that were equal to or higher than the observed mean nest correlation was used as an estimate of the probability (P) of obtaining the observed mean correlation by chance. Correlations were considered to be significant if this probability was smaller than 0.05. To avoid overestimating the significance of P, only one half of the distribution of simulated means was considered, negative or positive according to the value of the observed mean correlation. The same procedure was used to evaluate the relation between dominance rank and foundresses body size, size of

black clypeal marks and the proportion of full-sisters in the group. To explore the possibility that factors determining rank status are different for different parts of the rank hierarchy, these analysis were also performed considering only part of the dominance queue, i.e. rank1 and 2, and rank 1, 2 and 3.

Generalized Linear Models (GLMs) assuming quasipoisson error distributions were used to further examine the effect of the four potential explanatory variables, and their interactions, on rank. Wasps in the same nest cannot be considered independent data points, hence one foundress was randomly sampled from each group. Rank was considered as the response variable and order of arrival, body size and size of clypeal mark(s) as potential explanatory variables. Group size, site and year were also considered as potential covariates. This procedure (re-sampling and GLM) was repeated 2000 times to determine the probability of obtaining significant terms in the GLM only by chance. Explanatory terms were considered to be significant if P values smaller than 0.05 were obtained in at least 10% of the re-sampling events (n= 200), twice what would be obtained by chance alone. Two-way interactions and the effect of site and year were not included in the results unless significant.

Data from all 53 nests (no. of wasps: 177; 2004 and 2005) were used in all the analyses, unless stated otherwise. All analyses were carried out using the software R (version 2.4.1; http://www.r-project.org). The functions used to perform the simulations are described in the appendix (II).

5.3   Results

5.3.1 Rank and order of arrival

Overall, the order in which foundresses arrived at the nest was not significantly correlated with rank (mean r = 0.152, P = 0.117; Figure 2). The result was maintained when arrival times were rounded up to intervals of 3 days, to account for gaps between censuses so that the differences in order of arrival were reduced (mean r = 0.139, P = 0.125). When considering only rank 1, 2 and 3 wasps however, arrival order tended to be correlated with rank. Nevertheless, this correlation was not significant (mean r = 0.221, P = 0.061, no. of nests = 39, Figures 1 and 2), and the observed correlation values vary considerably in magnitude and direction between nests (Figure 2). Moreover, rank 1 wasps usually preceded rank 2 wasps on the nest. The correlation between rank and arrival order was stronger when considering only the two individuals of highest rank (mean r = 0.396, P = 0.006, no. nests = 47). These results were maintained when excluding wasps with clypeal marks from the analysis.

5.3.2 Rank and kinship

Closely related foundresses, i.e. full sisters, were present in 26 nests (out of 53). Overall, rank status was negatively correlated with the number of full-sisters foundresses had in their group (mean r = 0.252, P = 0.017; Figure 3). Highly-ranked wasps (rank 1 and 2) were less likely to share a nest with
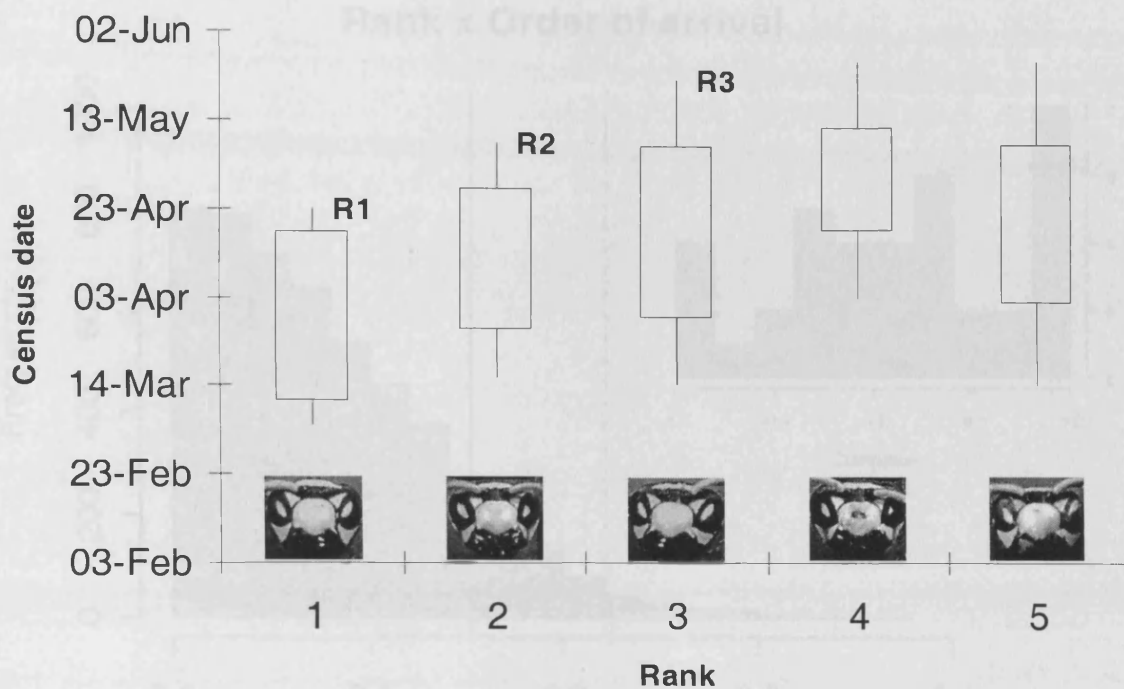
Figure 1 - Summary of nest history, for one of the 41 experimental nests (Nest 40 – Site 2). Lines indicate the first and last day each ranked wasp was observed on the nest. Area of boxes is proportional to the time each wasp was observed on the nest. Date and order of experimental removals are indicated at the top of boxes (**R**). Black clypeal marks (pictures), although more common on dominant (rank 1) wasps were also observed on subordinates.

Figure 2 - Distribution of positive mean correlation values (Kendall's tau) obtained with 10000 simulated groups where rank status was randomly determined with respect to order of arrival. Solid line indicates the observed mean correlation between rank and order of arrival when considering all ranked wasps (associated $P = 0.117$; 53 nests). Dashed line indicates the observed mean correlation value obtained when considering only rank 1, 2 and 3 (associated $P = 0.061$; 39 nests). Inset histogram shows the distribution of observed correlation values obtained in these 39 nests.

their full-sisters than wasps of lower rank (Figure 5). Rank 1 wasps, in particular, had full sister in their groups less frequently than any other ranked foundresses (14 out of 53 nests; Figure 5). Since proportional data were used instead of raw measurements of relatedness (regression estimates), this result is unlikely to be an artefact of group size. Moreover, the proportion of sisters for each individual was calculated independently of the rank status of it sister(s), i.e. if they were of a lower or higher rank than that particular individual. Hence, the number of wasps in the dominance queue had no direct effect on this result.

Foundresses rank was not affected by whether their full-sisters had a higher or lower position in the dominance hierarchy (Figure 4). The proportion of full-sisters of higher rank status was not significantly different from the proportion of sisters of lower rank status for any foundress of intermediate rank (rank 2: X^2 = 2.161, df = 1, P =0.141; rank 3: X^2 = 0.001, df = 1, P = 0.976; rank 4: X^2 = 0.836, df = 1, P = 0.361). For wasps of rank 1 and 5, respectively the highest and lowest rank considered, the distribution of relatives in the group was also balanced. The number of sisters of rank 1 wasps that were rank 2, 3, 4 or 5 was not significantly different (X^2 = 0.579, df = 3, P = 0.901). The number of rank 5 sisters that were rank 1, 2, 3 tended to be smaller than the number of rank 5 sisters that were rank 4 (X^2 = 7.0, df = 3, P = 0.071). However, when not considering rank 3, since no rank 5 sisters were rank 3, no significant differences were found between the remaining ranks (X^2 = 2.730, df = 2, P = 0.255).

When considering only the full-sisters on each nest, relative rank was not correlated with the order of arrival (mean r = - 0.145, P = 0.209, no. nests = 26), or the size of foundresses (mean r = 0.442, P = 0.169, no. nests = 26).

The significance of the correlation with the size of clypeal black marks was not calculated since only 7 nests had wasps with these marks. On 4 of these nests wasps with clypeal marks were the rank 1 wasps, and on both of the nests where more than one marked wasp with clypeal marks was present, the rank 1 wasp had the smallest clypeal mark.

Rank x Proportion of sisters

Figure 3 - Distribution of positive mean correlation values (Kendall's tau) obtained with 10000 simulated groups where rank status was randomly determined with respect to the proportion of sisters each individual had in its group. Dashed line indicates the observed mean correlation rank and proportion of sisters between for 26 nests were full-sisters were present (associated $P = 0.017$). Inset histogram shows the distribution of observed correlations between rank status and the proportion of full-siters in these 26 nests (excluding 4 nests were full-sisters had equal ranks).
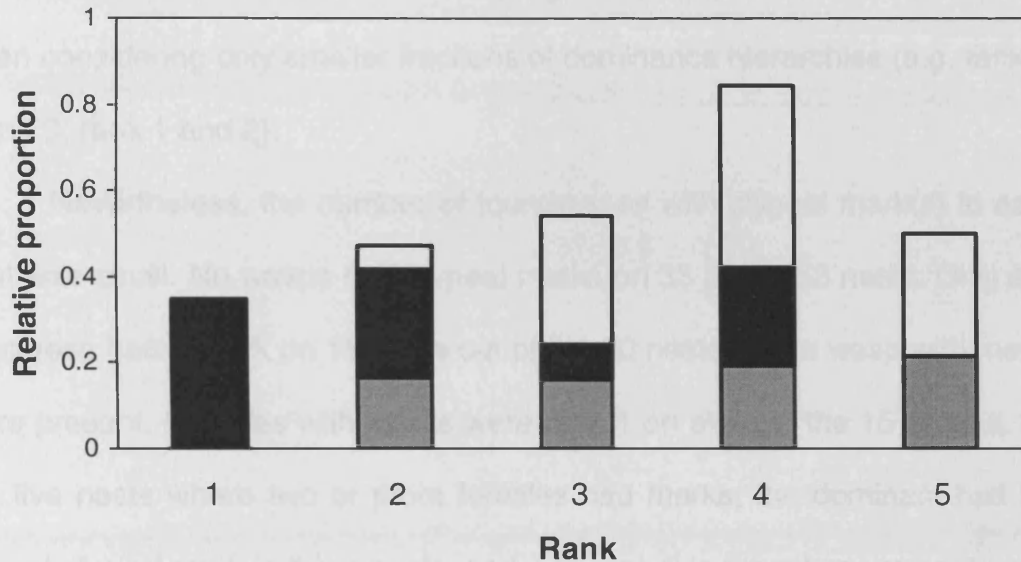
Figure 4 -Proportion of nests where full-sisters were present for wasps at each position in the dominance hierarchy (total length of bars). Black and white fraction of bars indicates the proportion of nests where all sisters had a lower and higher rank than their relatives, respectively. The grey fraction of the bars indicates the proportion of nests where relatives with lower and higher rank were present at the same time. For rank 5, the lowest rank considered, hence the grey fraction refers to nests where relatives with higher and equal rank were present at the same time.

5.3.3. Rank and morphological differences

Foundresses with larger black clypeal marks were more likely to be the dominants (rank 1). Rank was significantly correlated with the size of clypeal marks (mean $\tau = -0.379$, $P = 0.022$; Figure 5). This result was maintained when considering only smaller fractions of dominance hierarchies (e.g. rank 1, 2 and 3; rank 1 and 2).

Nevertheless, the number of foundresses with clypeal mark(s) in each nest was small. No wasps had clypeal marks on 33 out of 53 nests. Only one foundress had a mark on 15 nests out of the 20 nests where wasp with marks were present. Females with marks were rank 1 on eight of the 15 groups. On the five nests where two or more females had marks, the dominant had the largest clypeal mark in three nests, and was rank 3 in the other two nests.

Rank was not significantly correlated with foundresses body size (mean $\tau = 0.041$, $P = 0.622$, Figure 6). This result was maintained when considering only parts of the hierarchy (rank 1 and 2; rank 1 to 3).

5.3.4 GLM results

When considering all four potential determinants of rank together, the order of arrival of foundresses in the nest has no significant effect on rank (mean $z = -0.463$, $P < 0.05$ in only 3.7 % of the simulations). GLM results also confirmed that wasp body size has no significant effect on rank (mean $z = -0.371$, $P < 0.05$ in only 2.3 % of the simulations).
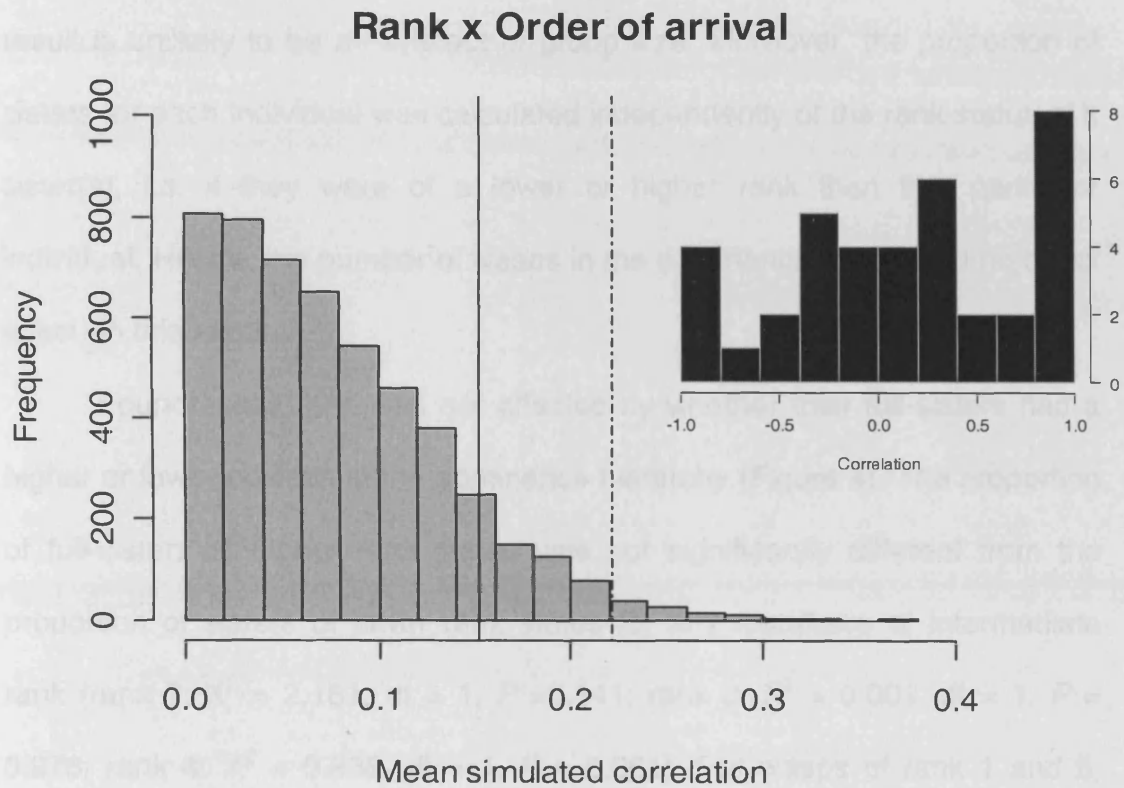
Rank x Clypeal mark size
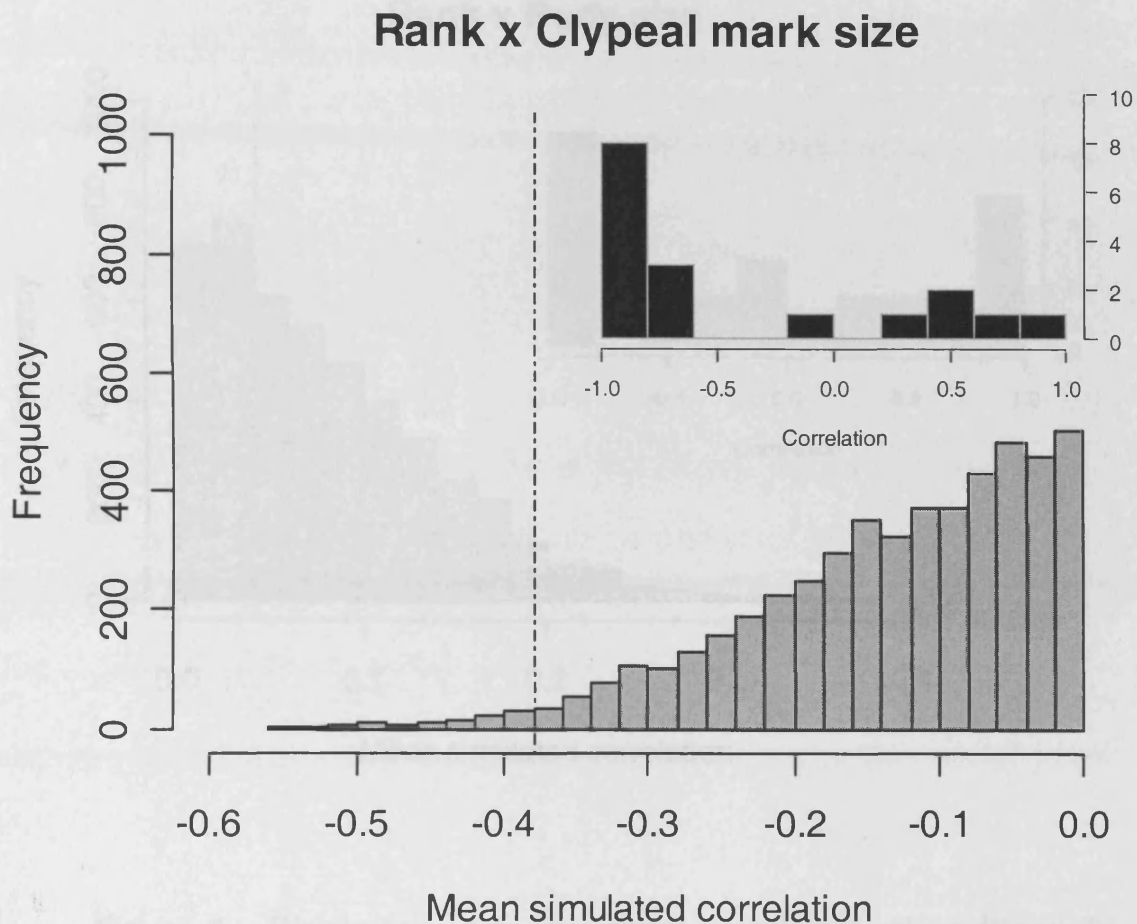
Figure 5 –Distribution (half) of mean correlation values (Kendall's tau) obtained with 10000 simulated groups where rank status was randomly determined with respect to the size of clypeal mark(s). Dashed line indicates the observed mean correlation value for 17 nest where at least one wasp with clypeal mark(s) was present (associated *P* = 0.022). Inset histogram shows the distribution of observed correlations between rank and the size of black clypeal marks in these 17 nests.

Interestingly, in contrast to the results of the previous studies, the size of clypeal marks has no significant effect of rank (mean $r = -0.172$, $P < 0.05$ in any 3.6 % of the simulations).

GLM results confirmed that the number of cells nutritive autonomous bees as a group had a significant effect on their rank status (mean $r = 0.106$, $P = 0.05$ in 15 % of the simulations, Figure 3). Group size was the only other variable that had a significant effect on rank (mean $r = 24.12$, $P = 0.05$ in 30 % of the simulations). Nest fidelity data supported these two highest [illegible]

[illegible] as [illegible] throughout the direct economic standable soundly revisited since residence the minimum rank that can be observed in each [illegible] the greater proportion of females and is [illegible] size not by [illegible]

simulations. These were also confirmed that the observed correlation between rank status and wasp body size is not an artefact of group size.

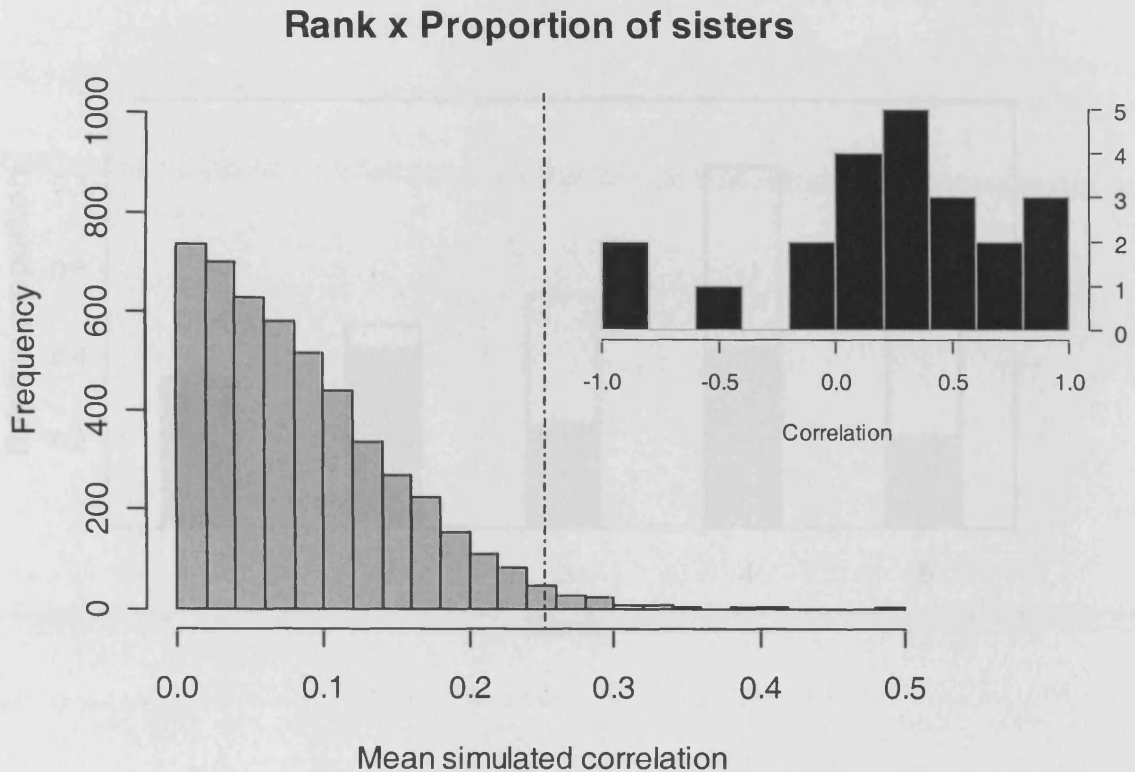**Figure 6** - Distribution of positive mean correlation values (Kendall's tau) obtained with 10000 simulated groups where rank status was randomly determined with respect to wasp body size. Dashed line indicates the observed mean correlation value for 53 nests (associated $P = 0.622$). Inset histogram shows the distribution of observed correlations between rank and wasp body size in these 53 nests.

Interestingly, in contrast to the results of the previous section, the size of clypeal marks has no significant effect of rank (mean $z = -0.172$, $P < 0.05$ in only 3.6 % of the simulations).

GLM results confirmed that the overall number of close relatives foundresses have in a group has a significant effect on their rank status (mean $z = 0.786$, $P < 0.05$ in 15 % of the simulations; Figure 3). Group size was the only other variable that had a significant effect on rank (mean $z = 2.112$, $P < 0.05$ in 53 % of the simulations). These results were maintained when considering only these two explanatory variables in the GLM. The effect of group size is expected since the number of wasps in each nest was highly variable and it directly determines the minimum rank that can be observed in each group. The interaction between the proportion of full-sisters and group size had no significant effect on rank (mean $z = -0.172$, $P < 0.05$ in 6 % of the simulations). These results also confirmed that the observed correlation between rank status and the proportion of full-sister is not an artefact of group size.

5.4   Discussion

5.4.1 Rank and order of arrival

Although limited to the initial fraction of the hierarchy (rank 1 to 3), the observed correlation between order of arrival and rank suggests that precedence may be important in the establishment of dominance in foundresses associations of *P. dominulus*. Seppä and co-workers (2002) reported a similar pattern for *Polistes carolina,* where the rank of the first three foundresses wasps in the hierarchy was frequently determined by their order of arrival on the nest. Seppä et al. (2002) argued that precedence is not vulnerable to the infiltration of outsiders, i.e. new arrivals do not affect the order of arrival of resident group members. Hence, precedence may promote group stability compared with morphological traits (e.g. body size), since if the latter were used to determine rank, outsiders (e.g. late joiners) could out-rank group members and consequently destabilize formed hierarchies (Seppä et al. 2002).

The importance of precedence has also been experimentally demonstrated by Pratte and Gervet (1992), who showed that prior residence can affect the outcome of dominance interactions. Resident rank 1 wasps were significantly more likely to retain their rank when potential usurpers (also rank 1 in their original nest) were introduced. They speculated that this residence effect may be associated with a stronger chemical signature of rank 1 on its nest. However, it has recently been shown that at the early stages of the nest foundation period, wasps cannot be distinguished by their chemical

signatures (Dapporto et al., 2005b). Thus, the importance of the order of arrival may not be correlated with differences in chemical profiles.

Although conventions based on truly arbitrary cues are theoretically possible, it is logical to expect that these cues should instead reflect true underlying differences between contenders (Nonacs, 2001). Nevertheless, for the two *Polistes* species where precedence in the nest is likely to be important, it remains unclear which true differences between foundresses are reflected by their order of arrival on the nest. One possibility is that precedence is a direct indicator of reproductive capacity. Variation in ovary size and juvenile hormone synthesis, both positively correlated with dominance, are observed in hibernating foundresses before nests are started (Röseler, 1991). Foundresses experimentally exposed to higher temperature and light levels at the end of the hibernation period had more developed corpora allata and ovaries, and were more likely to achieve dominance (Röseler et al., 1985; Röseler et al., 1986). Microclimatic variations are likely to occur between different hibernation sites since their exposure can vary considerably (see Chapter 4). In addition, temperature variations can also occur within large aggregations ($\geq$ 100 individuals), where females in the outer portion of the aggregation would be more exposed to microclimatic changes. Thus, if wasps exposed to early spring warmth leave their winter aggregations earlier, the order of arrival in the nest could reflect differences in reproductive capacity.

Overall, independently of which underlying traits are associated with arrival order, it is difficult to explain why precedence (and any associated trait) is important only in determining the highest positions of the hierarchy (rank 1 to 3). Conventions based on arbitrary cues are expected to be established

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more frequently when asymmetries between group members are small (Maynard Smith and Parker, 1976; Nonacs, 2001). Hence, if within-group asymmetries are structured, the relevance of any convention may also be unequally distributed within the group. In my study population foundresses associations are frequently formed by a mixture of full-sisters and non-sisters, thus within-group asymmetries are likely to be structured according to kinship. Since closely related individuals (i.e. full-sisters) frequently have a low rank status, it could be predicted that a convention based on arrival order would be more important at the end of the hierarchy. My results show the exact opposite, suggesting that arrival order is unlikely to be the basis of a convention.

5.4.2 Rank and kinship

My results show that social structure in foundresses associations. of *P. dominulus* is correlated with kinship. Whether relatedness directly determines rank or not cannot be established. Nevertheless, my results suggest that relatedness between group members is a fundamental determinant of the dominance hierarchy.

The prediction that subordinate wasps unrelated to the dominant would occupy higher ranks was not confirmed. Although high rank foundresses tended to have less close relatives in their groups than low rank females, these were not necessarily closely related to the dominant wasp (see Figure 5). Moreover, dominant wasps frequently had no full-sister in their nests.

Thus, is unlikely that dominants are offering higher ranks as an incentive to unrelated subordinates in order to retain them in the group.

As previously suggested, unrelated foundresses may form groups based on the prospects of nest inheritance (Queller et al., 2000). Since the likelihood of inheriting the nest decreases with decreasing rank, highly ranked wasps are more likely to obtain direct fitness benefits (Shreeves and Field, 2002). Hence, this could explain why in my study population foundresses with few or no close relatives in their groups are at higher ranks.

Nevertheless, inheritance alone cannot explain why subordinates foundresses unrelated to the dominant wasp accept positions near the end of the dominance queue. One possible alternative is that low ranked females obtain a combination of small reproductive benefits from two sources. Firstly, although reproductive skew is on average high in nests of P.dominulus, subordinates obtain a small share of the nest reproduction (ca. 10 - 20% of all offspring produced; Liebert and Starks, 2006; Queller et al., 2000). Secondly, even if this fraction of direct reproduction obtained by low ranked subordinates probably decreases with decreasing rank, subordinates with sisters in their nest (also subordinates) can also obtain a small indirect fitness benefits by helping their sisters to obtain their small share of the nest reproduction. This combination of small direct and indirect benefits could explain why low ranked wasps remain in their group.

Furthermore, my results also show that a subordinate's full-sisters do not necessarily have a lower rank status than their close relatives. Low ranked wasps, although unrelated to the dominant, are frequently full-sisters of rank 2 or 3 wasps, and so may obtain indirect fitness benefits when these inherit the dominant position. Overall, the combination of small direct and indirect

benefits could explain why low ranked wasps remain in their group. However, information on dominance turnover in naturally formed groups remains limited; consequently the full importance of nest inheritance cannot be assessed (Shreeves and Field, 2002).

5.4.3 Rank and morphological differences

My results suggest that body size has no strong effect on the establishment of dominance hierarchies. Evidence that body size is important to the establishment of dominance hierarchies in paper wasps foundresses remains limited (e.g. Nonacs and Reeve, 1995 in *P. dominulus*).

In contrast, the observed correlation between rank and the size of black clypeal marks suggests that facial colour patterns are important in dominance-subordinate interactions. It has been experimentally demonstrated that foundresses with more disrupted clypeal marks are frequently the dominant individual in paired contests of *P. dominulus* wasps (Tibbetts and Dale, 2004). The results presented here provide the first evidence that clypeal marks may also be important under field conditions, in naturally formed hierarchies.

Nevertheless, females with clypeal marks were relatively uncommon in my study population and usually only one foundress with mark(s) was present in each group (Chapter 2). The vast majority of groups was formed by foundresses with a completely yellow clypeus. Moreover, the GLM results shown that when different within-group kinship structure and group size were considered, the size of clypeal marks had no significant effect on rank.

Overall, these results suggest that although clypeal marks may be an honest and "respected" signal of quality they are not essential cues in the establishment of dominance hierarchies. Alternatively, clypeal marks may be a trait associated with the "sit-and-wait "nesting strategy, i.e. foundresses that instead of starting their own nest, "wait" and join established groups or adopt abandoned nests later in the season (Starks, 2001). Females that join nests later in the season are faced with established social structures. Hence, foundresses that clearly display their quality (e.g. fighting capacity) may have an increased chance of achieving a high rank status in established groups. In my study population, females with black clypeal marks are significantly more frequent at the end of the nest foundation period, suggesting that clypeal may be important for late joiners (Chapter 2).

5.5 Conclusions

The establishment of dominance hierarchies in foundresses associations of *P. dominulus* is likely to be bounded by within-group kinship structure. Foundresses rank status is significantly correlated with the proportion of group members that are its full-sisters.

In contrast with my prediction, concessions by the dominant individual, based on relatedness alone, are unlikely to be important in the establishment of the social structure. Dominant wasps often have no close relative in their groups. Conventions based on precedence, i.e. the order of arrival on the nest, are also unlikely to be essential determinants of rank order.

Potential morphological indicators of overall quality such as body size and facial colour patterns may have only a limited importance in the establishment of dominance-subordinate status. Nevertheless, clypeal black marks are likely to be functional badges of quality.

Finally, a combination of direct fitness benefits through nest inheritance, and marginal indirect benefits could explain the dominance hierarchy and kinship structure observed in nests of *P. dominulus.*

CHAPTER 6: Concluding remarks

Here I present a summary of the findings of each chapter (5.1 to 5.4), my suggestions for future studies on group formation in *Polistes* wasps (6.5) and my other considerations (6.6).

6.1   Genetic relatedness in early associations of *Polistes dominulus*

Within-group genetic relatedness in foundress associations of *P. dominulus* is very high in the earliest stages... the latest stages of the nest foundation period... dominance as the foundation period... colonies of *P. dominulus* do not prefer to...

Workers... finished and unfinished foundresses... in the same nest in the previous... two sisters in the same group. In contrast, early adopting as... frequently formed by closely related foundresses. This may be explained by the tendency of foundresses to return to their sister site... (Seppä et al. 2002; Sumana et al. 2005) combined with an overall higher abundance of full-sisters at this stage. Although groups formed exclusively by sisters were found throughout the nest foundation period, within-group relatedness was significantly lower in the later stages. The vast majority of stable associations... nests had at least one foundress which was behaviorally assessed as certainly related to the remaining members of the group... preferring on active nesting could...

Here I present a summary of the findings of each chapter (6.1 to 6.4), my suggestions for future studies on group formation in paper wasps (6.5) and my final considerations (6.6).

6.1 Genetic relatedness in early associations of *Polistes dominulus*

Within-group genetic relatedness in foundresses associations of *P. dominulus* is variable between groups and, between subsequent stages of the nest foundation period. Interestingly, within-nest relatedness decreases as the foundation period advances. This suggests that foundresses of *P. dominulus* do not prefer to found nests with their close relatives.

Winter aggregations were formed by mixtures of related and unrelated foundresses, suggesting that foundresses that emerged from the same nest in the previous year, frequently full-sisters, do not necessarily over-winter in the same group. In contrast, early spring nests were frequently formed by closely related individuals. This may be explained by the tendency of foundresses to return to their natal site, i.e. philopatry (Dapporto et al., 2004; Sumana et al., 2005) combined with an overall higher abundance of full-sisters at this stage. Although groups formed exclusively by sisters were found throughout the nest foundation period, within-group relatedness was significantly lower in the later stages. The vast majority of stable working-producing nests had at least one foundress which was completely unrelated (or distantly related) to the remaining members of the group. Local constraints on single nesting could

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explain why distantly related wasps associate, since they could obtain a small fraction of indirect fitness benefits (as opposed to no benefit when nesting alone). Nevertheless, a considerable proportion of groups was formed by completely unrelated co-foundresses. Hence, indirect fitness benefits alone are unlikely to explain the conundrum of *P.dominulus* associations.

6.2 Morphological cues and nest-mate choices in foundress associations

Considerable within-group variability in body size and facial colour patterns was observed in groups of *P. dominulus* foundresses. Wasp body size was significantly less variable on early unstable nests than in any other stage of the nest foundation period. This is more likely to be correlated to changes in within-group relatedness than to foundresses actively choosing to nest with similar sized wasps at the beginning of the nesting season and later choosing more dissimilar wasps. Thus, it is unlikely that foundresses of *P. dominulus* are using body size as an indicator of the general condition of potential nest-mates. Nevertheless, body size could be a cue used by foundresses of *P. dominulus* to detect close relatives.

Clypeal marks, although variable in size and shape, are a relatively rare trait in the studied population. Thus, foundresses of *P.dominulus* cannot rely exclusively on clypeal marks to select nest-mates. In addition, wasps with clypeal marks may preferentially adopt a "sit and wait" nesting strategy since they were significantly more frequent at the end of the nest foundation period, when most groups were already established.

6.3   Founders versus joiners: early fluctuations in group composition

Foundresses that hibernate in the same winter aggregation are likely to initiate a nest together, but groups formed exclusively by wasps coming from the same aggregation are rare. Thus, sharing the same winter refuge is unlikely to be the only factor determining nest-mate choices of *P. dominulus* foundresses.

Foundress disappearances and the arrival of joiners at established groups were the main causes of the fluctuations in group composition observed throughout the nest foundation process. Monogynic nests had a low rate of survival in the studied population, suggesting that this nesting strategy is locally less viable. Polygynic nest foundation and "sit and wait" were the most common nesting strategies observed. Foundresses that "sit and wait" tend to join established groups rather than adopting abandoned nests, as assumed by Starks (2001). Alternatively, 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.

Movements of foundresses between nests were relatively infrequent. Foundresses that were observed in more than one group tended to move to nests with higher within-group relatedness than their original group, but not necessarily a higher number of their own close relatives. This suggests that nest-mate choices may be determined by differences in kinship structure between potential groups. In addition, foundresses tended to move away from groups where the size of clypeal marks was variable. This suggests that clypeal marks are either effective visual cues used in selection of co-

foundresses or that wasps with marks are more successful at evicting unwanted co-foundresses.

6.4 Conventions, concessions and inheritances: dominance hierarchies in foundresses associations

The establishment of dominance hierarchies in foundresses associations of *P. dominulus* is likely to be determined by group kinship structure. The number of close relatives an individual has in its group has a stronger effect on its rank status than its arrival time in the group, its body size or its facial colour patterns.

Dominant wasps often have no close relative on their nests; hence based on relatedness alone, concessions by the dominant individual may not be the sole determinant of the social structure observed in foundresses associations. Moreover, despite their overall limited importance in the establishment of social structures, clypeal black marks are likely to be functional badges of quality. Foundresses with marks, although uncommon, tended to be highly ranked (rank 1 and 2).

A combination of direct fitness benefits through nest inheritance (more frequently obtained by highly ranked wasps), and small direct and indirect benefits (obtained by subordinate wasps) may explain the social structure observed in foundresses associations of *P. dominulus.*

6.5   Future studies on group formation in *Polistes dominulus*

6.5.1 Relatedness and group composition

The potential cause(s) of the variation in within-group relatedness observed throughout the nest foundation period need to be experimentally evaluated under field conditions. One possibility would be to re-locate early unstable groups (1-2 wasps) soon after they have been founded, before all group-members have arrived. This could be used to test whether the high within-group relatedness observed on early nests is due to philopatry alone or to foundresses actively selecting their close relatives as nest-mates.

Most importantly, future studies need to examine the importance of within-group relatedness as a determinant of dominance hierarchies. One possible experiment would involve altering the kinship structure of established groups. Subordinate wasps that rely on the presence of close relatives to remain in a group may be expected to abandon (or be evicted from) their group more frequently after their relatives have been experimentally removed. In addition, it may be expected that conflict over rank would increase if subordinates that "lost" relatives remained in that group. Non-lethal tissue collection for DNA extraction have been used successfully in other eusocial Hymenoptera (Châline et al 2004; Lopez-Vaamonde et al., 2004). Hence, it may be possible to establish the kinship structure of active nests.

6.5.2 "Sit, wait and join"

The success of the "sit, wait and join" strategy is based on the potential advantages that foundresses adopting this strategy may have over foundresses that initiate their own nests. One possible such advantage may be increased survival. Thus, future studies may test whether late joiners live longer than foundresses that founded their own nest, when both are kept under equal conditions in experimental enclosures.

6.5.4 Inheritance queue

Evidence that resource inheritance may be important in the establishment of paper wasps foundresses associations has now been presented in several independent studies (Cant and Field, 2001; Queller et al., 2000). Nevertheless, data on its occurrence under field conditions remain very limited. Future studies may address these problem using long-term surveys of individually marked wasps in established groups. Nests would need to be monitored until all foundresses disappeared so that all dominance turnover events could be recorded. Although time and resource-consuming, this type of study would provide critical information to elucidate the problem of unrelated foundresses in *P. dominulus.*

6.6   Final considerations

Overall, my findings help to clarify some fundamental aspects of the group formation process in eusocial wasps. In particular, they strongly suggest that within-group relatedness has a considerable effect on the establishment of groups. In contrast, other potentially important factors such as body size and badges of quality are likely to have only a limited effect on group formation.

My results show that foundress mortality during the nest foundation period is relatively high, particularly, at the earlier stages. Assuming that individual mortality rates vary according to group composition, it can be hypothesized that determinants of group composition, such as kinship, may also have an effect on individual mortality. Thus, it is possible that groups formed by more closely related individuals, common at the early stages of the nesting period, disappear more frequently than groups formed by a mixture of related and unrelated individuals. This, could explain why within-group relatedness decreases significantly between early nests and stable worker-producing nests.

Unexpectedly, my results also suggest that group size has no strong effect on individual foundress's decisions to join or leave a group. This suggests that foundress associations of *P. dominulus* may not be at their saturation point, the maximal group size above which a new member will not be accepted since this would lead to a decrease in the individual fitness of the existing members of the group (Reeve and Emlen, 2000). Thus, it may be hypothesised that group structure in foundresses associations is not

constrained by the number of wasps in the group. Interestingly, this contrasts with the idea that resource (nest) inheritance may be important in the formation of cooperative groups of unrelated foundresses (Cant and Field, 2001; Queller et al., 2000). Although the pay-off from inheriting a nest may be larger in larger and more productive groups (Shreeves and Field, 2002), the chances of subordinate wasps inheriting a nest are directly correlated to the size of the queue and hence to overall group size. One possible solution to this conundrum would be the existence of sub-structures within the established group social structure. What determines the size of these sub-groups, rather than the total number of individuals in the group, might be the fundamental determinant of group composition. One possible such group sub-structure could be based on genetic relatedness so that groups are sub-divided in a low relatedness fraction and a high relatedness fraction. My findings suggest that this may be the case in associations of *P. dominulus*. Foundresses with no or few close relatives in their groups tend to occupy higher ranks, while foundresses with one or more full sisters in their group are in the lower portion of the dominance queue.

Furthermore, relatedness between co-foundresses in stable groups is frequently asymmetric, i.e. different sibships are present in the same group, and these asymmetries are correlated to group social structure, i.e. dominance hierarchies. Considering that group stability/productivity depends on the establishment of social structures, it is possible that relatedness between group members has an effect on group stability. Interestingly, the observed patterns of foundresses movement between nests suggest that groups with different kinship structure are not equally stable. Foundresses

often left groups with lower relatedness to join group with higher within-group relatedness, but not the reverse.

Overall, my results strongly suggest that within-group relatedness has an effect on the formation of foundresses associations, but the direction of causality cannot be determining using my data. As suggested above, future research would be necessary to elucidate whether within-group relatedness asymmetries determine group composition and dominance structure or whether they are a by-product of their establishment, i.e. the result of disputes between group members or sibships over reproductive opportunities.

References

Alcock J, Simmons LW, Beveridge M, 2006. Does variation in female body size affect nesting success in Dawson's burrowing bee, *Amegilla dawsoni* (Apidae: Anthophorini)? Ecological Entomology 31: 352-357.

Arevalo E, Zhu Y, Carpenter J, Strassmann J, 2004. The phylogeny of the social wasp subfamily Polistinae: evidence from microsatellite flanking sequences, mitochondrial COI sequence, and morphological characters. BMC Evolutionary Biology 4:1-16.

Beani L, Cervo R, Lorenzi CM, Turillazzi S, 1992. Landmark-based mating systems in 4 *Polistes* species (Hymenoptera, Vespidae). Journal of the Kansas Entomological Society 65: 211-217.

Beani L, Turillazzi S, 1988. Alternative mating tactics in males of *Polistes dominulus* (Hymenoptera, Vespidae). Behavioral Ecology and Sociobiology 22: 257-264.

Bernasconi G, Strassmann JE, 1999. Cooperation among unrelated individuals: the ant foundress case. Trends in Ecology and Evolution 14: 477- 482.

Blanckenhorn WU, 2000. The evolution of body size: what keeps organisms small? The Quarterly Review of Biology 75: 385-407.

Blouin MS, 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology and Evolution 18: 503-511.

Bourke AFG, Franks NR, 1995. Social Evolution in Ants, Princeton University Press, 550 pp.

Bourke AFG, 1997. Sociality and kin selection in insects. In: Behavioural Ecology: An Evolutionary Approach, vol. 4th Edition (Krebs JR and Davies NB, eds.), pp: 203-227. Oxford: Blackwell.

Bourke AFG, 2005. Genetics, relatedness and social behaviour in insect societies. In: Insect Evolutionary Ecology (Fellowes MDE et al. eds.), pp: 1-30. CABI.

Burian RM, 1996. Some epistemological reflections on *Polistes* as a model organism, In: Natural History and Evolution of Paper Wasps (Turillazzi S and West-Eberhard MJ, eds.), pp: 319-337. Oxford, O.U.P.

Buston P, 2003. Social hierarchies: size and growth modification in clownfish. Nature 424: 145-146.

Camazine S, Deneubourg JL, Franks NR, Sneyd J, Theraulaz G, Bonabeau E, 2003. Self-organization in Biological Systems, Princeton University Press, 560 pp.

Cant MA, Field J, 2001. Helping effort and future fitness in cooperative animal societies. Proceedings of the Royal Society of London Series B-Biological Sciences 268: 1959-1964.

Cant MA, English S, 2006. Stable group size in cooperative breeders: the role of inheritance and reproductive skew. Behavioural Ecology 17: 560-568.

Cant MA, English S, Reeve HK, Field J, 2006a. Escalated conflict in a social hierarchy. Proceedings of the Royal Society B-Biological Sciences 273: 2977-2984.

Cant MA, Llop JB, Field J, 2006b. Individual variation in social aggression and the probability of inheritance: theory and a field test. American Naturalist 167: 837-852.

Carpenter JM, 1991. Phylogenetic relationships adn the origin of social behavior in the Vespidae. In: The Social Biology of Wasps (Ross KG and Matthews RW,. eds.), pp: 7-32. Ithaca, NY: Cornell University Press.

Cervo R, Lorenzi MC, 1996. Behaviour in usurpers and late joiners of *Polistes biglumis bimaculatus* (Hymenoptera, Vespidae). Insectes Sociaux 43: 255-266.

Cervo R, Zacchi F, Turillazzi S, 2000. *Polistes dominulus* (Hymenoptera, Vespidae) invading North America: some hypotheses for its rapid spread. Insectes Sociaux 47: 155-157.

Châline N, Ratnieks FRL, Badcock NS, Burke T, 2004 Non-lethal sampling of honey bee, *Apis mellifera*, DNA using wing tips, Apidologie 35: 311-318.

Choe JC, Crespi BJ, 1997. Explanation and evolution of social systems. In: The Evolution of Social Behaviour in Insects and Arachnids (Choe JC. and Crespi BJ, eds.), pp: 499-524. Cambridge, C.U.P.

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

Crawley MJ, 2005. Statistics: An Introduction Using R. John Wiley and Sons, Chichester UK, 342 pp.

Crespi BJ, Yanega D, 1995. The definition of eusociality. Behavioral Ecology 6: 109-115.

Curtis TR, Aponte Y, Stamp NE, 2005. Nest paper absorbency, toughness, and protein concentration of a native vs. an invasive social wasp. Journal of Chemical Ecology 31: 1089-1100.

Dakin EE, Avise JC, 2004. Microsatellite null alleles in parentage analysis. Heredity 93,5: 504-509.

Dani FR, Foster KR, Zacchi F, Seppa P, Massolo A, Carelli A, Arevalo E, Queller DC, Strassmann JE, Turillazzi S, 2004. Can cuticular lipids provide sufficient information for within-colony nepotism in wasps? Proceedings of the Royal Society of London Series B-Biological Sciences 271: 745-753.

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 pre-hibernating clusters of *Polistes dominulus*. Annales Zoologici Fennici 42: 135-139.

Dapporto L, Sledge FW, Turillazzi S, 2005b. Dynamics of cuticular chemical profiles of *Polistes dominulus* workers in orphaned nests (Hymenoptera, Vespidae). Journal of Insect Physiology 51: 969-973.

Downing HA, 1991.The function and evolution of the exocrine glands In: The Social Biology of Wasps (Ross KG and Matthews RW, eds.), pp: 540-569. Ithaca, NY: Cornell University Press.

Eichinger L, Pachebat JA, Glockner G, Rajandream MA, Sucgang R, Berriman M, Song J, Olsen R, Szafranski K, Xu Q, Tunggal B, Kummerfeld S, Madera M, Konfortov BA, Rivero F, Bankier AT, Lehmann R, Hamlin N, Davies R, Gaudet P, Fey P, Pilcher K, Chen G, Saunders D, Sodergren E, Davis P, Kerhornou A, Nie X, Hall N, Anjard C, Hemphill L, Bason N, Farbrother P, Desany B, Just E, Morio T, Rost R, Churcher C, Cooper J, Haydock S, van Driessche N, Cronin A, Goodhead I, Muzny D, Mourier T, Pain A, Lu M, Harper D, Lindsay R, Hauser H, James K, Quiles M, Madan Babu M, Saito T, Buchrieser C, Wardroper A, Felder M, Thangavelu M, Johnson D, Knights A, Loulseged H, Mungall K, Oliver K, Price C, Quail MA, Urushihara H, Hernandez J, Rabbinowitsch E, Steffen D, Sanders M, Ma J, Kohara Y, Sharp S, Simmonds M, Spiegler S, Tivey A, Sugano S, White B, Walker D, Woodward J, Winckler T, Tanaka Y, Shaulsky G, Schleicher M, Weinstock G, Rosenthal A, Cox EC, Chisholm RL, Gibbs R, Loomis WF, Platzer M, Kay RR, Williams J, Dear PH, Noegel AA, Barrell B, Kuspa A, 2005. The genome of the social amoeba *Dictyostelium discoideum*. Nature 435: 43-57.

Emlen ST, 1997. Predicting family dynamics in social vertebrates. In: Behavioural Ecology: An Evolutionary Approach, 4th ed. (Krebs JR and Davies NB, eds.), pp 228-253, Oxford: Blackwell.

Faraway J, 2004. Linear Models with R. Chapman and Hall/CRC, Boca Raton, Florida, USA, 240 pp.

Field J, Shreeves G, Sumner S, 1999. Group size, queuing and helping decisions in facultatively eusocial hover-wasps. Behavioral Ecology and Sociobiology 45: 378-385.

Field J, Shreeves G, Sumner S, Casiraghi M, 2000. Insurance-based advantage to helpers in a tropical hover wasp. Nature 404: 869-871.

Field J, Solis CR, Queller DC, Strassmann JE, 1998. Social and genetic structure of paper wasp cofoundress associations: tests of reproductive skew models. American Naturalist 151: 545-563.

Foster KR, Wenseleers T, Ratnieks FLW, 2006. Kin selection is the key to altruism. Trends in Ecology and Evolution 21: 57-60.

Frank SA, 1998. Foundations of Social Evolution. Princeton University Press, Princeton, USA, 280 pp.

Gadagkar R, 1990. Evolution of eusociality: the advantage of assured fitness returns. Philosophical Transactions: Biological Sciences 329: 17-25.

Gadagkar R, 1991. *Belonogaster, Mischocyttarus, Parapolybia,* and independent-founding *Ropalidia marginata.* In: The SocialBiology of Wasps (K. G. Ross and Matthews, R. W. eds.), pp: 149-190. Ithaca, NY: Cornell University Press.

Gadagkar R, 1991. Demographic predisposition to the evolution of eusociality: a hierarchy of models. Proceedings of the National Academy of Science 88: 10993-10997.

Gamboa GJ, Dropkin JA, 1979. Comparisons of behaviors in early vs late foundress associations of the paper wasp, *Polistes metricus* (Hymenoptera, Vespidae). Canadian Entomologist 111: 919-926.

Gamboa GJ, 2004. Kin recognition in eusocial wasps. Annales Zoologici Fennici 41: 789-808.

Gamboa GJ, Stump KA, 1996. The timing of conflict and cooperation among cofoundresses of the social wasp *Polistes fuscatus* (Hymenoptera: Vespidae). Canadian Journal of Zoology-Revue Canadienne De Zoologie 74: 70-74.

Goodnight KF, Queller DC, 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers, Molecular Ecology, 8 (7): 1231-1234.

Grafen A, 1984. Natural selection, kin selection and group selection, in: Behavioural Ecology: An Evolutionary Approach, 2nd ed. (Krebs JR and Davies NB, eds.), pp: 62-84. Blackwell Scientific Publications, Oxford.

Grafen A, 1991. Modelling in behavioural ecology. In: Behavioural ecology: An Evolutionary Approach, 3rd ed. (Krebs JR and Davies NB, eds.), pp: 5-31. Blackwell Scientific, Oxford.

Griffin AS, West SA, 2002. Kin selection: fact and fiction. Trends in Ecology and Evolution 17: 15-21.

Griffith SC, Owens IPF, Burke T, 1999. Environmental determination of a sexually selected trait. Nature 400: 358-360.

Guo SW, Thompson EA ,1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361-372.

Hamilton WD, 1964. The genetical evolution of social behaviour, I and II. Journal of Theoretical Biology 7: 1-16 and 17-52.

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

Higashi M, Yamamura N, 1993 What determines animal group size? Insider-outsider conflict and its resolution. American Naturalist 142: 553-563.

Hughes CR, Strassmann JE, 1988. Foundress mortality after worker emergence in social wasps (*Polistes*). Ethology 79: 265-280.

Hunt JH, 2006: Evolution of castes in *Polistes*. Annales Zoologici Fennici 43: 407–422.

Jeanne RL, 1991. The swarm-founding Polistinae. In: The Social Biology of Wasps (Ross KG and Matthews RW, eds.), pp: 191-231. Ithaca, NY: Cornell University Press.

Jeanne RL, Morgan RC, 1992. The influence of temperature on nest site choice and reproductive strategy in a temperate zone *Polistes* wasp. Ecological Entomology 17: 135-141.

Johnson RN, Starks PT, 2004. A surprising level of genetic diversity in an invasive wasp: *Polistes dominulus* in the northeastern United States. Annals of the Entomological Society of America 97: 732-737.

Johnstone RA, 2000. Models of reproductive skew: A review and synthesis (Invited Article). Ethology 106: 5-26.

Klahn JE, 1979. Philopatric and non-philopatric foundress associations in the social wasp *Polistes fuscatus*. Behavioral Ecology and Sociobiology 5: 417-424.

Kudo K, 2005. Effects of body mass on nest and brood development in the paper wasp, *Polistes chinensis* (Hymenoptera: Vespidae). Sociobiology 46: 647-654.

Kukuk PF, Sage GK, 1994. Reproductivity and relatedness in a communal halictine bee *Lasioglossum (Chilalictus) hemichalceum*. Insectes Sociaux V41:443.

Legendre P, Legendre L, 1998. Numerical ecology, Elsevier; 2nd ed. UK, 870 pp.

Lehmann L, Keller L, 2006. Synergy, partner choice and frequency dependence: their integration into inclusive fitness theory and their interpretation in terms of direct and indirect fitness effects. Journal of Evolutionary Biology 19: 1426-1436.

Liebert AE, Starks PT, 2006. Taming of the skew: transactional models fail to predict reproductive partitioning in the paper wasp *Polistes dominulus*. Animal Behaviour 71: 913-923.

Liebig J, Monnin T, Turillazzi S, 2005. Direct assessment of queen quality and lack of worker suppression in a paper wasp. Proceedings of the Royal Society B-Biological Sciences 272: 1339-1344.

Lopez-Vaamonde C, Koning JW, Brown RM, Jordan WC, Bourke AFG, 2004. Social parasitism by male-producing reproductive workers in a eusocial insect. Nature 430: 557-560.

Makino S, Sayama K, 1991. Comparison of intraspecific nest usurpation between 2 haplometrotic paper wasp species (Hymenoptera, Vespidae, *Polistes*). Journal of Ethology 9: 121-128.

Maynard Smith J, Parker GA 1976 The logic of asymetric contests. Animal Behavior 24: 159-175.

Maynard Smith J, Harper D, 2003 Animal signals. Oxford University Press, Oxford ,UK. 166 pp.

Mesnick S, Evans K, Taylor BL, Hyde J, Escorza-Treviño S, Dizon AE, 2003. Sperm whale social structure: why it takes a village to raise a child. In: Animal Social Complexity: Intelligence, Culture and Individualized Societies (de Waal FBM and Tyack PL, eds.), pp: 170-175, Harvard University Press, Cambridge, Massachusetts.

Nei M, 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, USA, 512 pp.

Nonacs P, Reeve HK, 1993. Opportunistic adoption of orphaned nests in paper wasps as an alternative reproductive strategy. Behavioural Processes 30: 47-60.

Nonacs P, Reeve HK, 1995. The ecology of cooperation in wasps - causes and consequences of alternative reproductive decisions. Ecology 76: 953-967.

Nonacs P, 2001. A life-history approach to group living and social contracts between individuals. Annales Zoologici Fennici 38: 239-254.

Nonacs P, 2002. Sex ratios and skew models: the special case of evolution of cooperation in polistine wasps. American Naturalist 160: 103-118.

Nonacs P, Reeve HK, Starks PT, 2004. Optimal reproductive-skew models fail to predict aggression in wasps. Proceedings of the Royal Society of London Series B-Biological Sciences 271: 811-817.

Nonacs P, Liebert AE, Starks PT, 2006. Transactional skew and assured fitness return models fail to predict patterns of cooperation in wasps. American Naturalist 167: 467-480.

Pardi L, 1942. Ricerche sui polistine. V. La poliginia iniziale in *Polistes gallicus*. (L.) Bollettino dell'Istituto di Entomologia della Università degli Studi di Bologna 14: 1-106.

Pardi L, 1946 Ricerche sui polistine. VII. La "dominazione" e il ciclo ovario annuale in *Polistes gallicus*. (L.) Bollettino dell'Istituto di Entomologia della Università degli Studi di Bologna 15: 25-84.

Pardi L, 1996. *Polistes*: analysis of a society. In: Natural history and evolution of paper-wasps (Turillazzi S and West-Eberhard, MJ, eds.) pp: 1-17. Oxford, O.U.P.

Pekkarinen A, Gustafsson B, 1999. The *Polistes* species in northern Europe (Hymenoptera: Vespidae). Entomologica Fennica 10: 191-197.

Pickett KM, Carpenter JM, Wheeler WC, 2006: Systematics of *Polistes* (Hymenoptera: Vespidae), with a phylogenetic consideration of Hamilton's haplodiploidy hypothesis. Annales Zoologici Fennici 43: 390–406.

Post DC, Jeanne RL, 1982. Recognition of former nestmates during colony founding by the social wasp *Polistes fuscatus* (Hymenoptera, Vespidae). Behavioral Ecology and Sociobiology 11: 283-285.

Pratte M, 1979. Mobilité des fondatrices de *Polistes gallicus*. CR UIEIS sec. française 133-140.

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

Pratte M, Gervet J, 1992. Effects of prior residence and previous cohabitation on the *Polistes dominulus* (Christ) dominance hierarchy. Ethology 90: 72-80.

Premnath S, Sinha A, Gadagkar R, 1996. Dominance relationship in the establishment of reproductive division of labour in a primitively eusocial wasp (*Ropalidia marginata*). Behavioral Ecology and Sociobiology 39: 125-132.

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

Pulliam HR, Caraco T, 1984. Living in groups: is there an optimal group size. In: Behavioural ecology: An Evolutionary Approach, 2nd ed. (Krebs JR and Davies NB, eds.), pp: 122:147. Blackwell Scientific Publications.

Queller DC, 1996. The measurement and meaning of inclusive fitness. Animal Behaviour 51: 229-232.

Queller DC, Peters JM, Solis CR, Strassmann JE,1997. Control of reproduction in social insect colonies: Individual and collective relatedness preferences in the paper wasp, *Polistes annularis*. Behavioral Ecology and Sociobiology 40: 3-16.

Queller DC, Zacchi F, Cervo R, Turillazzi S, Henshaw MT, Santorelli LA, Strassmann JE, 2000. Unrelated helpers in a social insect. Nature 405: 784-787.

Queller DC,1994. Genetic relatedness in viscous populations. Evolutionary Ecology V8: 70-73.

Queller, DC, Goodnight KF, 1989. Estimating relatedness using genetic markers. Evolution 43(2): 258-275.

Raymond M, Rousset F, 1995a. An exact test for population differentiation. Evolution 49: 1280-1283.

Raymond M, Rousset F, 1995b. GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. Journal of Heredity 86: 248-249.

Reeve HK, 1991. *Polistes.* In: The Social Biology of Wasps (Ross KG and Matthews RW, eds.), pp: 99-148. Ithaca, NY: Cornell University Press.

Reeve HK, 1992. Queen activation of lazy workers in colonies of the eusocial naked mole-rat. Nature 358: 147-149.

Reeve HK, Emlen ST, 2000. Reproductive skew and group size: an N-person staying incentive model. Behavioral Ecology 11: 640-647.

Reeve HK, Jeanne RL, 2003. From individual control to majority rule: extending transactional models of reproductive skew in animal societies. Proceedings of the Royal Society B: Biological Sciences 270: 1041-1045.

Reeve HK, Keller L, 2001. Tests of reproductive-skew models in social insects. Annual Review of Entomology 46: 347-385.

Reeve HK, Starks PT, Peters JM, Nonacs P, 2000. Genetic support for the evolutionary theory of reproductive transactions in social wasps. Proceedings of the Royal Society of London Series B-Biological Sciences 267: 75-79.

Richter MR, 2000. Social wasp (Hymenoptera: Vespidae) foraging behavior. Annual Review of Entomology 45: 121-150.

Röseler PF, 1991 Reproductive competition during colony establishment in: *The social biology of wasps* (Ross KG and Matthews RW, eds.), pp: 309-333. Ithaca, NY: Cornell University Press.

Röseler PF, Röseler I, Strambi A, 1985. Role of ovaries and ecdysteroids in dominance hierarchy establishment among foundresses of the primitively social wasp, *Polistes gallicus*. Behavioral Ecology and Sociobiology 18: 9-13.

Röseler PF, Röseler I, Strambi A, 1986. Studies of the dominance hierarchy in the paper wasp, *Polistes gallicus* (L) (Hymenoptera-Vespidae). Monitore Zoologico Italiano-Italian Journal of Zoology 20: 283-290.

Rousset F, Raymond M, 1995. Testing heterozygote excess and deficiency. Genetics 140: 1413-1419.

Rusina L, Gilev A, Skorokhod O, Firman L, 2006. Seasonal dynamics of the phenotypic structure of a population of the paper wasp *Polistes dominulus* (Christ) (Hymenoptera, Vespidae). Entomological Review 86: 168-175.

Seppa P, Queller DC, Strassmann JE, 2002. Reproduction in foundress associations of the social wasp, *Polistes carolina*: conventions, competition, and skew. Behavioral Ecology 13: 531-542.

Shreeves G, Field J, 2002. Group size and direct fitness in social queues. American Naturalist 159: 81-95.

Shreeves G, Cant MA, Bolton A, Field J, 2003. Insurance-based advantages for subordinate co-foundresses in a temperate paper wasp. Proceedings of the Royal Society of London Series B-Biological Sciences 270: 1617-1622.

Sledge MF, Boscaro F, Turillazzi S, 2001. Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. Behavioral Ecology and Sociobiology 49: 401-409.

Sledge MF, 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 PT, 1998. A novel 'sit and wait' reproductive strategy in social wasps. Proceedings of the Royal Society of London Series B-Biological Sciences 265: 1407-1410.

Starks PT, 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.

Starks PT, 2003. Natal nest discrimination in the paper wasp, Polistes dominulus. Annales Zoologici Fennici 40:53-60. Starks PT,Fefferman NH, 2006: Polistes nest founding behavior: a model for the selective maintenance of alternative behavioral phenotypes. Annales Zoologici Fennici 43: 456–467.

Strassman JE, Solis CR, Peters JM, Queller DC, 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 (Ferraris JD and Palumbi SR, eds.), pp: 163-178, Wiley-Liss, New York- USA.

Strassmann JE, 1981. Parasitoids, predators, and group-size in the paper wasp, Polistes exclamans. Ecology 62: 1225-1233.

Strassmann JE, 1993. Weak queen or social contract? Nature 363: 502-503.

Strassmann JE, Hughes CR, Queller DC, Turillazzi S, Cervo R, Davis SK, Goodnight KF, 1989. Genetic relatedness in primitively eusocial wasps. Nature 342: 268-270.

Strassmann JE, Zhu Y, Queller DC, 2000. Altruism and social cheating in the social amoeba Dictyostelium discoideum. Nature 408: 965-967

Strassmann J, 2001. The rarity of multiple mating by females in the social Hymenoptera. Insectes Sociaux V48: 1-13.

Sullivan JD, Strassmann JE, 1984. Physical variability among nest foundresses in the polygynous Social Wasp, Polistes annularis. Behavioral Ecology and Sociobiology 15: 249-256.

Sumana A, Liebert AE, Berry AS, Switz GT, Orians CM, Starks PT, 2005. Nest hydrocarbons as cues for philopatry in a paper wasp. Ethology 111: 469-477.

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 EA, 2002. Visual signals of individual identity in the wasp *Polistes fuscatus*. Proceedings of the Royal Society of London Series B-Biological Sciences 269: 1423-1428.

Tibbetts EA, Reeve HK, 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.

Tibbetts EA, Dale J, 2004. A socially enforced signal of quality in a paper wasp. Nature 432: 218-222.

Tibbetts EA, 2006. Badges-of-status in worker and gyne *Polistes dominulus* wasps, Annales Zoologici Fennici 43: 575- 582.

Tindo M, Dejean A, 2000. Dominance hierarchy in colonies of *Belonogaster juncea juncea* (Vespidae, Polistinae). Insectes Sociaux V47: 158-163.

Tsuchida K, 1991. Effects of female body size and timing of nest foundation on the reproduction of the Japanese paper Wasp, *Polistes jadwigae* Dalla Torre (Hymenoptera, Vespidae). Researches on Population Ecology 33: 361-366.

Turillazzi S, Beani L, 1985. Mating behavior in the paper wasp *Polistes gallicus*- (L) (Hymenoptera Polistinae). Monitore Zoologico Italiano-Italian Journal of Zoology 19: 169-170.

Turillazzi, S, West-Eberhard, MJ (eds), 1996. Natural History and Evolution of Paper Wasps, Oxford University. Press, Oxford. 416 pp.

Turillazzi S, Mastrobuoni G, Dani FR, Moneti G, Pieraccini G, la Marca G, Bartolucci G, Perito B, Lambardi D, Cavallini V, Dapporto L, 2006. Dominulin A and B: Two new antibacterial peptides identified on the cuticle and in the venom of the social paper wasp *Polistes dominulus* using MALDI-TOF, MALDI-TOF/TOF, and ESI-ion trap. Journal of the American Society for Mass Spectrometry 17: 376-383.

Ugolini A, 1983. Homing in the wasp *Polistes gallicus* (L): Information gained enroute and initial orientation. Monitore Zoologico Italiano-Italian Journal of Zoology 17: 212-213.

Wenzel JW 1991. Evolution of nest architecture. In: The Social Biology of Wasps (Ross KG and Matthews RW, eds.), pp: 480-519. Ithaca, NY: Cornell University Press.

West, MJ, 1967. Foundress associations in polistine wasps: dominance hierarchies and the evolution of social behavior. Science, 157: 1584-1585.

Wilson EO, 1971. The Insect Societies. Harvard University Press, Cambridge (Mass), 562 pp.

Wilson EO, 1975. Sociobiology: the New Synthesis, Harvard University Press, Cambridge (Mass), 720 pp.

Zacchi F, Cervo R, Turillazzi S, 1996. How *Polistes semenowi*, obligate social parasite, invades the nest of its host *Polistes dominulus* (Hymenoptera, Vespidae). Insect Social Life 1: 125-130.

Appendix 1

DNA extraction protocol

Modified from Strassman (1996)

1. Remove a maximum of 20 anterior thoraces from freezer and place on ice.

2. Place each thorax in a separate labelled tube and grind with a disposable micropestle in 100 μl of grinding buffer (0.1M NaCl; 0.1M Tris-HCl, h = 8.0, 0.05M EDTA; 0.05% SDS).

3. Spin briefly to collect tissue at the bottom of the tube.

4. Add 200 μl of grinding buffer and incubate at 65°C for 30 minutes.

5. Will tube still warm, add 43 μl of 8 M potassium acetate and mix well inverting tubes, then tap them to bring contents to the bottom.

6. Incubate on ice (4°C) for 30 minutes to precipitate salt and SDS.

7. Centrifuge tubes at 14000rpm for 15 minutes.

8. Transfer supernatant (~ 200 μl) to a a new set of labelled 1.5ml tubes.

9. Add 250 μl cold 100 % ethanol, mix well and incubate overnight at - 20°C to precipitate DNA.

10. Centrifuge tubes at 14000rpm for 15minutes.

11. Remove supernatant and allow pellet to dry (by air, no more then 10 minutes).

12. Resuspend pellet in 25 μl of ultra-filtered distilled water.

Appendix 2

R functions

a. Function 1 (fun1) <-* *random replacement of ranks status values**

```
function(x){d<-dim(x)[1]
res<-replace(x[[3]],1:d,sample(1:d))
res}
```

b. Function 2 (fun2) <- **calculate Kendall's tau**

```
function(x){
c1<-x[2]
c2<-x[4]
sco<-cor(c1,c2,method="kendall")
sco}
```

c. Function 3 <- **generate simulated distribution of mean correlations**

```
function(data,nperm){
res<-vector(length=nperm)
for(i in 1:nperm){gapply(data,FUN=fun1,groups=data$nest)->nr
unlist(nr)->nr
data.frame(data,nr)->xi
gapply(xi,FUN=fun2,groups=xi$nest)->nc
unlist(nc)->nc
na.omit(nc)->nc
res[i]<-mean(nc)}
res}
```

Obs: gapply function of *R* nlme library