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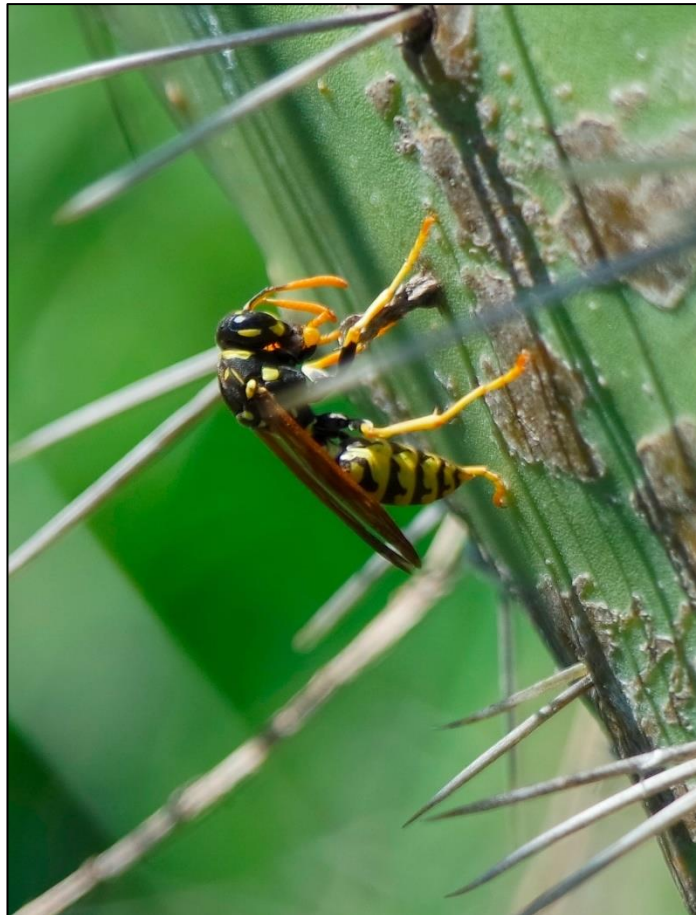
Alternative Reproductive Tactics in Social Wasps

Submitted by:

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to the University of Sussex as a thesis for the degree of
Doctor of Philosophy in Biological Sciences

2018



Declaration

I certify that all materials in this thesis that are not my own work have been identified, and that this thesis has not been and will not be submitted in whole or in part to another University for the award of any other degree.

Signature:

Christopher Francis Accleton

Alternative Reproductive Tactics in Social Wasps

Abstract

There is an abundance of different behavioural approaches, or tactics, employed by animals to maximise reproductive success in different situations. The best documented alternative reproductive tactics (ART) have mainly been those carried out by males. In this thesis I use field data and molecular genotyping to investigate ARTs carried out by females in two very different species of social wasps.

In **Chapter 1**, I explore the topic of alternative reproductive tactics in insects, and introduce my two study systems, *Polistes dominula* and *Ammophila pubescens*. In Part I of this thesis (**Chapters 2 - 3**), I focus on ARTs in foundress associations of the primitively eusocial paper wasp *P. dominula*. In these associations, there is a dominance hierarchy in which the rank 1 female lays most or all of the eggs. In **Chapter 2** I use data from a Spanish field site to investigate the rules involved in establishing the dominance hierarchy. Specifically, how two potential ARTs, where a female either initiates a nest of her own, or joins an existing nest, affect reproductive status. I show that being the female that first initiates the nest is the most important predictor of attaining rank 1 reproductive status within the *P. dominula* hierarchy, but that body size might also be important. Following this, in **Chapter 3** I compare characteristics of multi-female nests originally initiated by either a solitary foundress or a group of foundresses. I show that nest founding tactic may indirectly affect both productivity and survival through its effect on group size.

In Part II of this thesis (**Chapter 4**), I focus on ARTs in the solitary nesting subsocial digger wasp *A. pubescens*. *A. pubescens* females produce a sequence of nests, each containing a single offspring that is provisioned with paralysed caterpillars. In **Chapter 4** I investigate the factors influencing the occurrence of intraspecific parasitism, whereby a female removes the egg of a conspecific host and replaces it with an egg of her own. I show that parasites do not restrict their selection of host's nests to those in the locality of their own nests, but instead parasitize throughout the aggregation.

In **Chapter 5**, I first introduce a framework inspired by the well-developed sex-allocation literature, in which ARTs can be distinguished based on when and how tactic-specific phenotypic determination occurs. Next, I summarise my key findings and attempt to characterize the ARTs in my study systems within the aforementioned framework. Finally, I comment on future possible directions for research in this area.

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Author's declarations

All of the chapters presented in this thesis were written by Chris Accleton, with comments from Jeremy Field. Further contributors for each chapter are detailed below where relevant.

Chapter 1 and 5

No further contributions were made.

Chapters 2 and 3

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Chapter 4

The dataset used in this chapter was provided by Jeremy Field, having been collected over two years (2012 - 2013) by Jeremy Field, William Foster and Chris Accleton.

Chapter 1: General introduction

1.1 - Alternative reproductive tactics

Alternative reproductive tactics (ARTs) refer to the existence of two or more distinct ways individuals of the same sex and species achieve reproduction. In fish, for example, the most widespread ART is when large males monopolize resources and access to mates in their territories (i.e. guarding), while small males steal fertilisations (i.e. sneaking) without investing behaviourally in territory defence (Taborsky, 2008). For example, the deep-water plainfin midshipman (*Porichthys notatus*) spawns in the rocky shores of the intertidal zone, where limited nest sites leads to strong selection for male ARTs: the largest (guarding) males gain nest sites through aggressive defence, and attract females with their well-developed sonic muscles; whereas, the smaller (sneaking) males do not guard territories or attempt to call females, and have poorly developed sonic muscles. Smaller males instead steal fertilisations by either sneaking into a guarding male's territory when a female is present, or waiting just outside and fanning their sperm in (Cogliati *et al.*, 2014).

In social mammals it is common for a solitary, dominant male to monopolise a female during oestrus (Wolff, 2008). An ART in such a system is to form an alliance of two or more males. Cheetahs (*Acinonyx jubatus*) often employ this tactic, forming alliances of two or three males, often brothers. In this species, alliances typically hold and defend a territory, while lone males wander large areas avoiding territory holders, and attempting to copulate surreptitiously with females (Dalton *et al.*, 2013; Gottelli *et al.*, 2007; Wolff, 2008).

ARTs can occur at various stages in the reproductive process. In insects, where ARTs are commonplace and occur in most orders, male reproductive behaviour typically involves four stages: 1) locating a female; 2) gaining access to her; 3) copulating with her; and 4) engaging in post-copulatory behaviour to increase likelihood of sperm use by the female. For example, insects exhibit three general types of mate locating behaviour (stage 1): remain in current location and fight for / guard potential mates; advertise to attract a mate to own location; or disperse to a potential mate's location (Brockmann, 2008). Males of the neotropical ant *Hypoponera opacior* employ two of these behaviours. Winged males carry out nuptial flights, which is typical in ants. Wingless (apterous) males on the other hand, remain in their natal nests and mate with very young virgin queens still encased in their cocoon. Males carrying out this ART cling on to the queen's cocoon, and insert their genitalia through an opening which is used to expel stored faeces (meconium), with copulations lasting an average of 12 hours (Foitzik et al., 2010, 2002; reviewed in Kureck et al., 2013).

Once a female has been located, a male may have to compete with other males for access (stage 2), or by some mechanism cause the female to select him. Males of the spider *Pisaura mirabilis* typically offer a nuptial gift to prospective females, consisting of an insect prey wrapped in silk. The cost of copulation can be reduced, however, by offering a worthless gift (e.g. prey remains or plant matter) wrapped in silk. A male carrying out this ART will successfully copulate with the female, but will experience a 20% shorter copulation duration than males providing high value gifts (Albo et al., 2011; Ghislandi et al., 2014, 2017).

It is not only males that exhibit ARTs, however, but so far female ARTs are relatively understudied (Alonzo, 2008). One way in which female ARTs might arise is to avoid sexual conflict resulting from costly male behaviours, such as excessive mate guarding (Alonzo, 2008). For example, colour morphs in several damselfly species may have evolved to alleviate the costs of sexual conflict in the form of male mating harassment, in that a male-like 'andromorph' female mimics the blue males and so receives less male attention than the typical heteromorph. In *Enallagma* damselflies, heteromorphs have been found to accomplish the lessening of male harassment by use of a

different method, camouflage. Green / brown heteromorphs were less visible against vegetation, but once discovered were readily recognized as females. Andromorphs on the other hand, with their blue colouration, were obvious in the environment but not initially recognisable as female (Winfrey and Fincke, 2017).

ARTs can also be exhibited in by those living in cooperative groups (Koenig and Dickinson, 2008). For example, the common marmoset (*Callithrix jacchus*) is a small Neotropical primate endemic to Northeast Brazil. They live in social groups of between 3 and 15 individuals and usually contain only one breeding (dominant) pair, as well as subordinate males and females that care for the dominant pair's offspring, sub-adults and infants. Female reproductive dominance is maintained over subordinate females through interruption of copulations, as well as ovulation suppression by behavioural and olfactory signals. This suppression is not permanent, however, and subordinates regain their ability to reproduce once apart from the suppressing signals. Thus, subordinates do sometimes reproduce in the wild, but this often leads to infanticide or expulsion from the group (Schiel and Souto, 2017; Setchell, 2008). A subordinate's best chance of successful reproduction is through gaining a dominant position, either within their own group, or after migrating to another. Therefore, in this species the best female tactic is to be the reproductive dominant, whereas a subordinate position represents an ART in which females avoid wasting reproductive effort and stress while they await an opportunity to become a dominant themselves (Setchell, 2008).

Female ARTs often involve exploiting the maternal efforts of conspecifics. This could be through the usurpation of a conspecific's nest, or even through the depositing of eggs into a nest to be cared for by the owner or host. Thus, these intraspecific parasites save the costs accrued by one or more stages of brood care, and in some cases, increase the number of offspring produced (Taborsky and Brockmann, 2010). An example of this type of ART can be seen in the stingless bees, which form perennial swarm-founded colonies. Virgin queens typically mate and either supersede their mother as the queen, or head a swarm to found a new colony. In the stingless bee *Melipona scutellaris*, however, an alternative tactic is often utilised. This ART is termed intraspecific queen

parasitism, whereby young mated queens seek out nearby queenless colonies, entering the nest late in the evening when guarding efficiency is at its lowest, and attempt to takeover. Interestingly, this ART has been linked to the fact that species in the genus *Melipona* typically produce queens in excess of the colony's needs, unlike in other advanced eusocial bees. Queens and workers develop in identical brood cells, enabling females to self-determine their caste. Many of these excess queens are killed by workers on emergence, but over 50% successfully leave the nest (Van Oystaeyen *et al.*, 2013; Vollet-Neto *et al.*, 2018; Wenseleers *et al.*, 2010).

A major objective of evolutionary biology as a whole, and the study of alternative reproductive tactics more specifically, is to understand how alternative phenotypes such as those described above, are maintained within populations (Taborsky *et al.*, 2008). For example, ARTs can result from several sources of selection pressure (Taborsky *et al.*, 2008), such as Intrasexual, intersexual, and frequency dependent selection. Also of interest are the ecological, social, physiological and genetic factors that influence the tactic employed (Clutton-Brock, 2009; Cockburn, 1998a; Field *et al.*, 1998; Keller and Reeve, 1994). In order to dissect the processes involved in maintaining such discrete morphological, physiological and behavioural morphs, however, we must first characterize the patterns they make (reviewed in Taborsky *et al.*, 2008). The focus of this thesis is to develop an understanding of three examples of female alternative reproductive tactics, and then characterize them within an ART framework. To this end, I use field data and molecular genotyping to investigate ARTs carried out by females in two very different species of social wasps.

In Part I of this thesis (**Chapters 2 - 3**), I focus on ARTs in foundress associations of the primitively eusocial paper wasp *Polistes dominula*. In these associations, there is a dominance hierarchy in which the dominant rank 1 female lays most or all of the eggs, while other individuals work to rear the rank 1's offspring. Thus, there are large fitness implications in the tactics employed at this stage. In **Chapter 2** I use data from a Spanish field site to investigate the rules involved in establishing the dominance hierarchy. Specifically, how two potential ARTs, where a female either initiates a nest of her own, or joins an existing nest, affect reproductive status. Answering some of

the outstanding questions in this area, such as whether foundresses initiating nests are more likely to become the dominant than the subsequent joiners, would enable previously impracticable questions to be addressed. Of particular interest is the question of whether wasps that become the dominant are intrinsically different from subordinates.

Following this, in **Chapter 3** I use field data and molecular genotyping to compare characteristics of nests originally initiated by either a solitary foundress or a group of foundresses. Previous work in this area has exclusively focused on comparing solitary nesting females with those nesting in a group. This was of particular interest as it begged the question of why an individual would join a group as a subordinate, and so give up independent breeding. Yet the question of how the method of nest initiation, solitarily or by a group of foundresses, subsequently affects group characteristics and fitness outcomes remains unexplored. Do nests started by a group of foundresses, for example, have higher within-group relatedness? Are solitary or group founded nests initiated by morphologically distinct females?

In Part II of this thesis (**Chapter 4**), I use field data and molecular genotyping to investigate ARTs in the solitary nesting subsocial digger wasp *Ammophila pubescens*. *A. pubescens* females produce a sequence of nests alongside those of conspecifics in multi female aggregations, each nest containing a single offspring that is provisioned with paralysed caterpillars by the owner. In **Chapter 4** I investigate the factors influencing the occurrence of intraspecific parasitism, whereby a foreign female enters a conspecific's nest and removes the host egg, replacing it with an egg of her own. Intraspecific parasitism occurs in a wide range of taxa (Harris, 2008; Summers and Amos, 1997), birds (Lyon and Eadie, 2008; Yom-Tov, 2001), fish (Taborsky, 2008) and insects (Tallamy, 2004; Field, 1992), but has been best studied in birds. Advances in this field have only begun to be applied in insects, and the detailed nature of our field data allows us to investigate it at unusually fine scale. In **Chapter 5**, I first introduce a framework inspired by the well-developed sex-allocation literature (Brockmann, 2001), in which ARTs can be distinguished based on when and how tactic-specific phenotypic determination occurs (Engqvist and Taborsky, 2016). Next, I summarise my key findings

and attempt to characterize the ARTs in my study systems within the aforementioned framework. Finally, I comment on future possible directions for research in this area.

1.2 - Study systems

In the following sections I provide a general introduction to my two study systems and the sites at which they were based.

Polistes

Originally separated from *Vespa* as a genus in 1802 by Latreille, the genus *Polistes* now contains over 220 species in the family Vespidae, subfamily Polistinae (Hymenoptera, Vespidae, Polistinae). Most of these are free living eusocial species, but three are obligate social parasites (Arévalo *et al.*, 2004; Carpenter, 1991; Johnson *et al.*, 2013). The genus has a world-wide distribution, the majority of which is based in the tropics, but 10 species are found in Europe including *P. dominula*, *P. nimpha* and *P. gallicus* (Neumeyer *et al.*, 2014).

The genus *Polistes* is a well-known and important model system, used in a broad range of topics including the evolution of eusociality (Ferreira *et al.*, 2013; Hunt *et al.*, 2011; Jandt *et al.*, 2014; Leadbeater *et al.*, 2011, 2010; Rehan and Toth, 2015), invasion biology (Cervo *et al.*, 2000; Gamboa *et al.*, 2002, 2004; Höcherl and Tautz, 2015a; Käfer *et al.*, 2015; Liebert *et al.*, 2006), chemical communication (Cini *et al.*, 2009; Dani *et al.*, 2004; Dapporto *et al.*, 2007; Leadbeater *et al.*, 2014; Sledge *et al.*, 2004; Sledge *et al.*, 2001), visual communication (Cervo *et al.*, 2008; Green *et al.*, 2012, 2013; Izzo and Tibbetts, 2012; Sheehan and Tibbetts, 2009, 2011; Tibbetts and Dale, 2004), individual and nest thermoregulation (Höcherl and Tautz, 2015b; Hozumi *et al.*, 2008; Käfer *et al.*, 2015; Kovac *et al.*, 2017; Weiner *et al.*, 2010, 2011), and the endocrine system (Giray *et al.*, 2005;

Kelstrup *et al.*, 2015; Sasaki *et al.*, 2009; Tibbetts *et al.*, 2011a, 2011b; Tibbetts and Izzo, 2009; Tibbetts and Sheehan, 2012).

The popularity of the genus for research use is largely the combination of two factors. First, the genus exhibits a form of social living that is similar to that of the cooperative breeding vertebrates, and is known as primitive eusociality. Small paper nests are founded by either lone mated females (foundress) or small groups of co-foundresses. Unlike advanced eusocial species, in which workers are sterile, all *Polistes* co-foundresses and workers are able to reproduce. A division of labour and reproduction exists, creating a behaviour based caste system. The dominant foundress lays the majority of the eggs, while the subordinate co-foundresses, and later the workers, forage and care for the developing brood (Reeve, 1991). As such, primitive eusociality might be thought of as an intermediate between a solitary life history and advanced eusociality. Foundresses also do not appear to be physically constrained to adopt a particular nesting strategy (Pardi 1996; Reeve, 1991). A subordinate female can abandon its initial group to found her own nest, or usurp an established group (Cervo and Lorenzi, 1996; Makino and Sayama, 1991). Alternatively, a female might 'sit-and-wait' for another foundress to abandon her nesting attempt, then adopt the nest herself (Liebert *et al.*, 2005; Starks, 1998, 2001). This behavioural flexibility provides a great opportunity to investigate alternative reproductive tactics (Burian, 1996; Choe and Crespi, 1997; Starks and Fefferman, 2006).

The second reason for the popularity of *Polistes*, from a more practical standpoint, is its nesting characteristics. The paper combs are open and relatively small, which allows for observation of behaviour on the nest face, using a combination of direct observations and video recording. Adult wasps can be individually marked and easily identified with the use of distinct colour combinations of enamel paints (Figure 1, a). As well as this, each immature offspring is restricted to a single open cell, enabling accurate monitoring of individual development (Figure 1, b).



Figure 1 - a) Identification of individual *P. dominulus* wasp is achieved with unique combinations of enamel paint marks. **b)** Brood are restricted to open cells and thus their presence and development can be monitored.

Polistes dominula (Christ, 1791)

Polistes dominula is one of the most frequently studied *Polistes* species. It is a temperate species most common in the Mediterranean area of Europe and North Africa, though it is native in eastern Europe and as far east as China (Carpenter, 1996; Cervo *et al.*, 2000). Beyond its native range, *P. dominula* has been accidentally introduced to both South (Willink and Chiappa, 1993) and North America (Hathaway, 1982; Hathaway, 1981), as well as Australia (Richards, 1978). The species has spread particularly quickly in N. America (Borkent and Cannings, 2004; Hesler, 2010; Jacobson, 1986, 1991a, 1991b; Judd and Carpenter, 1996; Menke, 1993; Staines and Smith, 1995), although reasons underpinning this success remain contentious (Höcherl and Tautz, 2015a). Range expansion has also occurred to the north and east in Europe, with both global warming and urbanisation being implicated (reviewed in Höcherl and Tautz, 2015a).

The colony cycle of *P. dominula* is typical of other temperate *Polistes* species, alternating between nesting and non-nesting phases. At the conclusion of the nesting phase, mated female reproductive offspring (gynes) depart their natal nests and form hibernation clusters, consisting of between a few to hundreds of individuals, often behind features such as large nests from the previous year.

These clusters often comprise individuals from multiple natal nests (Dapporto and Palagi, 2006). Winter represents a serious challenge to most poikilotherms, due to the low temperatures and food scarcity (Storey and Storey, 2001). In fact, many gynes die over the hibernation period, likely due to cold (Dapporto and Palagi, 2006). The formation of these hibernation clusters appears to offer some protection from climatic extremes, as well as predation (Storey and Storey, 2001). The hibernation period is typically divided into three stages: pre-hibernation; diapause; and post-hibernation. As the cluster initially forms, during pre-hibernation, some foraging and dominance interactions occur. These dominance interactions appear to accurately reflect the dominance potential of the foundresses involved, in that in 85% of cases the dominance relationship of a given pair in pre-hibernation is the same as in the resulting spring foundress associations (Dapporto *et al.*, 2006). As the temperature drops, gynes move deeper into their shelter where they spend diapause. Then in early spring, as the temperature begins to rise again, wasps exit diapause. As with pre-hibernation, post-hibernation is characterised with foraging and dominance interactions. Post-hibernation concludes with the beginning of nest initiation (Dapporto and Palagi, 2006).

Nests are built (founded) in the spring, by either a solitary female (figure 2a) or small group of co-foundresses (figure 2b), usually less than 10. Most nests then gain a number of additional co-foundresses after the initial founding (joiners). Despite the intense research interest in the *P. dominula* dominance hierarchy (Jandt *et al.*, 2014), much ambiguity remains around the mechanisms of hierarchy establishment in the genus as a whole, and *P. dominula* specifically. Evidence in another temperate *Polistes* wasp, *Polistes carolina*, showed that foundresses that initiate nest founding almost always became the dominants in their nests (Seppä *et al.*, 2002). It is unclear how important this factor may be in the establishment of *P. dominula* dominance hierarchies (Zanette and Field, 2009), but aggression is heightened in co-foundress groups at this early stage (figure 2c), with many extended clashes which sometimes end in falling fights. From this early wrangling emerges a stable dominance hierarchy, with serious fighting replaced by ritualised

dominance behaviours, which are predominantly carried out on the individual below in the hierarchy (Pardi, 1948; Reeve, 1991).



Figure 2 - Nests are founded by **a)** single foundresses and **b)** groups of co-foundresses. Aggression is initially high, and **c)** grappling and even falling fights can be seen. **d)** A subordinate co-foundress foraging for the nest.

An individual's rank in this linear hierarchy is of great importance for a number of reasons. First among these is access to breeding, as reproductive skew is high and the dominant produces almost all of the brood at a given time (Leadbeater *et al.*, 2011). As well as this, co-foundresses move up in rank if any above them in the hierarchy are lost from the group. For example, a rank two individual may gain the dominant position. The direct fitness earned by becoming the dominant in this way, is

enough to explain the presence of unrelated individuals within co-foundresses associations (Leadbeater *et al.*, 2011). Rank in *P. dominula* is characterised not just by a pattern of aggression, or reproductive skew, but also by time spent off the nest. While a dominant female will almost never leave the comb, subordinate co-foundresses spend between 16% and 94% of their time off the nest (figure 2d). The amount of time increases with each drop in rank, and with this, there is a greater risk of predation (Cant and Field, 2001). Thus, rank has major consequences in terms of individual survival and direct fitness.

Not all solitarily founded nests gain a joiner, however, and the existence of these solitary nesting females enabled researchers to investigate why an individual would join a group as a subordinate, and so forego independent reproduction. Numerous studies have since shown that there are many benefits to group living in *P. dominula*, of primary importance being increased fitness (Höcherl and Tautz, 2015a; Leadbeater *et al.*, 2011; Queller *et al.*, 2000b; Shreeves *et al.*, 2003; Tibbetts and Reeve, 2003; Zanette and Field, 2011). As yet unexplored, however, is how the method of nest initiation, solitarily or by a group of foundresses, affects the characteristics and fitness outcomes of nests that subsequently become multi-female.

Nest founding concludes when the first generation of offspring eclose in early summer, and the majority of these offspring will be female. Most will remain on the natal nest as workers, carrying out tasks such as foraging and nest defence (Reeve, 1991). Workers join the bottom of the dominance hierarchy, and a worker will gain the dominant position in a nest only upon the death or disappearance of all the original co-foundresses (Monnin *et al.*, 2009; Reeve, 1991; Tibbetts and Huang, 2010). In mid-summer, the reproductive offspring begin to emerge. The reproductive phase lasts until early - mid autumn, when the colonies begin to collapse. Reproductive males and females do not participate in the activities of their natal nests, and typically mate away from it (Pardi, 1942).

Polistes wasps have a lek based mating system, in which males fight for dominance positions in the lek, and females select a male based on some aspect of the males phenotype. Leks are formed on discrete landmarks such as fence posts, roof tops and the tops of trees, with the most dominant

males, typically larger (Beani, 1996), guarding the highest part of the lekking site. In *P. dominula*, smaller males typically take up transient, non-territorial positions and tend to gain less copulations. There is some flexibility between these reproductive tactics, however, and mating success appears to be more closely related to the accessory gland, rather than body size, where those with larger accessory glands gain more mating success (Beani and Zaccaroni, 2015; Izzo and Tibbetts, 2012). Male mating success is also associated with sexually selected ornamentation, in the form of abdominal spots (Beani and Zaccaroni, 2015; Izzo and Tibbetts, 2012). Females fly through the lekking site or perch and observe males prior to choosing, and during this time transient males also attempt to intercept, grasp and mate with the female (Beani and Zaccaroni, 2015; Izzo and Tibbetts, 2012).

Study populations

The data that I present in the following chapters (Part I: Chapters 2-3) come from studies I carried out with *P. dominula* in south-west Spain. I studied this species at several rural sites near the coastal town of Conil de la Frontera, Cádiz Province, Andalucía (figure 3). The sites are comprised primarily of arable and pastoral farmland, and support high densities of *P. dominula* that nest mainly on hedges of prickly pear cactus (*Opuntia* sp.). Four field sites were used over three field seasons (2014 – 2016): in 2014, sites 1 (BF) and 2 (DCF) were used; in 2015, sites 2 (DCF), 3 (SDF) and 4 (SF) were used; and in 2016, sites 2 (DCF) and 4 (SF) were used (figure 4).



Figure 3 - Map showing the location of Conil de la Frontera, in Cádiz Province, Andalucía. Three field seasons were carried out in the local vicinity with *P. dominula*, between 2014 and 2016.



Figure 4 - Map showing the locations of the four *P. dominula* field sites used between 2014 and 2016. In 2014, sites 1 (BF) and 2 (DCF) were used; in 2015, sites 2 (DCF), 3 (SDF) and 4 (SF) were used; and in 2016, sites 2 (DCF) and 4 (SF) were used.

Ammophila

The genus *Ammophila* comprises over 200 species (Pulawski, 2009) in the family Sphecidae (Hanson and Menke, 2006; Melo, 1999), tribe Ammophilini (Hymenoptera, Apoidea, Sphecidae).

Ammophila are part of a group commonly known as digger wasps and are found across most continents (Bohart and Menke, 1976).

Compared with *Polistes*, *Ammophila* is not such a widely used study system. Thus, compared with *Polistes* there is limited information available about the genus. However, this system has provided an insight into parental investment and social evolution (Baerends, 1941; Field, 2005; Field *et al.*, 2007; Field and Brace, 2004; Strohm and Liebig, 2008), as well as intraspecific parasitism and alternative reproductive tactics (Field, 1989a, 1989b, 1992; Kurczewski and Spofford, 1998; Rosenheim, 1987; Weaving, 1989). They are most well-known, perhaps, for their use in insect navigation (Baerends, 1941; Collett *et al.*, 2013), along with other digger wasp species in the family Sphecidae (Tengö *et al.*, 1990; Tinbergen, N. and Kruyt, 1938).

Ammophila pubescens

A. pubescens Curtis (Sphecidae) is a solitary nesting subsocial digger wasp that within the UK is restricted to the southern counties in England (Edwards, 1997). The species produces a series of L-shaped nests or burrows, typically fairly close to each other, in open sand within heather-dominated heathland (figure 5). Each nest contains a single offspring, which the mother provisions with prey in the form of paralysed lepidopteran caterpillars. These prey are typically carried to the nest in flight, though relatively large items may be dragged, potentially limiting the distance they can be transported (Evans, 1962). *A. pubescens* is a progressive provisioner, which is uncommon among non-social insects (Baerends, 1941; Field, 2005; Field and Brace, 2004). Food is provided gradually as the offspring develops, as opposed to being provided in a single mass. As such, the owner makes repeated visits to the nest during development of her offspring; therefore, if the nest has

been parasitized by a conspecific, the mother of the original offspring will come across the brood of that foreign wasp.

Intraspecific parasitism has been observed in several species of *Ammophila*, including *A. pubescens* and the closely related *Ammophila sabulosa*; the behaviour is described in a review by Field (1992). Upon opening a conspecific's nest, a foreign wasp (parasite) will remove all the prey items and eat or discard the owner's (host) egg. This often involves considerable time spent walking around with the prey, as well as stinging and biting it. The parasite will then either replace the original prey, or return with fresh prey, laying an egg on the first item.

A typical nesting sequence in our study population proceeds as follows (Field *et al.*, 2007): on Day 1, the owner digs an L-shaped burrow. After this point, whenever the mother leaves the nest, she temporarily closes the entrance. Later that day, or on Day 2, she places a single prey item in the burrow and glues one of her eggs onto it. Provisioning then halts for between 2-8 days, during which time the egg hatches and the larva begins to feed on the prey. Toward the end of this time the mother carries out an assessment visit, in which she re-enters the burrow and appraises the state of her larva (Baerends, 1941). After this visit, the owner begins the 'main provisioning bout', over which time (1 – 7 days) she will finish provisioning with additional prey items and permanently close the entrance. Unusually, an *A. pubescens* female can have multiple nests in this active stage (i.e. between digging and permanently closing) simultaneously.

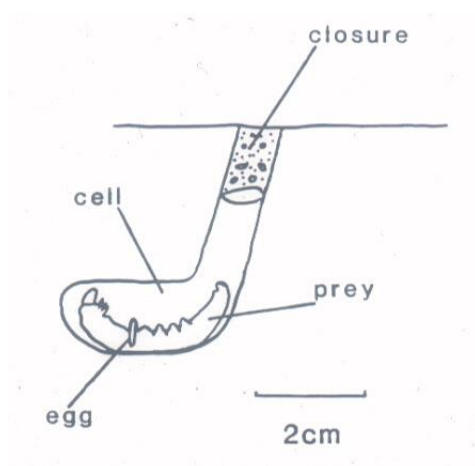


Figure 5 – Diagram of *A. pubescens* L-shaped burrow containing an egg attached to the first prey item. An adult will dig a series of these in her life time, each containing a single offspring which the mother provisions with insect prey (paralysed lepidopteran caterpillars). Diagram from Field and Brace (2004).

Study populations

In chapter 4 we analyse data collected by Jeremy Field, William Foster and Chris Accleton. Data were collected over two years (2012 – 13) at Witley Common, Surrey (51°09'06.4"N 0°40'53.9"W). This is a Site of Special Scientific Interest (SSSI) comprised of a large area of heath as well as woodland, in south-east UK (figure 6). We searched the site at the beginning of the 2012 field season (beginning of July) for dense aggregations of nesting *A. pubescens*. We selected an aggregation that was making use of an area of heath cut back to produce open soil for sand lizards to bask in (figure 7). During the two field seasons we observed an area of roughly 20m², this site was used for both years of the study.



Figure 6 – Location of the *A. pubescens* aggregation within Witley common, south-east UK.

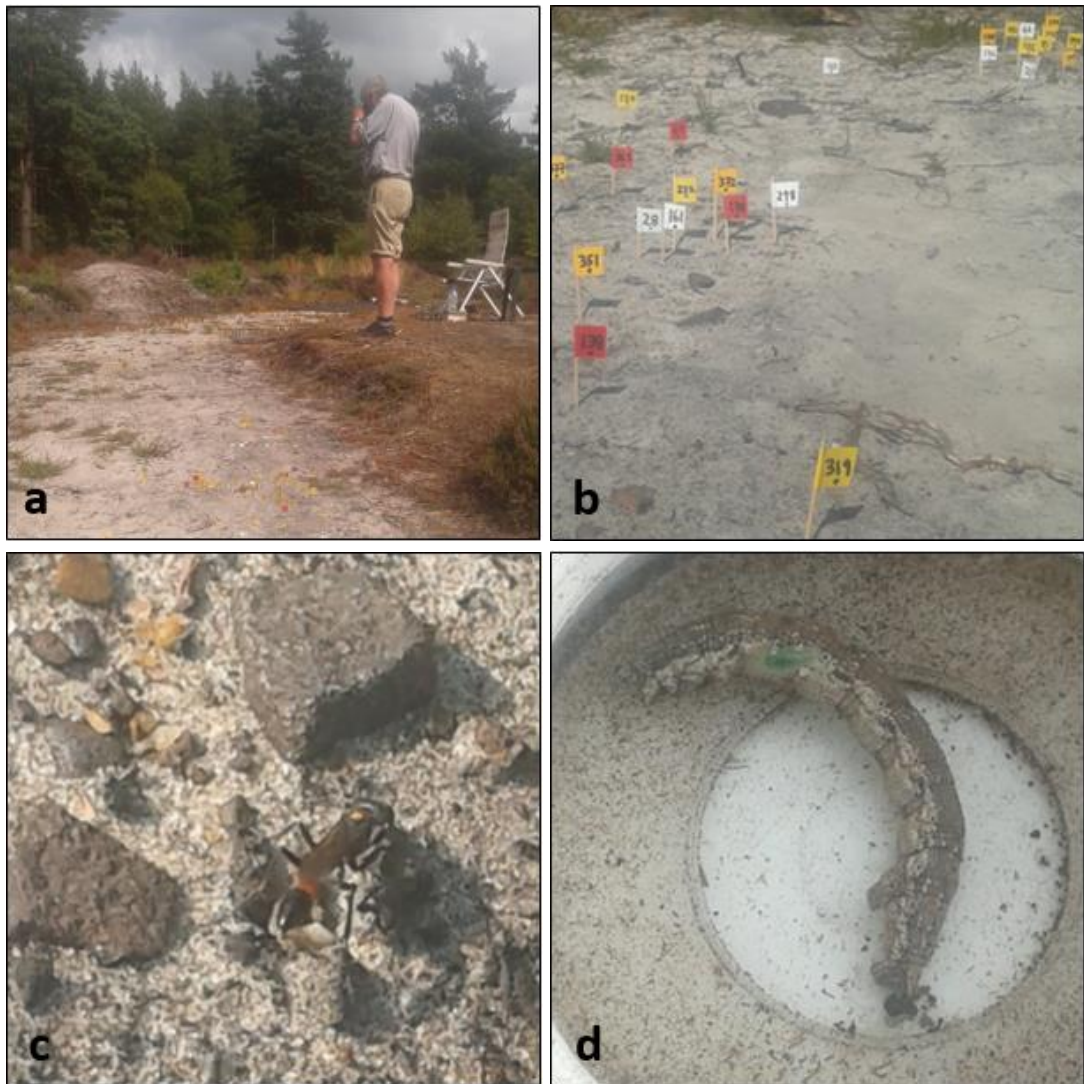


Figure 7 – a) William Foster observing activity at the aggregation; **b)** flags marking the location of each nest; **c)** an *A. pubescens* adult starting to dig a new nest; and **d)** a prey item with attached larva ejected by a parasite.

Part I: *Polistes dominula*

Chapter 2: What determines dominance in the paper wasp *Polistes dominula*?

2.1 - Introduction

First described in chickens (Schjelderup-Ebbe, 1922), dominance hierarchies are a common feature of animals living in social groups, and they share numerous similarities across social taxa (e.g. humans: (Boehm, 2009); primates: (Watts, 1998); mongooses: (Waser *et al.*, 1994); insects: (Hölldobler and Wilson, 1990; Reeve, 1991). The hierarchy represents an asymmetry in access to resources, reproduction and in the division of labour. The highest ranked individual, or dominant, typically produces the majority of offspring in the group and so maximises its direct fitness gains. Aggression in the group is normally directed from high-ranking individuals towards subordinates, except when a subordinate challenges for dominance. A dominant may also benefit from the aid of subordinates in several ways, including babysitting or brood care (Solomon and French, 1997), foraging (Cockburn, 1998b), as well as territory maintenance and defence (Grüter *et al.*, 2012; Taborsky, 1994).

Individuals in cooperatively breeding vertebrates and primitively eusocial insects (see Chapter 1: General Introduction), such as the polistine wasps, are all capable of reproducing and could become the dominant, yet only some do (Reeve 1991). Considering the benefits of high rank, considerable competition is expected over acquiring the dominant position. The benefits associated with group living, however, rely on stability in the hierarchy. Thus, the question of how dominance hierarchies

are established is clearly important. Firstly, with respect to understanding which individual in a group is likely to gain this valuable position. If we are able to predict the dominance status of an individual, we can ask how future rank affects current behaviour. Secondly, by studying the mechanisms of hierarchy establishment, we can predict the stability of the resulting hierarchy.

Hierarchies in the paper wasp *Polistes dominula*

In 1942, Pardi described the first linear dominance hierarchy in *Polistes dominula* (then *Polistes gallicus*, subsequently *Polistes dominulus*). In a linear hierarchy, each group member occupies a single rank and is always subordinate to individuals ranked above it and dominant to those ranked below. There are now numerous examples of linear hierarchies, with *Polistes* paper wasps remaining a key model genus. *P. dominula* is one of the best studied species in the genus, and the following summary of its life history is based on Reeve (1991). Nests are founded by a single mated female (foundress), or co-founded by a small group (co-foundresses) that have overwintered in sheltered hibernation clusters. During the nest founding stage (see Chapter 1: General Introduction), in which nest building begins, escalated conflict between co-foundresses is common, including grappling and falling fights. After initial construction of the nest has begun, most nests gain a number of additional foundresses (joiners). Some join almost immediately after the first cells are built, but joining continues to a lesser extent even after the nest founding stage has finished. As the founding stage ends, the heightened aggression is replaced with ritualised dominant – subordinate interactions. The dominants will go on to produce the majority of eggs on the nest, and most dominants retain their position throughout the season (Leadbeater *et al.*, 2011).

P. dominula groups are small (c. 1 to 10 co-foundresses) and it is common for a group to include one or more unrelated subordinates (Leadbeater *et al.*, 2011; Queller *et al.*, 2000a). Although the dominant produces the majority of eggs on the nest, all foundresses are able to reproduce and have the potential to take on the dominant position. Foundresses are also roughly the same age and size,

lacking fixed morphological differences found between castes in advanced eusocial insects (e.g. queens and workers; Reeve, 1991). The chemical profiles of different foundresses are also indistinguishable at the beginning of the nesting season (Dapporto *et al.*, 2007; Sledge *et al.*, 2001). Given the difficulty in determining the rank a female will later occupy, this species makes a fascinating subject to explore the rules by which dominance hierarchies are established. Understanding these rules has the potential to provide insight into the processes underlying early stages of eusocial evolution.

Rules of establishing dominance

The formation of a dominance hierarchy is effectively the process of dividing up the shared costs and benefits of group living. As with other contests over valuable resources, theory suggests several ways by which a hierarchy might be established, and the dispute settled (Cant *et al.*, 2006). The most obvious mechanism would be direct conflict, an example of which is the rut in the Red Deer stags. This is a mainly solitary species in which males compete over access to mates. Fighting typically occurs between males of a similar size, with dominance displays otherwise being sufficient (Clutton-Brock *et al.*, 1979). Escalated fighting can be costly, however, even for the winner. As well as an energetic cost (Briffa and Elwood, 2004; Hack, 1997; Rovero *et al.*, 2000), there can be increased risk of predation (Kelly and Godin, 2001) and serious injuries could be sustained (Siva-Jothy, 1987). Hierarchies based on agonistic ability may also be susceptible to upheaval by those challenging for a higher rank, inflicting further cost on the dominant and possibly affecting the productivity of the social group (Seppä *et al.*, 2002). For example, in the group living savannah baboon dominance rank is correlated with fighting ability, and other males can challenge for the dominant position, such as immigrants and younger individuals coming into their prime. Escalated conflicts during the nest founding stage are well documented in many polistine species, (Gamboa and Dropkin, 1979; *Ropalidia marginata* - Premnath *et al.*, 1996; *Polistes fuscatus* - Gamboa and

Stump, 1996; and *Belonogaster juncea juncea* - Tindo and Dejean, 2000), including *P. dominula* (Pardi, 1942). As yet, however, there is no direct evidence that this early fighting plays a part in the creation of the dominance hierarchy in *P. dominula*, or other polistine species.

Given the drawbacks of organising a hierarchy based on an individual's fighting ability (resource holding potential: RHP), it may be that all individuals involved do better under a conventional settlement. Such settlements may help to drastically reduce the costs of conflict, and may even increase the resilience of the hierarchy to potential challengers. Conventions may be based on a correlate of who is likely to win, or on some seemingly arbitrary variation between individuals (Seppä *et al.*, 2002).

Body size

To minimise the costs associated with conflict, animals should take into account (Smith *et al.*, 1988) the value of resources (Arnott and Elwood, 2008), and accurately assess the agonistic abilities of their would-be competitors (Searcy and Nowicki, 2005; Smith and Harper, 2003). Not all participants, however, are able to do this (Arnott and Elwood, 2008). In the case of animals that live in social groups largely comprised of relatives, such as *P. dominula*, selection should also favour limiting costs to the group, because of the effect on indirect fitness. The overall fighting ability of an animal often correlates with body size or mass (Arnott and Elwood, 2009) and so body size is commonly used as a proxy for RHP. Smaller combatants do sometimes win, however, especially when there is only a small size difference (Arnott and Elwood, 2009). The frequent correlation between fighting ability and body size might make body size a useful signal for this purpose, and is likely to result from the relationship between body size and muscle mass. Evidence in the polistine wasps broadly supports this (Cini *et al.*, 2011; Green and Field, 2011; Ortolani and Cervo, 2010; Tibbetts and Shorter, 2009). Evidence for a relationship between body size and hierarchical rank in these wasps, however, varies within and between species (Jandt *et al.*, 2014). In a Spanish

population of *P. dominula*, previous studies have found no significant correlation between body size and hierarchical rank (Zanette and Field, 2009). In Italy, however, the opposite appears to be the case (Cervo *et al.*, 2008; Pardi, 1948). Given the small mean effect (0.11mm), however, and the choice of statistical test (multiple subordinates from the same nest are not independent samples), the biological importance of this result may be questionable (Cervo *et al.*, 2008). Like *P. dominula*, studies of *Polistes annularis* and *Polistes bellicosus* have both produced conflicting results (Jandt *et al.*, 2014). In the case of *Polistes instabilis* and *Polistes carolina*, however, no evidence of a size relationship has been found (Jandt *et al.*, 2014; Seppä *et al.*, 2002).

In this chapter, we will test in *P. dominula* whether 1) rank correlates with within-group relative body size; 2) the ranks gained by the solitary initiating female in each nest, correlates with their within-group relative body size; and 3) there is a difference in absolute body size between solitary initiating females and joining foundresses.

Status badge

Another way in which competitors may gain information about an opponent's RHP is to make use of conventional signals. Also known as status signals or status badges in *Polistes*, they are small patches of colour that provide information about competitive ability without being causally linked to it. They are also relatively low in cost to produce (Smith and Harper, 2003), and as such have aroused controversy as to how signal reliability could be maintained (Senar, 1999; Whiting *et al.*, 2003). Early support for the importance of status signals comes mainly from birds (Qvarnström, 1997; Senar and Camerino, 1998) and lizards (Whiting *et al.*, 2003). More recently, considerable research on this topic has been carried out in the polistine wasps, though again, the results have been somewhat mixed (Jandt *et al.*, 2014). Studies on invading populations of *P. dominula* in the U.S. have shown that the 'brokenness' (a measure of spatial disruption [Tibbetts, 2010] of the melanin pattern on the facial clypeus; figure 1) is a signal of status in dominance and competitive

interactions (Tibbetts and Dale, 2004; Tibbetts and Lindsay, 2008; Tibbetts, 2010). Moreover, it has been shown to be an honest signal of RHP, with reliability being maintained through social costs (Tibbetts and Dale, 2004). Within the native European range of *P. dominula*, however, there is only limited evidence for a relationship between clypeal markings and dominance (Zanette and Field, 2009). In their study on an Italian population of *P. dominula*, Cervo et al. (2008) found no evidence of a relationship between the status badge and i) body size, ii) probability of surviving winter, iii) social rank in spring foundress associations, and iv) health status. Moreover, many individuals in the native range have a completely yellow clypeus (60% - Cervo et al., 2008), and many groups lack individuals with badges altogether (Zanette and Field, 2009). The geographic variation in the use of status badges in *P. dominula* may be the result of variation in its expression. The temperature experienced during pupal development affects the expression of the badge trait, with pupae housed at 20°C (e.g. N. America) emerging with a higher brokenness index than those at 30°C (e.g. Spain - Green et al., 2012).

In this chapter, we will test in *P. dominula* whether 4) presence / absence of a clypeal mark on the solitary initiating female in each nest, affects the likelihood of them gaining the dominant position; 5) solitary initiating females and joining foundresses differ in the proportion with / without clypeal marks.

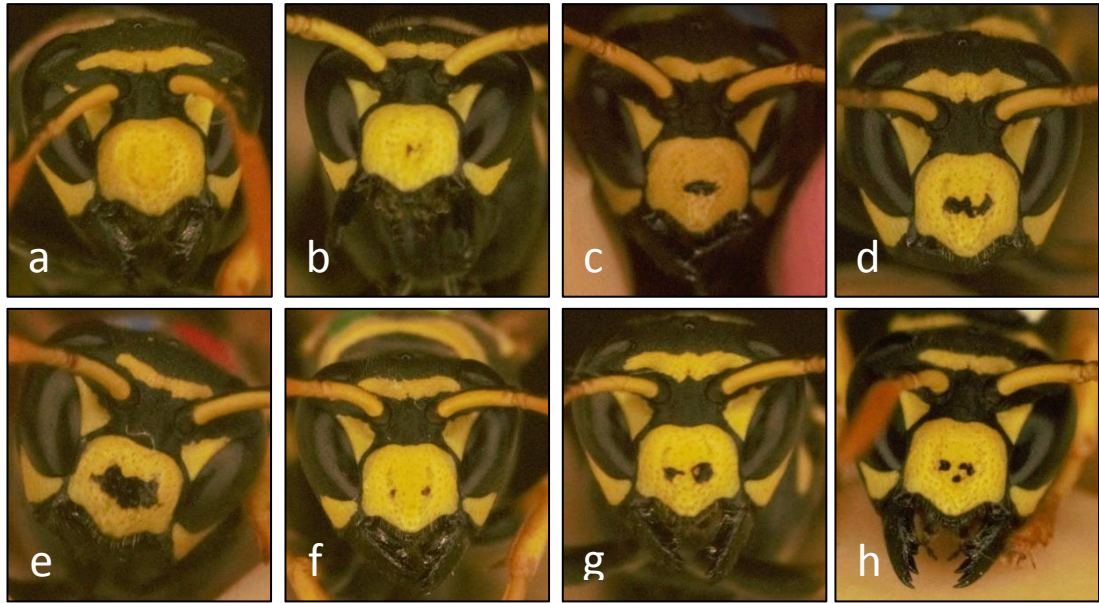


Figure 1 – Portraits of 8 *P. dominula* foundresses collected in southern Spain in the 2016 field season, and arranged in order of increasing brokenness of the clypeal mark (a – h). Brokenness increases with number of spots (i.e. 0 spots: a; 1 spot: b-e; 2 spots: f, g; 3 spots: h), as well as size of spot (e.g. e > b; and g > f), and finally, with pattern disruption (e.g. d > c, despite both having a single similar size spot).

Order of arrival

Order of arrival is a cue that could be considered stochastic in nature. In other words, the cue may single out an individual as the dominant, without reflecting on its quality (Bridge and Field, 2007). In this scenario, each new joiner would peacefully take the lowest rank, rather than entering into conflict over rank with the current members. A hierarchy based on this cue would likely drastically reduce the costs of conflict, as well as increase the stability of the hierarchy. In such a system, which wasps hold the dominant position might be expected to be random with respect to their RHP (Seppä *et al.*, 2002). There is some evidence for order-of arrival as a determinant of rank in the polistine wasps. Seppä and colleagues (2002) found that 11 of 12 first-arriving *P. carolina* foundresses became the dominant on their nest, a striking result. In contrast, Zanette and Field (2009) found that, overall, there was no significant effect of order-of-arrival on rank in a Spanish population of *P. dominula*. When considering ranks 1 and 2 in isolation, however, the correlation was

significant. Whether an individual is the first-arriver (begins nest building/founding by herself), or a joiner (joins the group after nest building/founding has already begun) could be key to their finding. Zanette and Field (2009) may not have fully captured this because their nests were already fairly well-developed when first discovered (1–8 foundresses [mean \pm s.e. = 2.5 ± 1.6] and 1–18 cells [mean \pm s.e. = 5.7 ± 5.2]).

There is also very clear evidence that age is another seemingly arbitrary cue in primitively eusocial insects. Age has been found to determine rank in both the ant *Pachycondyla sublaevis* (Higashi *et al.*, 1994), and the hover wasp *Liostenogaster flavolineata* (Bridge and Field, 2007). *L. flavolineata* lives in the South-East Asian tropics on perennial nests, in groups of typically between 1 and 5 individuals. A single female, or dominant, lays most of the eggs in the group. When fully developed brood reach adulthood, they join the group, and can begin foraging as quickly as 4 days after eclosing (Field *et al.*, 1999). Bridge and Field (2007) showed that groups adhere to a strict gerontocratic (age-based) inheritance queue, with the oldest female in the group becoming the dominant 87% of the time. Thus, as new adults emerge, they join the end of the cue. A parallel can be seen here with order-of-arrival, in that newly emerging *L. flavolineata* adults could be considered to be ‘arriving’. As a temperate species, *P. dominula* is unlikely to be applying the same rule, as all foundresses are of a similar age. Furthermore, the variation in age that does exist between *P. dominula* foundresses will be less apparent than in the case of *L. flavolineata*, where current group members will often witness adult maturation. Existence of a possible relationship between emergence date the previous year, and hierarchical rank the following spring, remains to be tested.

In this chapter, we will test in *P. dominula* whether 6) the solitary initiating female in each nest (first-arrivers) gain the dominant position more often than expected by chance. As well as, 7) which of the following factors best explain whether or not an individual becomes the dominant: solitary initiating female (first-arriver; Y / N), clypeal mark (Y / N), relative body size, year, or group size?

How arbitrary is order of arrival?

Although the use of direct correlates of RHP (e.g. body size) or conventional signals (e.g. status badges) to determine dominance might remove the costs of some conflict, the hierarchy would still be susceptible to upheaval from new arrivals that exceed and/or closely match the dominants in the relevant trait. This contrasts with the use of order of arrival, where the hierarchy would not be susceptible in the same way. However, it may be the case that order of arrival, or arriving first, is in some way correlated with fitness. In this scenario, order of arrival could be based on RHP, rather than being an arbitrary cue. Comparing the traits of first-arrivers with those of subsequent joiners can help us to understand factors that influence hierarchy formation.

In *Polistes*, one determinant of order of arrival is likely to be the timing of when an individual exits diapause, whereby wasps exiting diapause earlier may arrive first at a nest site. Tibbetts et al. (Tibbetts *et al.*, 2011a) found, though only in a subset of their data, a significant relationship between body weight and the initiation of post-diapause activity, such that heavier wasps became active at lower temperatures. They also found that wasps apparently advertising higher quality (through facial patterns) became active at lower temperatures. They suggest that this relationship may be one mechanism that maintains the reliability of how facial patterns advertise information about an individual's agonistic ability. Furthermore, that it might help to explain the geographic variation in reliability of the facial pattern signal. This relationship remains to be investigated in the wild.

Finally, in this chapter, we will test in *P. dominula* 8) which of the following factors best explain the founding date of a solitary initiating female (first-arriver): clypeal mark (Y / N), or absolute body size; and 9) whether the founding date of solitary initiating females (first-arriver) correlates with rank?

In this chapter we explore the rules of establishing dominance hierarchies in the extensively studied Spanish population of *P. dominula*. Specifically, we aim to fill in gaps in our current understanding of this system by asking: 1) Does rank correlate with within-group relative body size; 2) Do the ranks gained by the solitary initiating female in each nest (first-arriver) correlate with their within-group relative body size; 3) Is there a difference in absolute body size between solitary initiating females (first-arrivers) and joining foundresses (joiners); 4) Does the presence / absence of a clypeal mark on the solitary initiating female in each nest (first-arriver) affect the likelihood of them gaining the dominant position; 5) Do solitary initiating females (first-arrivers) and joining foundresses (joiners) differ in the proportion with / without clypeal marks; 6) Does the solitary initiating female in each nest (first-arriver) gain the dominant position more often than expected by chance; and 7) Which of the following factors best explain whether or not an individual becomes the dominant: solitary initiating female (first-arriver; Y / N), clypeal mark (Y / N), relative body size, year, or group size? Finally, we also explore a potential non-stochastic mechanism underlying order of arrival by asking: 8) Do the following factors correlate with the founding date of a solitary initiating female (first-arriver): clypeal mark (Y / N), or absolute body size; and 9) Does the founding date of solitary initiating females (first-arriver) correlate with rank?

2.2 - Methods

Identifying first-arrivers

We first aimed to identify and mark the first foundress to reach the nest site and initiate each nest. Data were collected over three consecutive field seasons: 2014 to 2016. In total, four field sites were used over the three years, though not all sites were used every year (see Chapter 1: General Introduction). Between February and April in 2015 and 2016, sites were searched daily for new nests that had only a single wasp present. In 2014, sites were searched for 2 weeks at the beginning of

March. Nests were found on prickly pear cactus (*Opuntia* sp.) at rural sites surrounding Conil de la Frontera in south-west Spain (36° 16' 41"N, 6° 5' 12"W). *P. dominula* nests have an open comb, lacking a nest envelope. This structure allows observations to be made from a distance, minimising disturbance. Nest searches were carried out in the early morning and late afternoon/evening when the wasps are not active due to the low temperature. All group members typically return to their nest by the end of the active period. Thus, a new nest (8 cells or less) with only a single wasp could be considered singly founded, and the wasp the 'first-arriver'. Each site was visited at least once per day, except in inclement weather, when wasps would not have been active. Then, using one of two specially constructed painting tools (figure 2a), the first-arriver was given a unique enamel paint mark while still on the nest (figure 2b). This was done by applying a small blob of enamel paint to the end of the painting tool (pin head), and then aiming to touch the thorax with the pin head. Most wasps remained on their nest after marking. Marking was carried out without removing the first-arriver from her nest to minimise disturbance, as nest abandonment seems to be common at this early stage. A single colour was used (a new colour was chosen for every c.10 wasps marked at a site to aid unique identification), and only one or two spots were applied. The colour, location and size of these marks was then recorded (in 2016 a photo was also taken; figure 2b).



Figure 2, a - d – a) tool for paint marking first-arrivers on the nest (scale = cm); b) a newly marked first-arriver next to her nest, consisting of a single cell with egg; c) a collected first-arriver, ready for confirmation of identity and application of a four spot paint mark; d) a newly painted four spot paint mark.

Nest monitoring, and morphological data collection

Group size was recorded on new nests for the 3 (2014 – 15) and 10 (2016) consecutive evenings immediately following first discovery, when the wasps were inactive. Presence, and the description, of a paint-marked wasp was recorded if this could be seen without causing a disturbance. If a nest was recorded as unoccupied for 3 consecutive evenings, then the nest was considered abandoned and no longer censused. In the 2016 field season the number of eggs was also recorded if a nest was unoccupied.

After this initial stage of monitoring, nests were checked every 2 to 4 days. On each occasion a cell and wasp count was carried out, and cells were checked for hatched eggs. Once a nest had either at least 20 cells or 1 hatched egg, the group was considered ready to collect for individual paint marking. This arbitrary cut-off point was used because nests with more cells and greater brood development were considered more stable and less likely to abandon the nest if disturbed. Wasps were collected from their nests before dawn, and stored at c. 4 degrees Celsius. A detailed description of the first-arriver's initial paint mark was recorded. All wasps were then given a unique combination of four enamel paint spots on the thorax (figure 2c). Wing length was measured using callipers (RS Pro digital calliper), which were accurate to 0.01 mm. Due to limitations of human accuracy, however, wing length was recorded to nearest 0.1 mm. Our measurement of wing length was used as a proximate measure of body size, as it is known to correlate with overall body size (Sullivan and Strassmann, 1984). The presence / absence of clypeal mark was also noted. In the 2016 field season, photos were taken of a first-arriver's initial paint mark (figure 2d), as well portraits of all wasps (figure 1). After marking and taking morphological measurements, wasps were again stored at c. 4 degrees Celsius. Wasps were then released close to their nests before 11am of the same morning as collection, when they would normally begin to be active. After marking, nests were censused for brood (number of eggs, larvae [small, medium and large] and pupae) and cell number every 1 to 2 weeks (see Chapter 1: General Introduction). When possible, the paint ID of each wasp on a nest was also recorded on the same day as each brood census, which was carried out in the evening when the wasps were inactive.

Determining hierarchical rank

The amount of time *P. dominula* co-foundresses spend off the nest, presumably foraging, correlates strongly with hierarchical rank (Cant and Field, 2001). Typically, rank 1 spends >70% on the nest, while lower ranks spend progressively more time off the nest. Thus, rank can be determined

relatively easily by recording the proportion of censuses in the active period (11.00 to 16.00, on sunny days) that individuals are off the nest. During April and May, before worker emergence, nests were censused in the active period and paint marks recorded. For each group, a minimum period of 30 minutes separated each census.

Nests were censused between 7 and 32 times, over 2 to 6 days, until all ranks were clearly differentiated, seemed unlikely to be, or the nest had failed. We aimed to separate all ranks by a minimum of 3 census visits; however, in many cases this was not possible despite making numerous visits. Thus, in this chapter, ranks are considered resolved if they differ by a minimum of 1 census visit. This means that for 2 individuals to be erroneously reversed in rank, a deviation of at least 2 census visits from the expected must occur. Ranks differed by between 0 and 14 census visits (mean \pm s.e. = 3.38 ± 0.34). In cases where ranks could not be resolved, they were given an intermediate .5 value (e.g. joint ranks 1 and 2 = 1.5).

2.3 - Statistical analysis

All statistical analyses were carried out using R Studio version 1.0.136 (<https://www.rstudio.com>) and R version 3.2.3 (<http://www.r-project.org>).

Multiple comparisons

When setting a significance threshold (or alpha) of 0.05, for example, it is accepted that there is 5% chance the observed result may have come about even though the null hypothesis is true, otherwise known as a false positive result. Conducting multiple statistical tests, or comparisons, on a dataset can lead to an increase in false positive results (Forstmeier *et al.*, 2016). I take this into account in this chapter by moderating my conclusions on analyses where P values come just within the acceptance threshold.

Correlates of dominance

Permutation method

Several analyses in this chapter share the same general permutation method. Thus, I will describe the first of these in detail, and highlight only the differences between methods used for subsequent analyses.

Body size

The aim of this analysis was to assess the potential importance of body size (relative to the largest group member) in determining the ranks gained by all group members (aim 1). Ranks of individuals within the same group, however, cannot be considered independent of each other. So, following the same general approach as Zanette and Field (2009), to assess the relationship between relative body size and rank across all group members, the observed mean within-nest correlation (τ) was compared with that obtained using simulated groups, in which rank was randomized (Pinheiro *et al.*, 2017). Permutations were carried out by 1) calculating the observed mean within-nest correlation (τ), 2) rank was then randomly permuted within groups and 3) the new mean within-nest correlation (τ) was calculated. Stages 2) and 3) were then repeated 10 000 times to produce a null distribution of means. A two-sided P value was then estimated based on the proportion of simulated (absolute) values, equal to or greater than the observed (absolute) value. Correlations were considered significant if $P \leq 0.05$.

The aim of this second analysis was to assess the potential importance of body size (relative to the largest group member) in determining the ranks gained by first-arrivers (aim 2). To do this we compared the observed correlation (τ) between first-arriver rank and within-nest relative body size, with those attained by randomly permuting rank. Thus, the dataset consisted of one sample per nest (the first-arriver), each with rank and relative body size within the group.

In this next analysis, we aimed to ascertain whether a difference exists in absolute body size between first-arrivers and joiners (aim 3). To that end, we compared the observed difference in body size of first-arrivers and joiners with those attained by randomly permuting foundress type (i.e. first-arriver vs joiner) - a positive difference indicating that first-arrivers are larger, and vice versa. All joiners from each nest were included in this analysis.

Status badge

In this analysis we aimed to determine whether presence / absence of a clypeal mark on a first-arriver affected the likelihood of them gaining the dominant position (aim 4). In order to achieve this, we summed the number of rank 1 positions gained by those with a clypeal mark, subtracted this from the summed number of rank 1 positions gained by those without a clypeal mark, and compared this value against simulated values in which presence / absence of a clypeal mark was randomly permuted among first-arrivers. Once again, the same permutation test approach was employed to ascertain whether first-arrivers and joiners differed in the proportion that had clypeal marks (aim 5).

Order of arrival

Following Seppa et al (2002), the question of whether first-arrivers gained the dominant rank 1 position more often than expected by chance was analysed using a G-test with William's correction (aim 6). To take into account the effect of group size, the expected value was calculated as $1/\text{average group size}$: if dominance is determined randomly with respect to order of arrival, the first-arrivers chance of becoming rank1 decreases in larger groups.

Dominance and predictors

To account for possible correlations between the 3 main potential determinants of rank investigated, we took a slightly different approach to that of Zanette and Field (2009). We examined their combined effect on whether an individual became the dominant (binary variable) using a Generalized Linear Model (GLM), with a binomial error distribution (aim 7). Group members, however, cannot be considered independent samples. Therefore, a random individual was selected from each co-foundress association (Pinheiro *et al.*, 2017) and the subset used in the analysis. Dominant (Y / N) was the response variable. The potential explanatory variables were: first-arriver (Y / N); clypeal mark (Y / N); and relative body size. Year and group size were also considered as potential co-variables.

Parameter estimates and model selection can be adversely effected by collinearity between predictor variables (Freckleton, 2011). Thus, potential collinearity was assessed by calculating variance inflation factors (VIF) for each variable (Frank, 2014). The value calculated for each variable represents the extent to which the variance of its regression coefficient is inflated due to correlation with the other variables. Variables with VIFs exceeding 5 or 10 are thought to suggest excessive collinearity (Graham, 2003; Montgomery *et al.*, 2015). VIFs were very low (first-arriver (Y / N) = 1.08, clypeal mark (Y / N) = 1.19, relative body size = 1.29, year = 1.57, and group size = 1.34), so all explanatory variables were initially included. Explanatory terms were considered significant if $P \leq 0.05$.

How arbitrary is order-of-arrival?

Did larger first-arrivers nest earlier in the season?

To investigate the relationship between first-arriver founding date and absolute body size (aim 8), a Generalized Linear Model (GLM) was carried out, with quasi-poisson error distribution to account for

over-dispersion. The response variable was first-arriver founding date, and the potential explanatory variables were absolute body size and clypeal mark (Y / N). Year and mean temperature were also considered as potential co-variates. Explanatory terms were considered significant if $P \leq 0.05$.

Founding date and dominance

Finally, the permutation test approach introduced above for testing the relationship between body size and rank was employed to assess the relationship between the founding date of first-arrivers and rank (aim 9). Thus, the observed correlation (Kendall's tau τ) between hierarchical rank and founding date was compared against simulated groups in which the rank was randomly permuted among group members.

2.4 - Results

Rules of establishing dominance

Hierarchical rank was established for 39 complete foundress associations that were each started by a solitary wasp, and that each subsequently gained at least one joiner (3 nests in 2014, 14 nests in 2015, 22 nests in 2016). These nests had a mean cell count when they were first discovered ranging from 1 to 8 (mean \pm s.e. = 3.67 ± 0.273). At the point when hierarchical rank was established (i.e. when the group composition had stabilised) the 39 nests comprised 150 foundresses with a group size ranging from 2 to 9 (mean \pm s.e. = 3.85 ± 0.317). Founding date, the mean ambient temperature that day, as well as morphological data (presence/absence of clypeal mark and body size) was collected for 78 first-arrivers over 2 years (39 in 2015 and 39 in 2016). Roughly half of nests initiated by a first-arriver failed prior to us establishing hierarchical rank.

Body size

There is some tendency for relatively large individuals in a group to have a higher rank (aim 1). The mean observed correlation between rank and relative body size was not significantly different to that obtained by randomly permuting rank within groups (mean $\tau = -0.203$, $n = 148$, $P = 0.072$; figure 3a).

First-arrivers that were large relative to their joiners had a higher rank (aim 2). The mean observed correlation between first-arriver rank and relative body size (within group) was significantly larger than that obtained by randomly permuting rank between first-arrivers ($\tau = -0.3$, $n = 39$, $P = 0.027$; figure 3b).

First-arrivers were significantly larger than joiners (aim 3). The mean observed difference in absolute body size between first-arrivers (mean = 11.69 mm) and joiners (mean = 11.42 mm) was 0.27 mm. There was a significant difference between the observed difference in size (mean = 0.27 mm) and the value obtained by randomly permuting foundress category (mean = 0.028 mm, $n = 148$; $P = 0.022$; figure 3c).

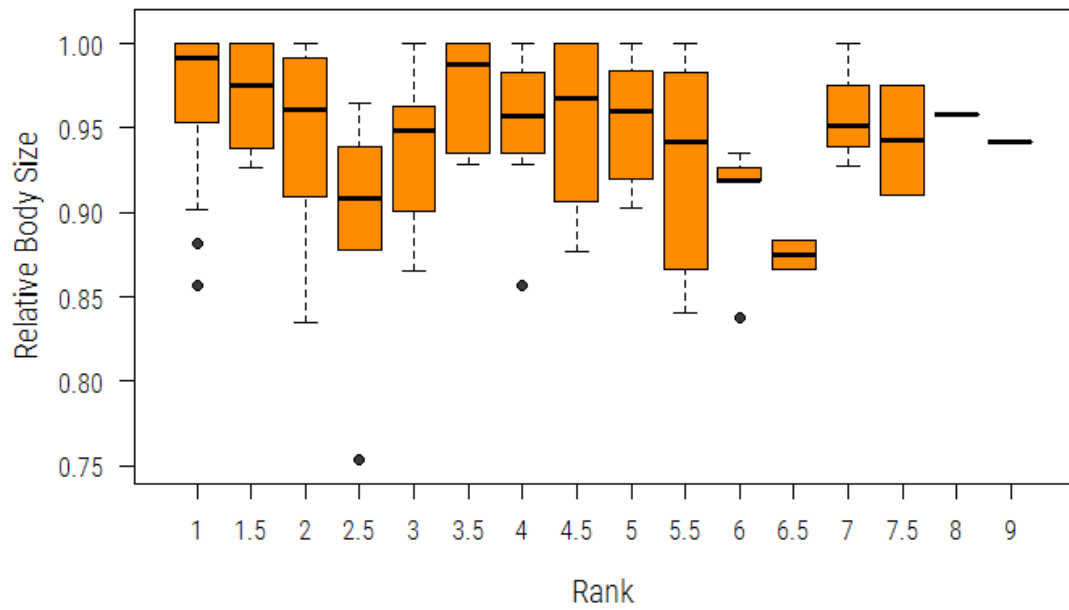


Figure 3a – Boxplot showing the median relative body size of each rank, with rank 1 being the dominant position. Body size is relativized within group to the largest member, i.e. wasp size / largest wasp size. Rank values ending in .5 are joint ranks.

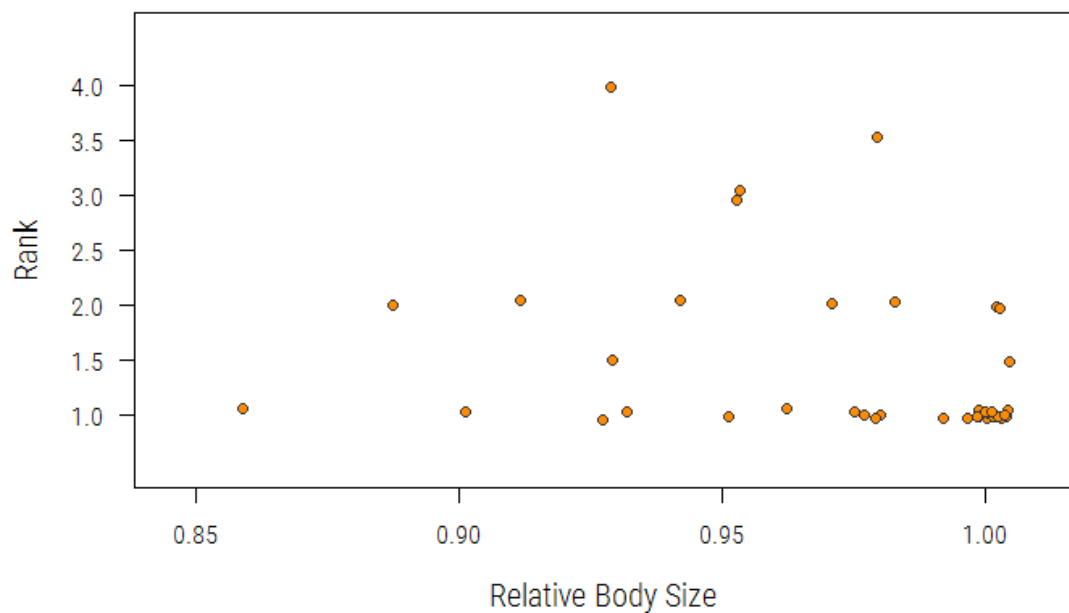


Figure 3b – Relative body size of first-arrivers and the rank they attained on the nest, with rank 1 being the dominant position. Body size is relativized within each group to the largest member, i.e. wasp size / largest wasp size. Rank values ending in .5 are joint ranks.

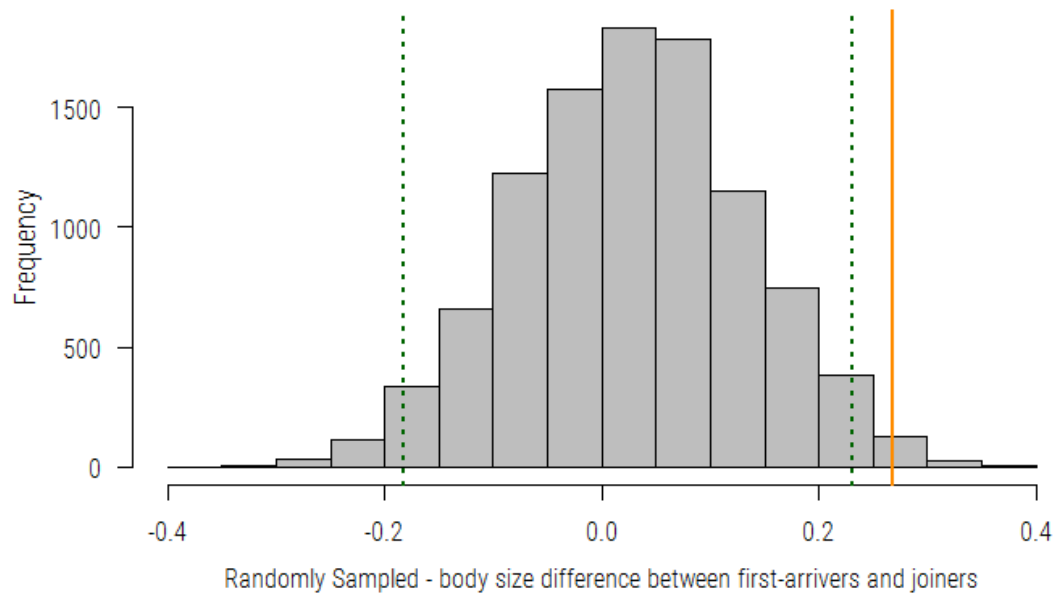


Figure 3c – Frequency distribution of mean absolute body size difference between first-arrivers and joiners.

The orange line shows the mean observed value. The dark green dotted lines show the upper and lower limits of the two-tailed permutation test. The test is significant when the orange line is above the upper limit, or below the lower limit.

Status badge

First-arrivers with clypeal marks are not more likely to be the dominant than first-arrivers without clypeal marks (aim 4). In our study, 81% (13/16) of first-arrivers with clypeal marks gained the dominant position, compared to 57% (13/23) without clypeal marks. This was not a significant difference $n = 39$, $P = 0.16$; figure 4a and 4b).

First-arrivers (16/39; 41%) and joiners (32/111; 29%) do not differ in the proportion that had a clypeal mark (aim 5). The mean observed difference in the proportion with clypeal marks did not differ significantly from those values attained by randomly permuting presence / absence of clypeal marks within groups ($n = 150$, $P = 0.24$; figure 4c)

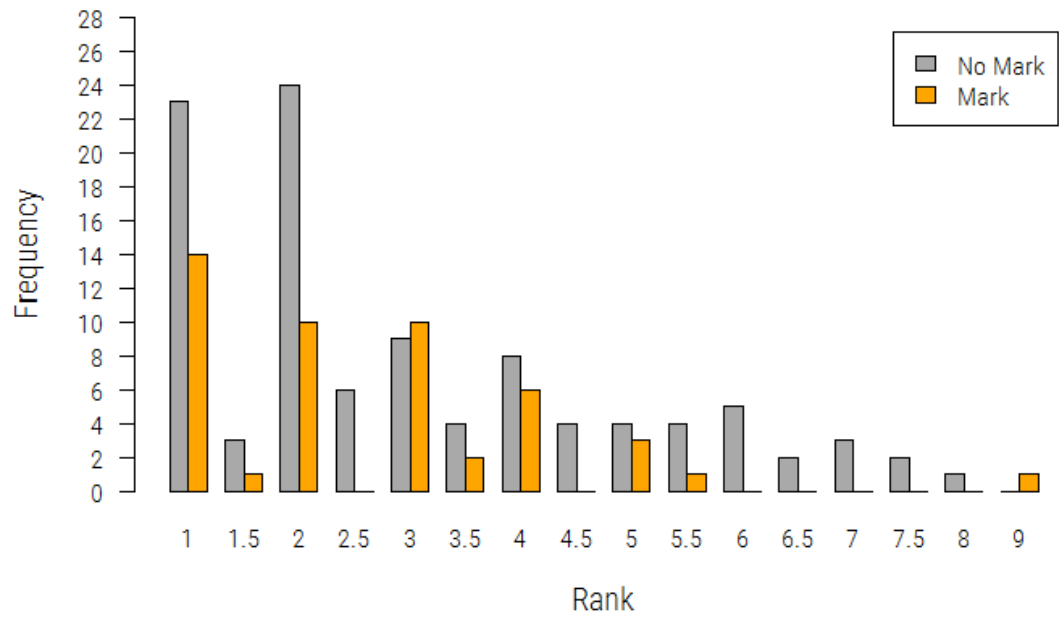


Figure 4a – Frequency distribution of ranks gained by foundresses with clypeal marks (orange) and without (grey). Rank values ending in .5 are joint ranks.

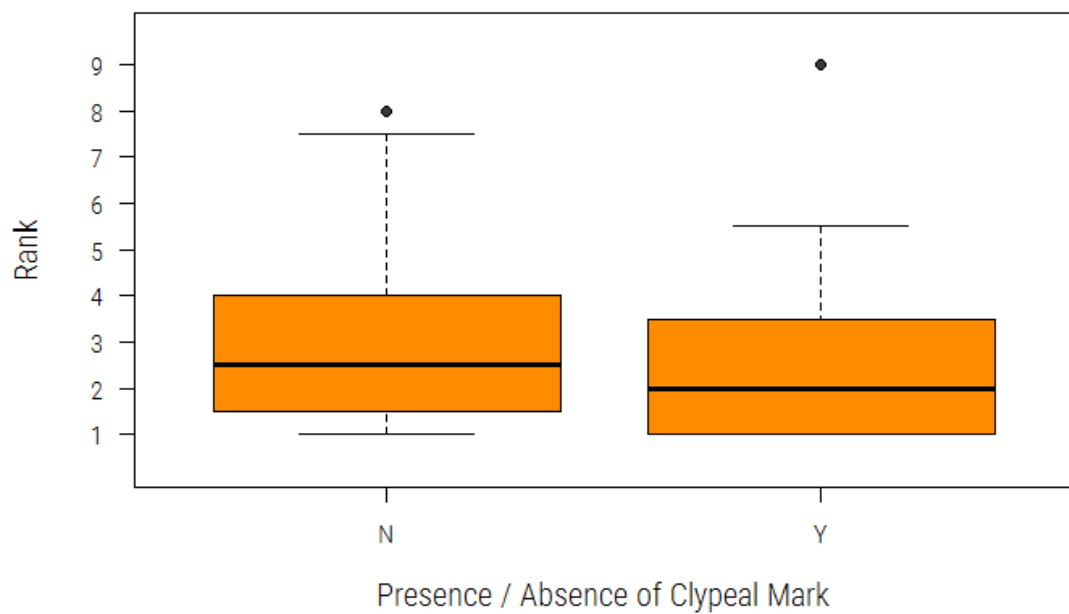


Figure 4b – Median ranks gained by foundresses with and without clypeal marks.

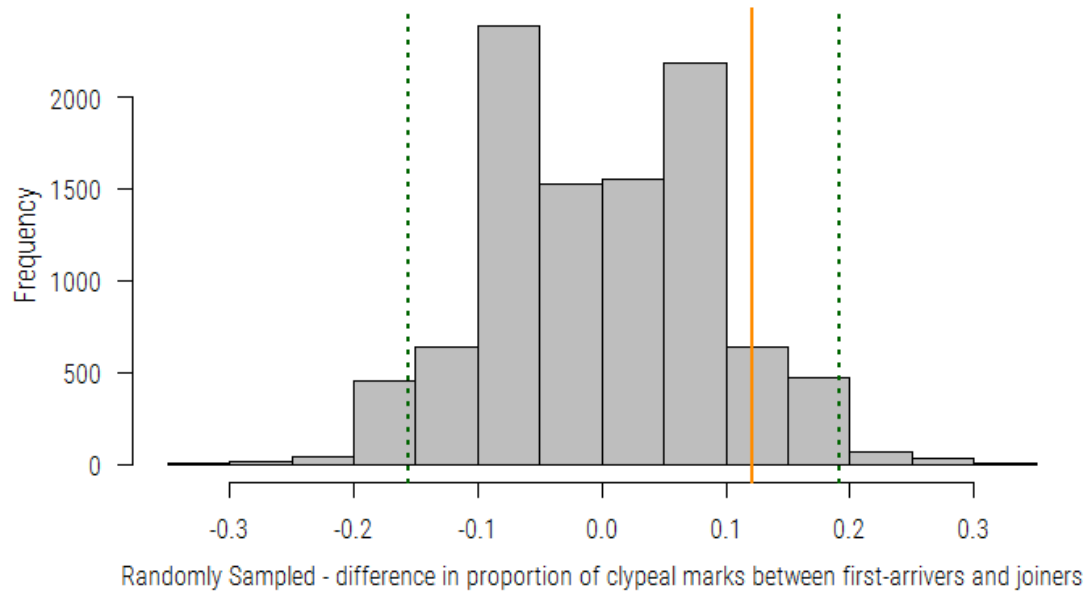


Figure 4c –Frequency distribution of mean difference in proportion of clypeal marks between first-arrivers and joiners. The orange line shows the mean observed value. The dark green dotted lines show the upper and lower limits of the two-tailed permutation test. The test is significant when the orange line is above the upper limit, or below the lower limit.

Order of arrival

First-arrivers were more likely than expected to become rank 1 dominants. 26 of the 39 first-arrivers became the dominant on their nest. Following the approach of Seppa et al. (2002), if hierarchical rank were random with respect to order of arrival, then 10 of 39 would have been expected by chance alone (mean group size = 3.76), which is significantly different from our observation (G-test with Williams correction, $n = 39$, $G = 27.871$, $P = 1.3e-07$; figure 5). In 2 of the 13 not-dominant outcomes, the first-arriver was found to be joint rank 1 with a joiner. These were considered not-dominant to be conservative.

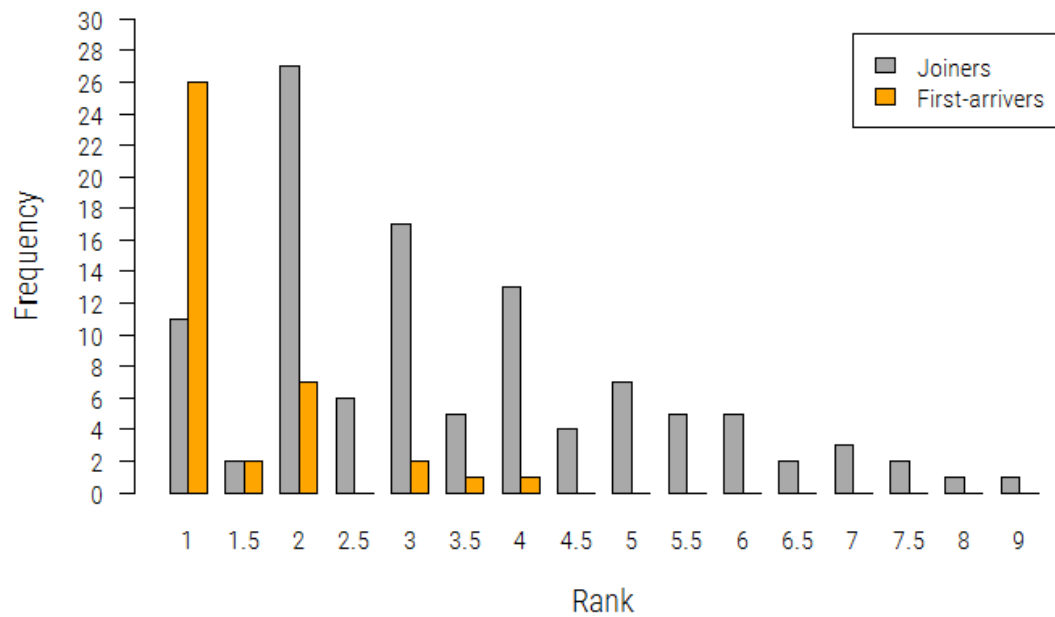


Figure 5 – Frequency distribution of ranks gained by first-arrivers (orange) and joiners (grey). Rank values ending in .5 are joint ranks. To be conservative, the two first-arrivers with joint 1st and 2nd ranks were considered not dominant in the analysis.

Dominance and predictors

Whether or not an individual is the first-arriver is the best predictor of whether or not they will gain the dominant position (aim 7). When using a GLM to simultaneously consider the three main predictors of dominance, being a first-arriver has a significant effect on whether an individual becomes the dominant ($n = 39$, $\chi^2_1 = 54$, $P = 0.002$). This supports the result found in the previous section, when the effect of being a first-arriver on dominance was explored in isolation.

Whether an individual has a clypeal mark has no effect on becoming the dominant ($n = 39$, $\chi^2_1 = 43$, $P = 0.92$). We found the same result as when the effect of clypeal mark on dominance was examined in isolation. Finally, there was no affect of relative body size on which individual gained the dominant position. This is somewhat at odds with the significant result we previously identified when considering only first-arrivers, and investigating the effect of relative body size on hierarchical

rank in isolation. However, it is more consistent with the non-significant trend between relative body size and hierarchical rank among all group members regardless of first-arriver status.

Year ($n = 39$, $\chi^2_1 = 43$, $P = 0.74$) and group size ($n = 39$, $\chi^2_1 = 44$, $P = 0.33$) were included in the global model to control for their effects, neither was found to effect whether an individual became the dominant.

How arbitrary is order-of-arrival?

Founding day and predictors

The year best explained the founding date of a first-arriver (aim 8). Year had a significant effect on founding date ($n = 78$, $\chi^2_1 = 268.36$, $P = 0.00039$). There is a near significant trend of absolute body size, with larger first-arrivers founding their nests later in the season ($n = 78$, $\chi^2_1 = 244.15$, $P = 0.051$; figure 6). Presence or absence of clypeal mark has no effect on founding day ($n = 78$, $\chi^2_1 = 233.66$, $P = 0.75$).

Founding day and dominance

First-arrivers that found their nests earlier in the season gain a significantly higher rank ($\tau = 0.292$, $P = 0.027$; aim 9; figure 7).

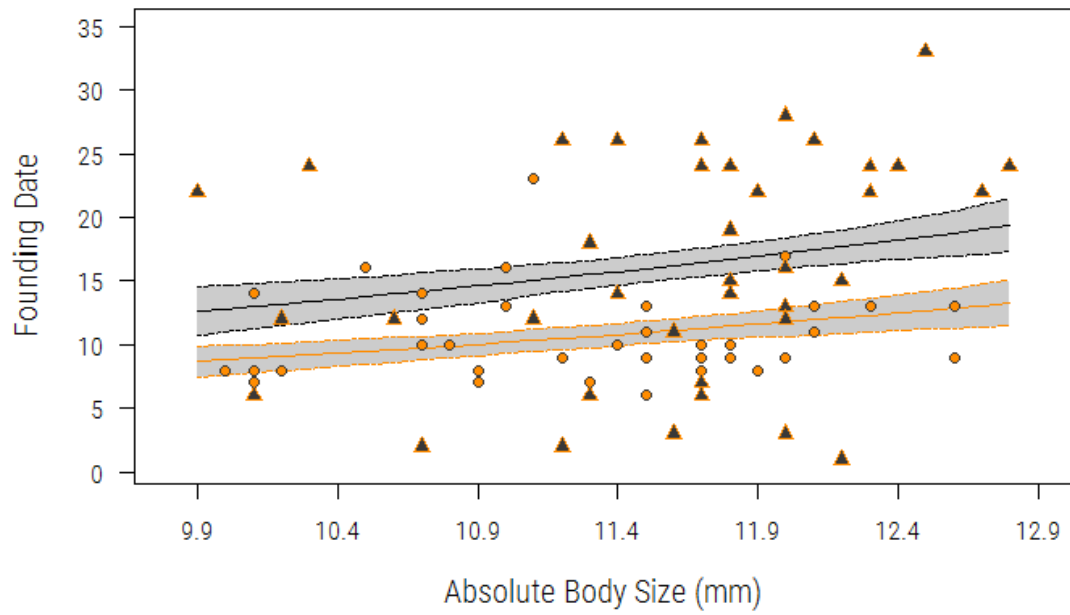


Figure 6 – Relationship between absolute body size of first-arrivers and the number of days after 16th of February that the nest was founded. Length of the wing (mm) was used as a measure of body size. Data from 2016 and 2015 are represented by black triangles and orange circles, respectively. The black and orange lines surrounded by grey shading, show the model predictions with standard error for 2015 and 2016 respectively.

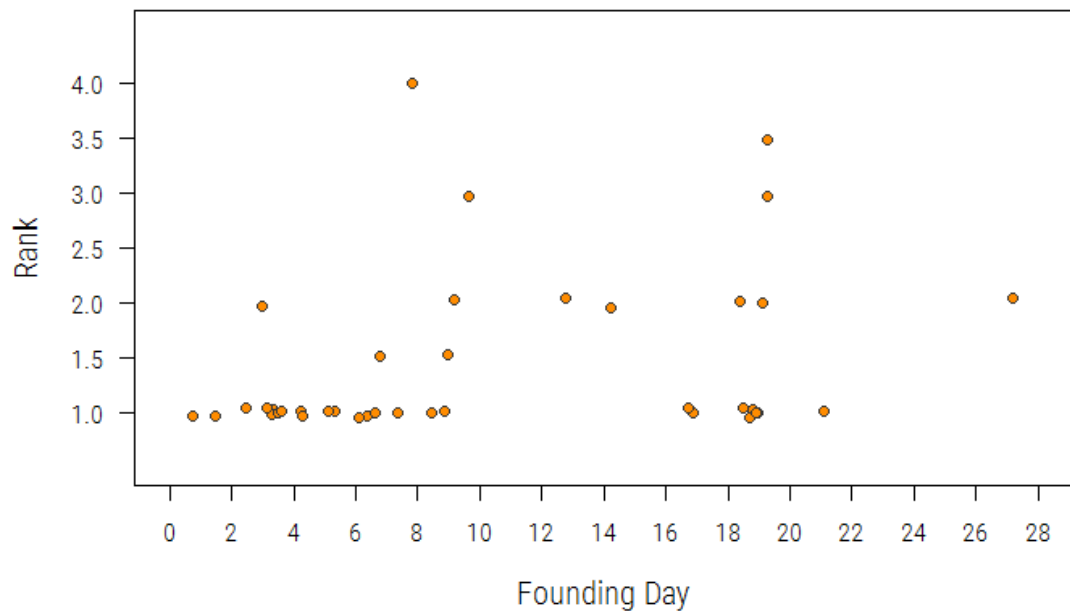


Figure 7 – Relationship between number of days after 16th of February that the nest was founded, and the rank attained.

2.5 - Discussion

We found that arriving first to initiate the nest led to a *P.dominula* foundress being significantly more likely to become the eventual dominant once groups had stabilised. Relative body size, but not presence / absence of a clypeal mark, seemed to have some effect on dominance status. Smaller first-arrivers also seem to be more likely to start founding their nests when the mean temperature was lower. Presence / absence of a clypeal mark had no effect. Those first-arrivers that initiated nest founding earlier were significantly more likely to become the dominant.

Rules of establishing dominance

Body size

Our mixed results suggest that further work is required to understand whether body size is an inconsistent correlate of one or more important traits involved in dominance, as well as under what conditions body size itself may be important in hierarchy formation. We found a non-significant trend for relatively larger individuals within a first-arriver group to have a higher rank (aim 1). We also found that the ranks gained by first-arrivers correlated significantly with their relative (within-group) body size (aim 2), and that first-arriving wasps are, on average, significantly larger than joiners (aim 3). However, when all potential factors were considered together with a single wasp randomly selected from each group, relative body size also did not have a significant effect on the likelihood of an individual becoming the dominant in a first-arriver group (aim 7). Despite the inconsistent results between our analyses, our findings suggest the existence of a relationship between body size and rank that has not previously been seen in the Spanish population (Cant *et al.*, 2006; Leadbeater *et al.*, 2011; Zanette and Field, 2009).

We asked how the relative size of first-arrivers affected their rank (aim 2) and how the absolute size of first-arrivers compared to joiners (aim 3), and found significant relationships in both cases. Aside from a study by Zanette and Field, these specific questions have not been asked before in *P. dominula*, as the first-arriver in each group has not been definitively known. Zanette and Field observed the order in which all group members arrived, but did not test whether there was a relationship between order of arrival and body size. Instead, they asked whether there was a correlation between body size and rank, in which they had a negative result. When all potential factors were considered together, we similarly found that relative body size had no affect on whether an individual became the dominant. Though, when considered in isolation, we did find a non-significant trend for relatively larger individuals within groups to have a higher rank.

Existing evidence of body size influencing the establishment of the dominance hierarchy in *P. dominula* is limited and not without issue (Italy - Cervo et al., 2008; US - Tibbetts and Izzo, 2009). As discussed above, the use of an inappropriate statistical test by Cervo and colleagues (2008) leaves an element of doubt regarding the reliability of their comparison of the body sizes of dominant and subordinate individuals. Tibbetts and Izzo (2009) investigated the relationship between juvenile hormone (JH) application, size and dominance in *P. dominula*. JH is a hormone thought to affect dominance phenotypes in Hymenoptera. Tibbetts and Izzo (2009) found that larger body size positively influenced the effect that JH had on dominance. The effect was tested using lab-based paired contests, leaving some question about how well the result translates to natural group formation in wild populations. Despite the fact that there is not a consistent pattern in the relationship between body size and rank in *P. dominula*, as well as within and between other species of paper wasps (Jandt et al., 2014), a significant positive relationship between the two has been shown on several occasions, suggesting its importance under some circumstances.

Contest outcomes can be influenced by numerous other factors in addition to body size, including experience, position, development of weaponry and physiological state (Arnott and Elwood, 2009). A possible explanation for the inconsistent pattern in the relationship between body size and rank in

paper wasps may be that, though body size may tend to co-vary with some of these factors, it might be too coarse a metric by which to solely assess an opponent. An alternative is to calculate an RHP index, taking multiple variables into account (Arnott and Elwood, 2009; Stuart-Fox *et al.*, 2006). Stuart-Fox *et al.* (2006) approached this by first measuring numerous morphological traits in their population of Cape dwarf chameleons (*Bradypodion pumilum*). A series of paired contest was then carried out to determine the best predictor/s and calculate RHP scores (Firth and Turner, 2012). Measurements of the selected phenotypic characters can then be taken from new individuals in future studies (Stuart-Fox, 2006), and the original model used to calculate RHP scores for each individual. Application of this approach to *P. dominula* should be quite feasible, since the use of one-on-one contests is well established (Tibbetts and Dale, 2004; Tibbetts and Izzo, 2009). Once the best fitting predictors have been established, future studies of the hierarchy would require measurements of just these traits, from which RHP scores could be calculated for each wasp.

Status badge

Our results, that the presence / absence of a clypeal mark on a first-arriver does not affect the likelihood of them gaining the dominant position, either when considered in isolation (aim 4), or when controlling for other factors such as body size and whether they are a first-arriver or joiner (aim 7), are largely in line with other studies in European based populations. In Italy, Cervo *et al.* (2008) found no evidence of a correlation between pattern brokenness and hierarchical rank. Moreover, there was no evidence of a link between pattern brokenness and health or survival, two variables that might correlate with rank. In Spain, Zanette and Field (2009) found a correlation between brokenness and rank when considered in isolation, but not after controlling for other potential determinants. It could be argued that the importance of this trait may not have been captured in our study because of our use of a binary (Y / N) measure. Given the low proportion of wasps with clypeal marks in European populations, however, as well as the strong evidence for a climatic influence in its expression (Green *et al.*, 2012), it seems likely that clypeal markings do not

have an important influence on the dominance hierarchy in our study population. Our finding that first-arrivers and joiners do not differ in terms of the proportion with / without clypeal marks further supports this conclusion.

Order of arrival

Wasps that arrived at the nesting site first in spring were significantly more likely to be the eventual dominant on the nest: 67% of first-arrivers became the dominant compared with only 26% expected by chance. This relationship is found both when first-arrivers were assessed in isolation (aim 6), and when all potential determinants were considered together with randomly selected individuals from each group (aim 7). The importance of arriving first is clear when it is considered that 85% of initial *P. dominula* dominants retain their rank until the end of the colony cycle, and that on average they produce 74% of the reproductive brood (Leadbeater *et al.*, 2011). Furthermore, of the 13 first-arrivers that did not become the dominant, 2 were joint rank 1, and 7 were rank 2. High ranking wasps are, by definition, in the best position to later inherit the dominant position if the initial dominant dies. Our result, that 67% (26/39) of first-arrivers become the dominant approaches the 91% (11/12) found in *P. carolina* (Seppä *et al.*, 2002), but suggests that arriving first is relatively more important in *P. carolina*. One possible explanation for the difference is within-group relatedness. *P. carolina* co-foundresses are predominantly sisters, while in *P. dominula* completely unrelated co-foundresses are common (Field and Leadbeater, 2016).

Zanette and Field (2009) found no effect of order of arrival on dominance in the same Spanish population of *P. dominula*, which contrasts with our findings. This discrepancy, however, is likely to be the result of differences in methodology. Observation on all nests in this study began with just a single wasp on the nest, and just 1 – 8 cells (mean \pm s.e. = 3.67 ± 0.273), compared with 1 - 8 foundresses (mean \pm s.e. = 2.5 ± 1.6) and 1 - 18 cells (mean \pm s.e. = 5.7 ± 5.2) in Zanette and Field's study. Furthermore, Zanette and Field removed wasps from their nest to mark them, increasing

disturbance. Despite these factors, when considering just ranks 1 and 2, Zanette and Field found a significant relationship between dominance and order of arrival. Further indirect evidence that order of arrival is important comes from Pratte and Gervet (1992). They found that when *P. dominula* dominants were experimentally re-homed on new nests, the first-arriving wasps of a pair was more likely to take the dominant position. This was not the case, however, when this was repeated with rank 2 individuals. The question of how far down the hierarchy order-of-arrival remains important requires further study. It may be that censusing the nests every other day for new joiners, the method employed by Zanette and Field (2009), does not produce data that is on a fine enough scale to capture any such relationship. In our study, we refrained from attempting to record the order of arrival of joiners for two reasons. First, given the importance of the dominant position and our prediction that first-arrivers would gain it, it seemed most important to clarify this relationship. Second, we were concerned about the high level of disturbance that would result from checking every day for new joiners, as well as marking them. This may in fact be possible, but would require considerable resources, as searching for new nests of such small size is very time consuming and each new nest would require a daily visit. By marking wasps in-situ as we have in this study, however, and taking a photograph to support later identification, the disturbance is much reduced.

How arbitrary is order-of-arrival?

A key question for understanding the formation of *P. dominula* hierarchy formation is: do first-arrivers gain the dominant position by convention, or are they better quality individuals? We found a near significant effect of body size on the initiation of nest founding, with smaller individuals tending to start founding their nests earlier in the season. Presence / absence of a clypeal mark, however, had no effect on nest founding date (aim 8). Also, first-arrivers that initiated nest founding earlier in the season, were significantly more likely to gain the dominant position in the nest (aim 9).

As smaller first-arrivers seem more likely to arrive earlier in the season and attain the dominant position on the nest, this could reflect that smaller individuals are more likely to exit diapause sooner and subsequently build nests earlier in the season compared to larger individuals. There is evidence to suggest that temperature affects diapause exit as well as post diapause activity, though some of the findings are conflicting. In final instar larvae of weevils (Matsuo, 2006; Menu and Desouhant, 2002), the Douglas-fir cone moth (Sahota and Ibaraki, 1991), and the desert bee (Danforth, 1999), smaller individuals have tended to exit diapause earlier, results that are in line with our findings. Interestingly, it is thought that the early exit from diapause of smaller individuals is due to limited metabolic resources, and gives them opportunity to gather additional resources (Matsuo, 2006). The same could be true in overwintering adult *P. dominula* gynes, in which we found smaller individuals tended to become active earlier. In contrast to our findings, however, a lab-based study of *P. dominula* diapause exit date by Tibbetts et al. (2011a) found that, at least in a sub set of their data, heavier wasps exited diapause earlier. Currently, we are unable to resolve our results with those of Tibbetts, but given that overall body weight was not related to diapause exit date in their dataset (and the association was weak in the subset of their data) it seems an unconvincing result. Thus, it seems that those *P. dominula* first-arrivers that begin nest founding earlier, may be of lower quality than later founding first-arrivers. This is surprising, given our result that first-arrivers that initiated nest founding earlier in the season were significantly more likely to gain the dominant position in the nest.

Our results, that smaller first-arrivers tend to found nests earlier, and that those earlier founding females are more likely to become the dominant on their nest, also appears to contradict with some of our own findings; first-arrivers gain joiners which are typically smaller than themselves, and first-arrivers are more likely to become the dominant if they are larger compared to their joiners. As we only marked first-arrivers as they founded nests, and not joiners, we have information only on the relationship between body size and founding / joining date for first-arrivers. Moreover, group composition is very fluid during the nest founding stage, with joiners frequently moving between

nests. Thus, we do not know whether joiners follow a similar pattern in their relationship between body size and activity to what seems to be the case of first-arrivers. If order-of-arrival was all that dictated rank, and all wasps follow the same pattern in activity seen in first-arrivers (smaller wasps are active earlier), we might expect first-arrivers to be smaller than joiners; yet we see the opposite. The absolute body size of first-arrivers was significantly larger than that of joiners. We also found a non-significant trend for rank to correlate with relative body size within groups. It is not clear how these seemingly contradictory findings might come about, but it seems likely that smaller first-arrivers found nests earlier and then gain smaller joiners. Based on our findings, body size does seem important in formation of the hierarchy, perhaps as a correlate of RHP. Rank may be initially based on the convention of order-of-arrival, but when disputes with joiners occur, first-arrivers that are relatively large are able to outcompete. Our results suggest that order of arrival might be influenced by both arbitrary factors (founding date), and by the quality of individuals (RHP).

Another component of *P. dominula* biology that seems to add an element of stochasticity to which individual becomes the dominant is the cycling of the corpora allata gland. The corpora allata gland produces juvenile hormone (JH), which is positively associated with increased dominance and ovarian development in *Polistes* (*P. annularis* - Barth *et al.*, 1975; *P. dominulus* - Röseler *et al.*, 1985, 1980; Turillazzi *et al.*, 1982). The gland goes through a cycle of increased and decreased size throughout the winter (Strambi, 1969). If the gland is, by chance, at the larger stage of the cycle when the warm spring weather begins, it will give that individual a better chance of gaining the dominant position (higher JH titer, larger ovaries, increased dominance behaviour).

Microclimate can also affect how developed the corpora allata gland is. It is unclear, however, whether this adds another stochastic influence to the order-of-arrival, as was suggested by Seppä *et al.* (2002). Wasps exposed to 1 hour of warmth and light per day, for 10 days prior to end of hibernation, had more developed glands. And were 86% more likely to become the dominant (Röseler *et al.*, 1985). Thus, wasps exposed to early spring warmth, perhaps because they are in less protected hibernation sites or on the edge of their hibernation cluster, appear to gain

considerable benefit in terms of likelihood of gaining the dominant position. In fact, Zanette and Field (2009) suggested that if those individuals exposed to the early spring warmth leave their winter aggregations first, this might reflect order of arrival. There are risks associated with a position at the edge of the hibernation cluster, however, including exposure to cold and/or dryness (Storey and Storey, 2001) and increased metabolic costs (Dapporto and Palagi, 2006), that perhaps only high quality females can cope with. Thus, survival on the edge of the cluster might not be arbitrary, as it could be influenced by the 'quality' of an individual. It is also possible that preferential locations within the hibernacula might be fought for during pre-hibernation. For example, positions that both minimise edge-risks, and increase the chance of exiting diapause sooner, would be most favourable. Dominance interactions in the pre-hibernation stage may allow assessment, or even improvement, of a wasp's dominance potential in the founding stage. In fact, in 85% of cases, dominance relationships of a given pair of females was the same in the pre-hibernation cluster as in the nest founding stage in the spring (reviewed in Dapporto and Palagi, 2006). Perhaps dominance interactions in the pre-hibernation stage also represent competition over location within the hibernacula. If this is the case, then order-of-arrival is at least partially founded on individual quality, though severity of winter weather conditions would obviously add a random element to this. It is also feasible that the choice between safety and dominance represents different strategies.

Conventions vs correlates

The use of a convention or 'rule of thumb', where an individual's role and behaviour is dictated by an asymmetry unconnected with its quality, could conceivably be based on numerous factors (Nonacs, 2001). These include actual / perceived level of genetic similarity (Nonacs and Hager, 2011), age (Bridge and Field, 2007; Higashi *et al.*, 1994; Shreeves and Field, 2002) and order of arrival (Seppä *et al.*, 2002). Logic suggests, however, that these conventions should actually reflect underlying differences in quality between individuals, such a system being more robust against attempted

cheating. In the case of hierarchies in social groups, it may be that group level costs, such as reduction in productivity, may be a reason enough to keep members honest (Field and Cant, 2009). This is even more likely where there is high relatedness between group members, and therefore greater potential for loss of indirect fitness through conflict. An example of this group level cost in *P. dominula* is the reduction in egg laying by a succeeding dominant as she undergoes the physiological development required before attaining full reproductive capacity (Strassmann *et al.*, 2004). Use of certain correlates of quality, however, such as body size or status badge, would increase the likelihood of such costs being incurred. A new arrival with larger body size, for example, would take over the dominant position. On the other hand, in the context of a system with an annual life cycle, such as *P. dominula* (as opposed to a perennial lifecycle like *Liostenogaster flavolineata*), a convention based on age is unlikely to differentiate between individuals, as they are all roughly the same age. Thus, in such a system, order of arrival may be the only available conventional cue that could intrinsically increase group stability. If order of arrival is purely stochastic, however, then on average the quality of the dominant individual will be lower. Revealing the way that the hierarchy is established in *P. dominula* enables us to better understand the selection pressures acting in the early stages of eusocial evolution, in this and similar systems.

Summary

Arriving first appears to be the most important factor influencing the attainment of a rank 1 reproductive status within the *P. dominula* hierarchy. First-arrivers are, on average, larger than joiners, and the specific rank attained by a first-arriver may be further mediated by their relative body size. It is unknown, however, whether this is a reflection of an individual's agonistic ability. Presence or absence of a clypeal mark (status symbol) does not affect a first-arriver's rank. We suggest an approach to find the best morphological correlates of RHP in *P. dominula*, to enable more accurate assessment of its relationship with hierarchical rank in the future. A priori knowledge

of which foundresses will become the dominant would enable previously impracticable questions to be addressed. Of particular interest is the question of whether wasps that become the dominant are intrinsically different from subordinates.

The first field-based investigation of a mechanism underlying order of arrival was carried out. We found that smaller first-arrivers tend to start founding their nests earlier, but that presence or absence of a clypeal mark, had no effect on founding date. First-arrivers that initiated nest founding earlier were more likely to become the dominant in the group. Possible explanations for these unexpected results were discussed, such as competition over preferential locations within the hibernation cluster.

Chapter 3: The effects of solitary versus group nest initiation on co-foundress associations

3.1 - Introduction

There are an abundance of different behavioural approaches, or tactics, employed by animals to maximise reproductive success in different situations. A classic example is the sneaker / fighter reproductive tactics employed by some male invertebrates (Morris *et al.*, 2013). An important question in the study of social evolution is why some individuals forgo independent reproduction to become subordinates in cooperatively breeding groups, when will reduce their direct fitness payoff (Leadbeater *et al.*, 2011; Tibbetts and Reeve, 2003). Less attention appears to have been paid, however, to the different ways (or tactics) by which a social group may be formed, and what impact this might have on the characteristics and outcome of those groups.

Differences in group composition, such as whether the male helper in a cooperatively breeding group remained on its natal nest or joined from elsewhere, has been shown to correlate with differences in behaviours such as mobbing (Maklakov, 2002), vigilance (Griesser, 2003; Valdez, 2010) and foraging effort (Magrath and Whittingham, 1997; Magrath and Yezerinac, 1997). For example, the cooperatively breeding Arabian babbler (*Turdoides squamiceps*) live in family or complex groups of between 2 and 16 individuals, subordinates helping with provisioning and nest defence through mobbing. Family groups are comprised of a breeding pair and their descendants, while complex groups have at least one additional breeder, typically male, who is unrelated. When

presented with a life like model of one of their natural predators, the horned viper (*Cerastes gasperettii*), complex groups spent significantly less time mobbing than family groups, which is though likely to be due to differences in group relatedness (Griesser, 2003). Thus, taking into account the history of each group, i.e. the way in which it was formed, may be important to explain variation in characteristics and outcomes. These differences may be the result of the tactics employed by each group member, and the choice of tactic can be influenced by ecological, social, physiological and genetic factors (Clutton-Brock, 2009; Cockburn, 1998; Field *et al.*, 1998; Keller and Reeve, 1994). Intra-sexual selection can favour a repertoire of behavioural solutions/physical morphs to maximise fitness in different environmental and social contexts. For this chapter, we define an alternative reproductive tactic (ART), or tactic, as a discontinuous behavioural trait selected to maximise fitness in 2 or more alternative ways in the context of intra-sexual competition (Taborsky *et al.*, 2008; Taborsky and Brockmann, 2010).

In primitively eusocial wasps, all individuals retain the ability to reproduce independently (see Chapter 1: General Introduction), as well as some level of plasticity regarding reproductive tactics (O'Donnell, 1996; Ross and Matthews, 1991; Starks, 2001; Starks and Fefferman, 2006; West, 1967). In general, foundresses (mated females; see Chapter 1: General Introduction) in seasonal habitats share 4 main reproductive tactics. First, they can initiate nest founding (construction; see Chapter 1: General Introduction) alone; or second, they initiate nest founding in a group; third, they can join an established nest / group; and finally, they can usurp / adopt an existing nest (Ross and Matthews, 1991). The choice of tactic can have a dramatic influence on an individual's fitness (Field and Leadbeater, 2016; Leadbeater *et al.*, 2011; Liebert *et al.*, 2005; Queller, 1996; Reeve, 1991; Tibbetts and Reeve, 2003), and females are expected to respond in a way that maximises payoff, given their current condition (Gross, 1996).

Factors affecting the choice of reproductive tactic could include condition dependant costs of independent nesting (Clouse, 1995; Field and Leadbeater, 2016; Leadbeater *et al.*, 2011; Liebert *et al.*, 2005; Queller, 1996; Tibbetts and Reeve, 2003), as well as a limited availability of nesting sites

(Field *et al.*, 1998; Gunnels, 2007; Seppä *et al.*, 2012). Furthermore, tactic choice might be influenced by likely indirect and direct fitness outcomes. A foundress choosing to join a nest comprised of individuals closely related to her, in particular the dominant, will maximise her indirect fitness gains (Hamilton, 1964; Seppä *et al.*, 2012). Many subordinates of the tropical species *Polistes canadensis* actually drift between several closely related colonies, helping on each of them (Sumner *et al.*, 2007). Direct fitness is best achieved by gaining the dominant position, an outcome largely dictated by the order that each individual arrives on the nest in at least two temperate *Polistes* species (Seppä *et al.*, 2002), but by age in the hairy-faced hover wasp *Liostenogaster flavolineata* (Bridge and Field, 2007). Subordinate foundresses may still gain direct fitness, however, either by inheriting the dominant position or by laying eggs as a subordinate (Bridge and Field, 2007; Leadbeater *et al.*, 2011; Queller *et al.*, 2000; Shreeves and Field, 2002; Sumner *et al.*, 2010). Overall, however, the likelihood of inheriting the dominant position will decrease as group size increases (Leadbeater *et al.*, 2011; Shreeves and Field, 2002).

Variation in morphological characteristics and physiological state can be important factors in tactic choice (Gross, 1996; Knott *et al.*, 2010; Leary *et al.*, 2005; Lidgard *et al.*, 2005; Martin and Festa-Bianchet, 2010; McCauley, 2010; Mora-Kepfer, 2011). The fighting ability of an animal often correlates with body size or mass (Arnott and Elwood, 2009), and so body size is commonly used as a proxy for resource holding potential. There is also some limited evidence of a relationship between body size and position within the hierarchy in some primitively eusocial wasps (reviewed in Chapter 2; and Jandt *et al.*, 2014). In such a system a relatively small individual may do better to accept a subordinate role than challenge for the dominant position, so avoiding the associated cost of such a competition. Another morphological trait with some evidence of association with hierarchical rank in *Polistes* is the clypeal mark. These are small patches of colour that, in some cases, provide information about competitive ability without being causally linked to it (reviewed in Chapter 2; and Jandt *et al.*, 2014).

The *Polistes* paper wasps, and *P. dominula* in particular, have proved to be important model systems for exploring questions relating to different reproductive tactics (Gamboa *et al.*, 1992; Klahn, 1988; Leadbeater *et al.*, 2010, 2011; Liebert and Starks, 2006; Queller *et al.*, 2000; Seppä *et al.*, 2012; Starks, 2001; Starks and Fefferman, 2006; Tibbetts and Reeve, 2003; Zanette and Field, 2011). Spring foundresses can choose between the 4 nesting options introduced above: i) initiating a nest alone; ii) initiating a nest in a group, iii) joining a nest / group; and iv) sit-and-wait to usurp / adopt a nest. Thus, both solitary and group founded nests may later gain a number of additional foundresses (joiners), and in the resulting co-foundress association, a hierarchy will be established. This hierarchy dictates the division of reproduction and labour within the group (Reeve, 1991). The first-arriving foundress at a prospective *P. dominula* nest will typically gain the dominant position, the chance may increase if she is large relative to her nest mates (see Chapter 2). The benefits to *P. dominula* foundresses of being even a subordinate in a co-foundress association, as opposed to remaining alone, are well established (Field and Leadbeater, 2016; Leadbeater *et al.*, 2011; Queller, 1996; Reeve, 1991; Tibbetts and Reeve, 2003). How the method of nest initiation subsequently affects group characteristics and fitness outcomes remains unexplored. Do nests started by a group of foundresses, for example, have higher within-group relatedness? Are solitary or group founded nests initiated by morphologically distinct females?

Aims

In this chapter, we will investigate the effect of nest founding tactic (solitary / group), as well as other variables, on various characteristics and outcomes of *P. dominula* co-foundress associations. Specifically, we will ask: 1) **Relatedness** - Which of the following factors best explains variation in within-group relatedness: founding tactic (solitary / group), group size, or the time since nest initiated; 2) **Productivity** - Which of the following factors best explains variation in nest productivity: founding tactic (solitary / group), mean body size, proportion of group members with clypeal marks,

group size, or the time since nest initiated; 3) **Survival** - Which of the following factors best explain variation in nest survival: founding tactic (solitary / group), mean body size, time before the first joiner arrives, or site; 4) **Group size** - Which of the following factors best explain variation in group size: founding tactic (solitary / group), days since nest initiated, time before the first joiner arrives, or site; and finally 5) **Founding females** - Which of the following morphological factors best differentiate between solitary founding and group founding females: clypeal mark (Y / N); or absolute body size?

3.2 - Method

Identifying newly founded nests

We first aimed to find and mark nests newly initiated by both lone foundresses and groups of co-foundresses. Data were collected from 3 field sites in 2015 (see Chapter 1: General Introduction). Sites were searched daily for new nests between February and April. Nests were found on prickly pear cactus (*Opuntia* sp.) at these rural sites surrounding Conil de la Frontera in south-west Spain (36° 16' 41"N, 6° 5' 12"W). *P.dominula* nests have an open comb, lacking a nest envelope. This structure allows observations to be made from a distance, minimising disturbance. Each site was visited at least once per day, except in inclement weather, when wasps would not have been active. Nest searches were carried out in the early morning and late afternoon/evening when the wasps are not active due to the low temperature. All group members typically return to their nest by the end of the active period. Thus, new nests discovered during these searches (with 8 cells or less) were considered solitary or group founded, when either one or multiple foundresses, respectively, were present. Then, using one of two specially constructed painting tools (figure 1a), a single wasp was given a unique enamel paint mark while still on the nest (figure 1b). This was done by applying a small blob of enamel paint to the end of the painting tool (pin head), and then aiming to touch the

thorax with the pin head. In group founded nests, only one of the founding wasps was marked, this individual was randomly selected from those present. Most wasps remained on their nest after marking. Marking was carried out while the foundress was still on the nest to minimise disturbance, as nest abandonment seems to be common at this early stage. A single colour was used (a new colour was chosen for every c.10 wasps marked at a site to aid unique identification), and only one or two spots applied. The colour, location and size of these marks was then recorded (figure 1b). Different colours were used for solitary founded females (e.g. figure 1d) vs group founded (e.g. figure 1b) in order to further differentiate them.



Figure 1, a - d – a) tool for paint marking first-arrivers on the nest; b) a newly marked foundress on a group founded nest, consisting of five cells; c) a collected first-arriver, ready for confirmation of identity and application of a four spot paint mark; d) a newly painted four spot paint mark.

Nest monitoring, and morphological data collection

Group size was recorded on new nests for the 3 consecutive evenings immediately following first discovery, when the wasps were inactive. Presence, and the description, of a paint-marked wasp was recorded if this could be seen without causing a disturbance. If a nest was recorded as unoccupied for 3 consecutive evenings, then the nest was considered abandoned and no longer censused.

After this initial stage of monitoring, nests were checked every 2 to 4 days. On each occasion a cell and wasp count was carried out, and cells were checked for hatched eggs. Once a nest had either at least 20 cells or 1 hatched egg, the group was considered ready to collect for individual paint marking. This arbitrary cut-off point was used because nests with more cells and greater brood development were considered more stable and less likely to abandon the nest if disturbed. Once stabilised, wasps were collected from their nests before dawn, and stored at c. 4 degrees Celsius. A detailed description of the first-arriver's initial paint mark was recorded. All wasps were then given a unique combination of four enamel paint spots on the thorax (figure 1c). Wing length was measured using callipers (RS Pro digital calliper), which were accurate to 0.01 mm. Due to human limitations, however, wing length was effectively recorded to the nearest 0.1 mm. Our measurement of wing length was used as a proximate measure of body size, as wing length is known to correlate with overall body size in *Polistes* (Sullivan and Strassmann, 1984). The presence / absence of a clypeal mark was also noted, and, a DNA sample taken by removing the tarsus of the right centre leg using micro scissors. Tarsai were stored in alcohol for later DNA extraction. After marking and taking morphological measurements, wasps were again stored at c. 4 degrees Celsius. Wasps were then released close to their nests before 11am of the same morning as collection, when they would normally begin to be active. After marking, nests were censused for brood (number of eggs, larvae [small, medium and large] and pupae) and cell number every 1 to 2 weeks (see Chapter 1: General Introduction). When possible, the paint ID of each wasp on a nest was also recorded on the same day as each brood census, this was carried out in the evening when the wasps were inactive.

Genotyping and relatedness

We follow a similar protocol to previous studies, with minor variations (Grinsted and Field, 2017; Leadbeater *et al.*, 2010, 2011). To Extract DNA, tarsi samples were bathed in 50 µl of squishing buffer (Gloor *et al.*, 1993). Samples were incubated at 57°C for 40 minutes, then at 95°C for 2 minutes, to denature the Proteinase K. Samples were then genotyped at nine microsatellite loci (Grinsted and Field, 2017; Tsuchida *et al.*, 2003), amplified in a single multiplex reaction, using the Qiagen multiplex PCR kit (Qiagen, Venlo, The Netherlands). 5.5 µl reactions were performed, comprised of 15–150 ng of template DNA, 2 µl of 2 × Multiplex master mix (3 mM MgCl₂) and 2 µl of primer mix. The primer mix consisted of 0.807 µmol of the primer pairs Pdom1 and Pdom20, and 0.484 µmol each of the primer pairs Pdom2, Pdom7, Pdom25, Pc68, Pc63, Pbe128TAG, and Pdom140. A drop of mineral oil was added to prevent evaporation. PCR was performed in a G-storm GS2 thermal cycler with a temperature profile of 95 °C for 15 min; 35 cycles of 94 °C for 30 seconds, 57 °C for 90 seconds and 72 °C for 60 seconds; followed by a final extension step of 60 °C for 30 min. PCR products were separated by size using a 48-well capillary Applied Biosystems 3730 Sequencer, compared with a size standard (Applied Biosystems GeneScan LIZ 500) and visualized using Applied Biosystems GeneMapper analysis software. Tests for linkage disequilibrium, null alleles and deviations from Hardy-Weinberg equilibrium revealed no significant deviations from chance expectations for these loci (Raymond and Rousset, 1995). Relatedness 5.0.8 software was used to calculate average relatedness between nest residents (Queller and Goodnight, 1989).

3.3 - Statistical Analysis

All statistical analyses were carried out using R Studio version 1.0.136 (<https://www.rstudio.com>) and R version 3.2.3 (<http://www.r-project.org>).

Multiple comparisons

When setting a significance threshold (or alpha) of 0.05, for example, it is accepted that there is 5% chance the observed result may have come about even though the null hypothesis is true, otherwise known as a false positive result. Conducting multiple statistical tests, or comparisons, on a dataset can lead to an increase in false positive results (Forstmeier *et al.*, 2016). I take this into account in this chapter by moderating my conclusions on analyses where P values come just within the acceptance threshold.

Relatedness

To investigate the relationship between within-group relatedness and founding tactic, a General Linear Mixed Model (GLMM) was carried out (aim 1). The response variable was within-group relatedness, and the fixed effects were founding tactic, group size, and the number of days since nest initiated. Parameter estimates and model selection can be adversely effected by collinearity between predictor variables (Freckleton, 2011). Thus, potential collinearity was assessed by calculating variance inflation factors (VIF) for each variable (Frank, 2014). The value calculated for each variable represents the extent to which the variance of its regression coefficient, is inflated due to correlation with the other variables. Variables with VIFs exceeding 5 or 10 are thought to suggest excessive collinearity (Graham, 2003; Montgomery *et al.*, 2015). VIFs were very low (group size = 1.22, days since nest initiated = 1.21, founding tactic = 1.03), so all explanatory variables were initially included. To account for the repeated measures of nests through the season, nest ID was included as a random effect. This factor was nested within the other random effect, site. Explanatory terms were considered significant if $P \leq 0.05$.

Productivity

To investigate the relationship between productivity and solitary / group founded nests, a Generalised Linear Mixed Model (GLMM) with a poisson error distribution was carried out (aim 2). The response variable was number of cells, and the fixed effects were founding tactic, mean body size, proportion with clypeal mark, and a two way interaction between group size and days since nest initiated. VIFs were low (group size = 1.65, days since nest initiated = 1.35, founding tactic = 1.03, mean body size = 1.22, proportion with clypeal mark = 1.07), so all considered explanatory variables were initially included. To account for the repeated measures of nests through the season, nest ID was included as a random effect. This factor was nested within the other random effect, site. Explanatory terms were considered significant if $P \leq 0.05$.

Survival

To investigate the relationship between survival (binary variable) and solitary / group founded nests, a Generalized Linear Model (GLM) with a binomial error distribution was carried out (aim 3). Nests were considered to have failed if all foundresses disappeared before worker emergence or the last census. The potential explanatory variables were: founding tactic, mean group size, number of (3 potential) initial evening censuses without a first joiner. Site was considered as a potential co-variate. VIFs were very low (mean group size = 1.22, number of (3 potential) initial evening censuses without a first joiner = 1.06, founding tactic = 1.04, site = 1.21), so all considered explanatory variables were initially included. Explanatory terms were considered significant if $P \leq 0.05$.

Group size

To investigate the relationship between productivity and solitary / group founded nests, a Generalised Linear Mixed Model (GLMM) with a poisson error distribution was carried out (aim 4).

The response variable was group size, the potential explanatory variables were: founding tactic, days since nest initiated and number of (3 potential) initial evening censuses without a first joiner. Site was considered as a potential co-variate. VIFs were very low (days since nest initiated = 1, number of (3 potential) initial evening censuses without a first joiner = 1.01, founding tactic = 1.01), so all considered explanatory variables were initially included. To account for the repeated measures of nests through the season, nest ID was included as a random effect. Explanatory terms were considered significant if $P \leq 0.05$.

Founding females

To investigate the relationship between the marked founding females from solitary / group founded nests and their morphological traits, a Generalized Linear Model (GLM) with a binomial error distribution was carried out (aim 5). The response variable was founding tactic (binary), the potential explanatory variables were: absolute body size and presence / absence of a clypeal mark. Site was considered as a potential co-variate. VIFs were very low (absolute body size = 1.06, presence / absence of clypeal mark = 1.02, site = 1.06), so all considered explanatory variables were initially included. Explanatory terms were considered significant if $P \leq 0.05$.

3.4 - Results

170 nests were initially recorded and observed until either failure, or the end of the observation period. 118 of these nests were solitarily founded, 62 of which gained at least 1 joiner during the observation period. 52 of the 170 nests were group founded. The mean number of foundresses initiating group founded nests was 2.5 (\pm s.e. = ± 0.13). 26 group founded and 50 solitary founded nests were still active when we collected wasps for morphological and genetic data sampling.

Relatedness

Relatedness of foundress associations was higher early in the season (aim 1; figure 2a and b).

Within-group relatedness decreased significantly with days since nest initiation ($n = 214$, $\chi^2_1 = 4.1$, $P = 0.043$) between the early nest stage and the stable nest stage. Relatedness did not differ between nests founded by a single foundress and a group of foundresses ($n = 214$, $\chi^2_1 = 0.55$, $P = 0.46$).

There was also no significant effect of group size ($n = 214$, $\chi^2_1 = 0.69$, $P = 0.41$) or site ($n = 214$, $\chi^2_1 = 0$, $P = 1$) on relatedness.

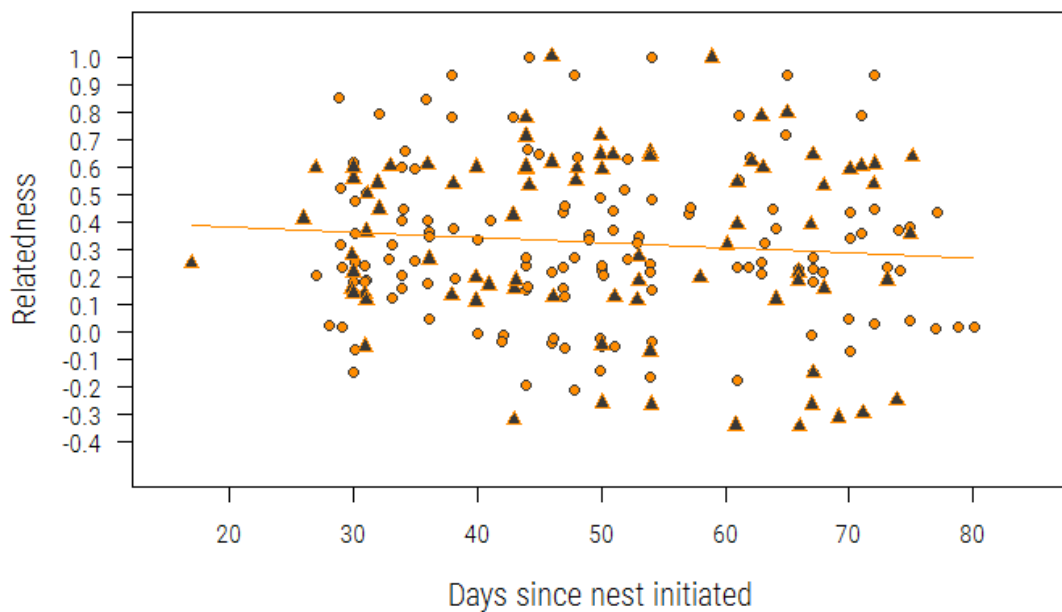


Figure 2a – Relationship between with-group relatedness and days since nest initiated. Orange circles are solitary founded nests and black triangles are group founded nests. The orange line represent the model prediction.

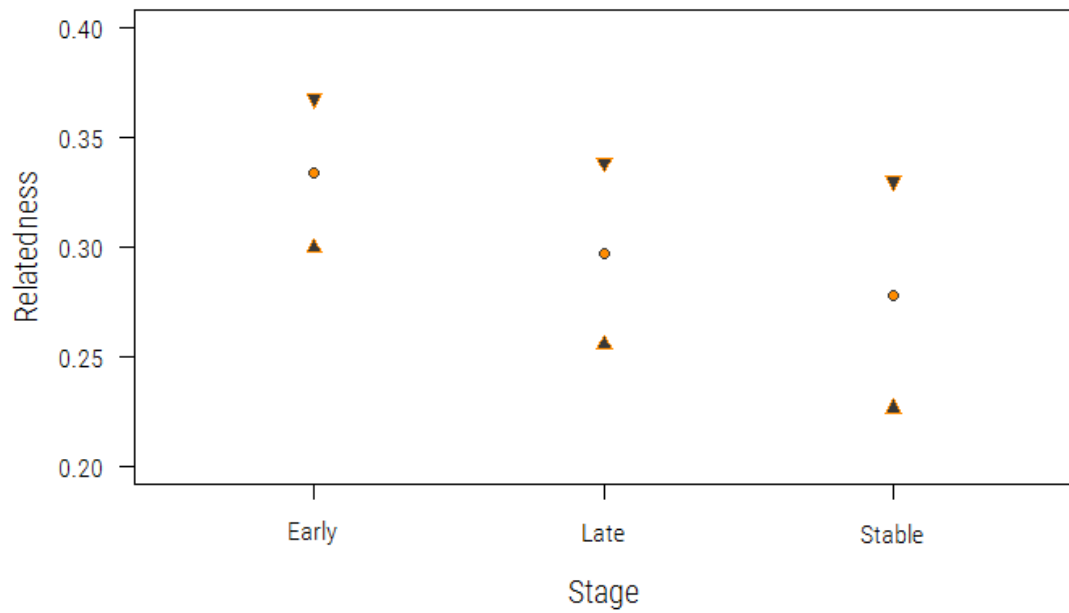


Figure 2b – Relationship between within-group relatedness and nest stage. Orange circles are the mean values from raw data for Early (days 15 to 25), Late (days 35 to 45), and Stable (days 55 to 65). Black triangles represent the standard errors for those data. This plot is included to aid in visualising the data, and comparison with Zannete and Field's (2008) results.

Productivity

Nests with a higher mean body size and a larger proportion of foundresses with clypeal marks were more productive. As the season progresses, large groups produce significantly more cells (aim 2; figure 3). Mean body size ($n = 149$, $x^2_1 = 4.3$, $P = 0.037$) and proportion of foundresses with a clypeal mark ($n = 149$, $x^2_1 = 5.8$, $P = 0.016$) both had a significant effect on cell count. Furthermore, there was a significant interaction between group size and days since nest initiated ($n = 149$, $x^2_1 = 59$, $P = 1.578e-14$), with the difference in nest size (between larger and smaller groups) increasing as the season progressed. Neither founding tactic ($n = 149$, $x^2_1 = 1.9$, $P = 0.17$) or site ($n = 149$, $x^2_1 = 0.15$, $P = 0.7$) affected nest productivity, after controlling for group size.

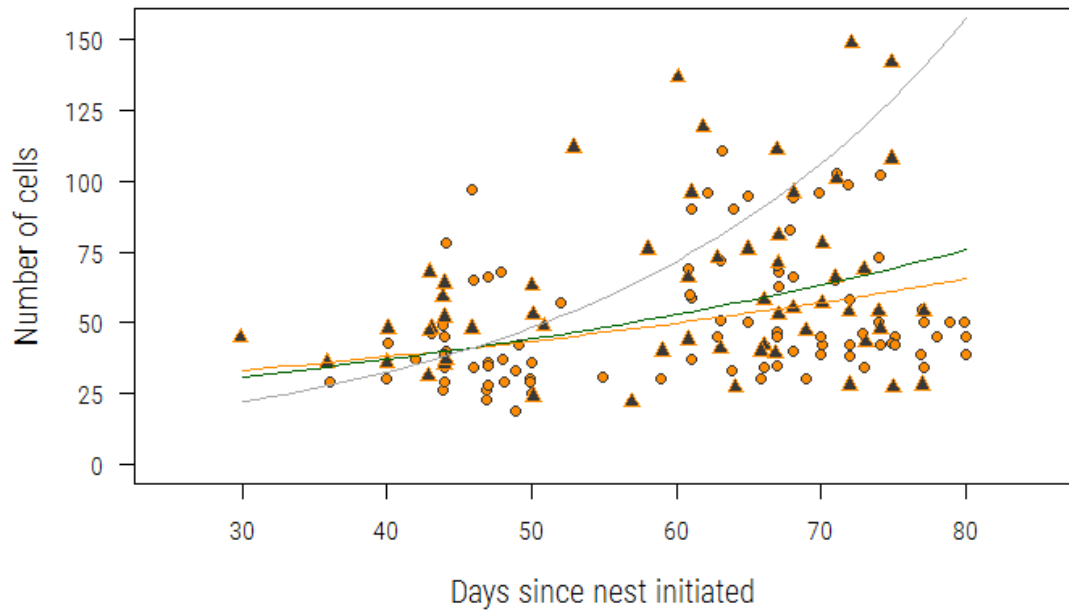


Figure 3 – Relationship between number of cells and days since nest initiated. Orange circles are solitary founded nests and black triangles are group founded nests. The different lines represent different group sizes: orange = 2 (lowest); green = 3 (mean); and grey = 8 (highest).

Survival

Larger groups have increased survival (aim 3; figure 4). Average group size was the only factor to have a significant effect on nest survival ($n = 80$, $\chi^2_1 = 9.7$, $P = 0.0018$). Thus, neither founding tactic ($n = 80$, $\chi^2_1 = 0.36$, $P = 0.55$), number of (3 potential) initial evening censuses without a first joiner ($n = 80$, $\chi^2_1 = 1.04$, $P = 0.31$), nor site ($n = 80$, $\chi^2_2 = 4$, $P = 0.14$) had a significant effect on survival.

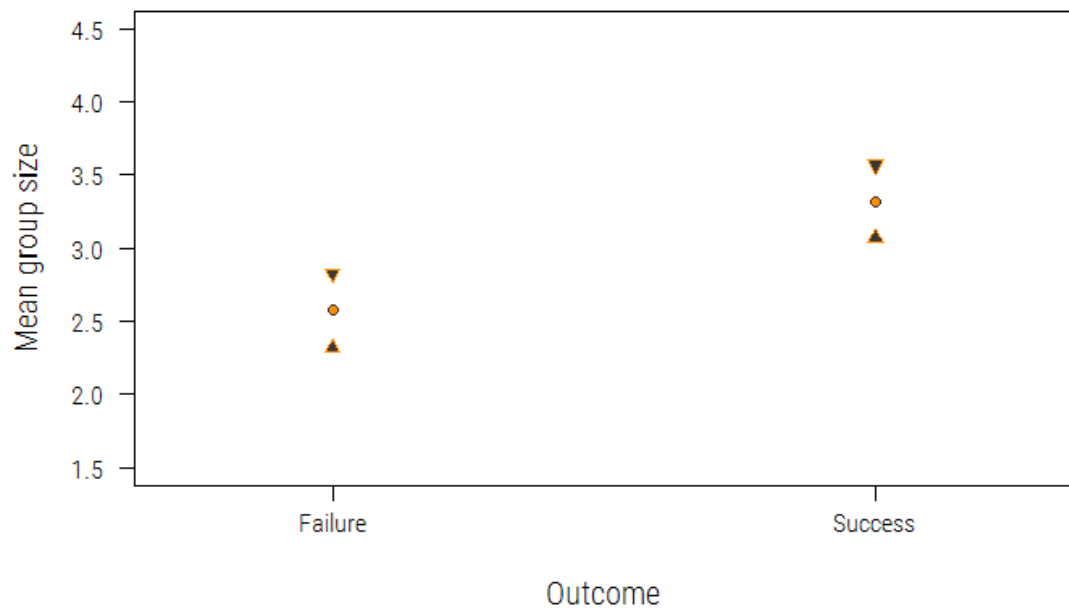


Figure 4 – Relationship mean group size and nest survival. Orange circles are the mean values from raw data and black triangles represent the standard errors.

Group size

Group founded nests have larger group size than singly founded nests (aim 4; figure 5). Founding tactic has a significant effect on groups size ($n = 262$, $\chi^2_1 = 5.1$, $P = 0.024$), with group founded nests predicted to have c.1 additional joiner, compared to solitary founded, on day 30, day 50, though just c.0.6 by day 70. Days since nest initiated also had a significant effect on group size ($n = 262$, $\chi^2_1 = 29.2$, $P = 6.439\text{e-}08$), with group size predicted to drop by c. 2.3 wasps over the observation period. However, there is no significant interaction between days since initiated and founding tactic ($n = 262$, $\chi^2_1 = 0.031$, $P = 0.86$). There is also no effect of site ($n = 262$, $\chi^2_1 = 0$, $P = 1$) or number of (3 potential) initial evening censuses without a first joiner ($n = 262$, $\chi^2_1 = 1.51$, $P = 0.22$).

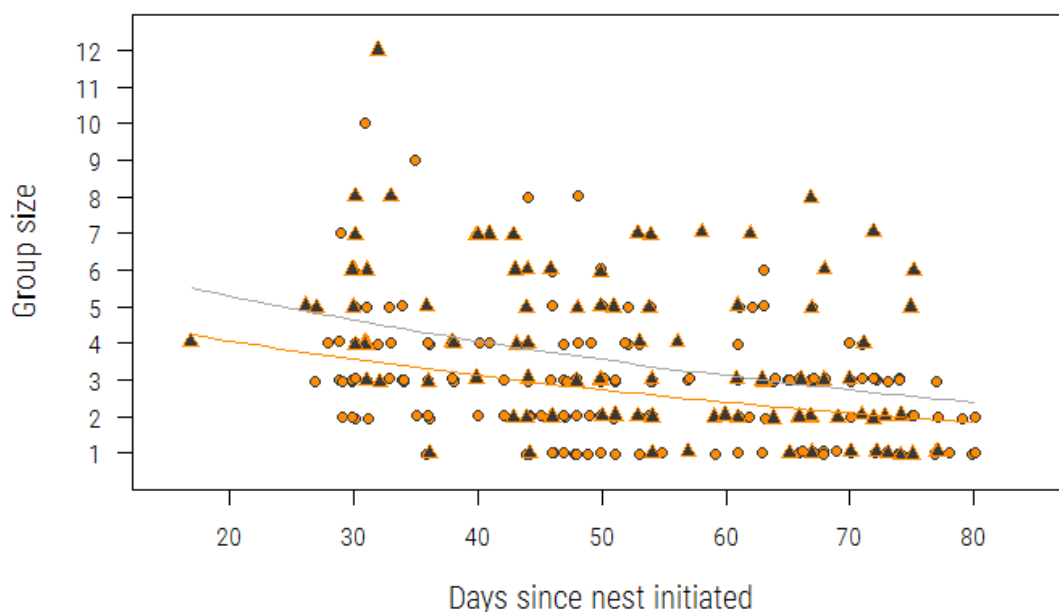


Figure 5 – Relationship between group size and days since nest initiated. and solitary / group founding. Orange circles are solitary founded nests and black triangles are group founded nests. The orange and grey lines represent the model predictions for solitary and group founded nests respectively.

Founding females

We found an interacting effect of body size and clypeal mark on founding tactic, in that group founding females are either large with a clypeal mark or small without, while solitarily founding females do not differ in size depending on whether they have a clypeal mark (aim 5; figure 6a -b). There is a significant interaction between body size and clypeal mark on solitary / group founding females ($n = 76$, $\chi^2_1 = 4$, $P = 0.045$; see figures 5a – 5c). Site has no significant effect on whether an individual is a solitary / group founded females ($n = 76$, $\chi^2_2 = 0.21$, $P = 0.9$). There is a higher proportion of clypeal marks in solitary (mean \pm s.e. = 0.55 ± 0.07) founding wasps compared to group (mean \pm s.e. = 0.4 ± 0.1) founding. There is a marked difference in body size between group founding foundresses with (mean \pm s.e. = 11.5 ± 0.13), and without (mean \pm s.e. = 10.9 ± 0.16) a clypeal mark. Body size does not differ between those solitary founding females with (mean \pm s.e. = 11.3 ± 0.13), and without (mean \pm s.e. = 11.4 ± 0.14) a clypeal mark.

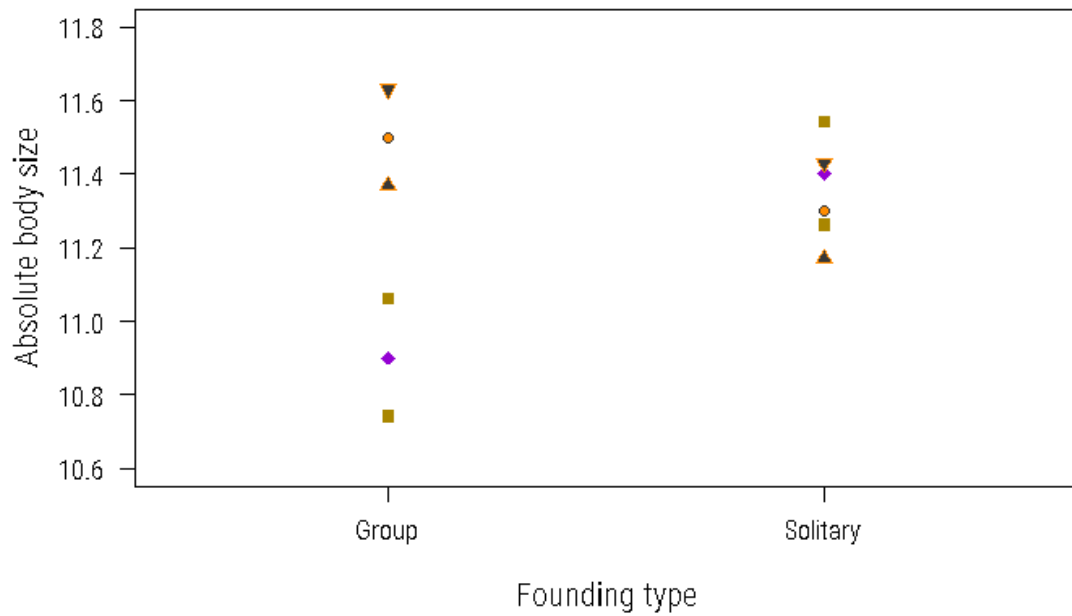


Figure 6a – Relationship between founding type and absolute body size, for founding females with and without a clypeal mark. Purple diamonds and gold squares are, respectively, mean and standard error values for females without clypeal. Orange circles and grey triangles are, respectively, mean and standard error values for females with clypeal.

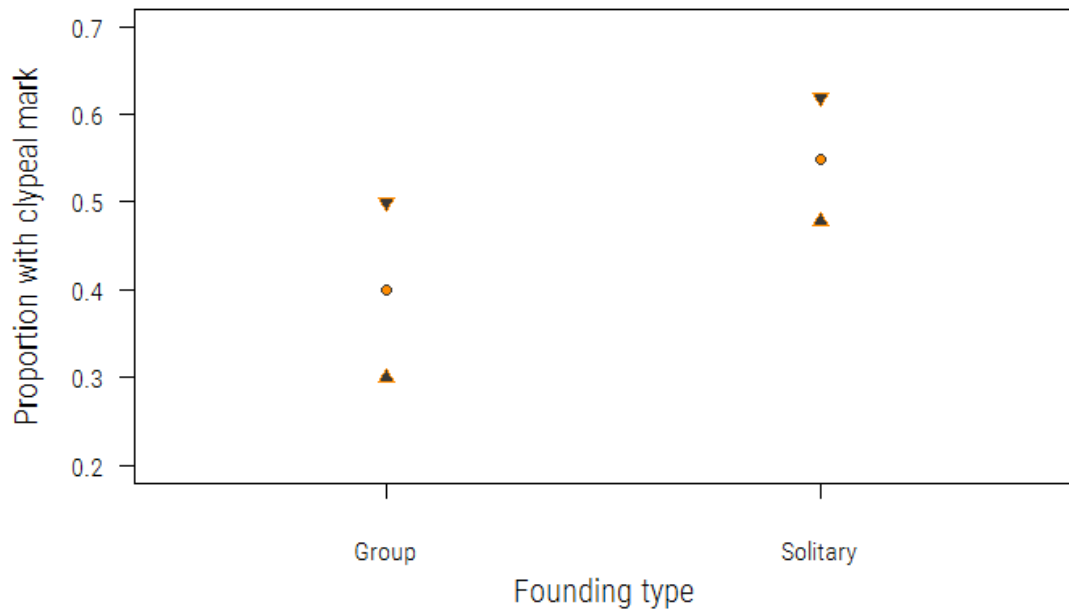


Figure 6b – Relationship between founding type and proportion of founding females with a clypeal mark.

3.5 - Discussion

In summary, group founded nests had larger overall groups sizes than solitary founded nests, but relatedness, productivity and survival did not differ between founding tactics when controlling for group size. Through an increase in group size, however, we found that group founded nests had greater survival and productivity than solitarily founded nests. Finally, we found an interacting effect of body size and clypeal mark on founding tactic of founding females.

Relatedness

In our study we found that, in general, within-group relatedness was low, and there was no evidence of a difference in relatedness between group and solitary founded nests (aim 1). We might have expected group founded nests to have higher genetic relatedness than solitary founded nests if founders tended to be close relatives (maximising indirect fitness: Hamilton, 1964), and joiners tended to be less or unrelated. Our result suggests this is not the case though, which is consistent

with previous work by Zanette and Field (2011), in which they found within-group relatedness was unaffected by late arriving joiners.

Within-group relatedness in *P. dominula* is typically relatively low (0.13 – 0.53) compared to other *Polistes* species (c.0.6), which are mainly comprised of full sisters (*P. dominula* - Leadbeater *et al.*, 2011, 2010; Liebert *et al.*, 2005; Queller *et al.*, 2000; Zanette and Field, 2008; *P. bellicosus* - Field *et al.*, 1998b; *P. fuscatus* - Reeve *et al.*, 2000; *P. carolina* - Seppä *et al.*, 2012, 2002; but see *P. aurifer* - Liebert *et al.*, 2005). In fact, between 15% (Spain - Leadbeater *et al.*, 2010) and 30% (Italy - Queller *et al.*, 2000) of *P. dominula* foundress nestmates are unrelated. In our results, we also found that within-group relatedness decreased between early spring, when nests have only recently been formed, and early summer, when groups have become stable; this result is supported in earlier work by Zanette and Field (2008). That within-group relatedness is unaffected by late arriving joiners (see above), but is decreasing through the course of the season, suggests that closely related individuals are being lost. This further implies that lower ranked group members have higher relatedness, as predation while foraging appears to be greatest threat to *P. dominula* foundresses (Nonacs and Reeve, 1995; Strassmann, 1981), and it is those of lowest rank that spend most time foraging. This is corroborated by Zanette and Field (2009), who showed that low rank wasps are frequently full sisters of rank 2 or 3 wasps., though typically unrelated to the dominant.

The relatively low within-group relatedness that we, and others, have found in *P. dominula* is unusual in eusocial insects (reviewed in Field and Leadbeater, 2016). By joining a group with high mean relatedness, foundresses can maximise their indirect fitness. In fact, in the related species *P. carolina*, foundresses actually monitor other groups within the population, sometimes making a permanent move and preferentially joining natal nestmates on new nests, which is most likely a result of kin recognition (Seppä *et al.*, 2012). In order to avoid exploitation, group living species should at least be able discriminate between group members and non-group members (Hölldobler and Wilson, 1990). Most species can discriminate between several features of conspecifics, which can include sex, reproductive status, dominance rank, kinship and individual identity. Individual

recognition, however, is likely to be much more complex, though it may bring significant benefits (Thom and Hurst, 2004). *P. dominula* is known to exhibit nestmate recognition using cuticular hydrocarbons (CHC; Gamboa, 1996). However, there is mixing of these chemicals between unrelated individuals within the hibernacula during winter (Zanette and Field, 2008). It is therefore unlikely that kin recognition occurs using CHCs in this species (Dapporto *et al.*, 2004); the findings of several studies support this hypothesis (Sheehan and Tibbetts, 2010; Sledge *et al.*, 2004; Sledge *et al.*, 2001). However, a recent study by Leadbeater and colleagues (Leadbeater *et al.*, 2014) has found some evidence of within-nest kin discrimination in *P. dominula*, making use of an as-yet-unknown cue.

Despite low relatedness within *P. dominula* nests, Zanette and Field (2008) found that genetic relatedness was significantly higher in newly founded nests compared to winter aggregations. Thus, group formation does not seem to be a random sampling of the hibernacula. It is possible that this increase in relatedness could come about from a tendency to return to the location of your natal nest (female natal philopatry; Jeanne and Morgan, 1992; Klahn, 1979; Queller, 1994; Starks, 2003; Sumana *et al.*, 2005). Which of these mechanisms, natal philopatry or preferentially joining groups with natal nestmates, is responsible for the increase in relatedness as nest founding begins is currently unknown.

Productivity

Productivity did not differ between founding tactics in this study after controlling for groups size. One reason why productivity might be expected to differ between founding tactics in *P. dominula*, is if the relatedness structure of the resulting groups differ between tactic types. Even groups of the same size can differ in their productivity, as a result of differences in their composition (Magrath and Whittingham, 1997; Magrath and Yezerinac, 1997; but see Cockburn, 1998). Thus, *P. dominula* subordinates may increase foraging effort when nesting with relatives to maximise indirect fitness,

and reduce foraging effort in low related nests. By reducing foraging effort, an individual could increase their chance of inheriting the dominant position, as there is a high risk of predation while foraging (Cant and Field, 2001). However, in the previous section we show that there is no difference in relatedness between the individuals of group founded and solitary founding nests, which might explain why productivity does not differ between founding tactics.

We also found that larger groups produced significantly more cells compared to smaller groups as the season progressed (aim 2). Positive effects of group size on productivity are common in cooperative breeders (Boland *et al.*, 1997; Brown *et al.*, 1982; Emlen and Wrege, 1991; Hatchwell *et al.*, 2004; Heg *et al.*, 2005; Kingma *et al.*, 2010; Komdeur, 1994; Mumme, 1992; but see Legge, 2000; Leonard *et al.*, 1989; Walters, 1990). This pattern continues in primitively eusocial insects (Field *et al.*, 1999; Jeanne and Nordheim, 1996; Packer, 1993; Schwarz *et al.*, 1998; Schwarz, 1988; Shakarad and Gadagkar, 1995; Shreeves and Field, 2002; but see Smith *et al.*, 2007), including *P. dominula* (Leadbeater *et al.*, 2011; Tibbetts and Reeve, 2003; but see Höcherl and Tautz, 2015a). Thus, our finding that there was an interacting effect of group size and days since nest initiation on productivity, supports current research. The use of correlative evidence to explore the effects of group size is questionable, however, because additional factors (e.g. territory size and quality of group members) that might correlate with group size can influence productivity. Unlike many cooperative breeding birds (Brown, 2014; Komdeur, 1992; Russell *et al.*, 2007), however, nests of primitively eusocial insects do not typically have feeding territories. The quality of the nearby food patches (e.g. food availability and predation risk) can vary, of course, and can sometimes be quantified (Seppä *et al.*, 2012). Obvious distinctions in quality cannot always be made, however, as was the case in our field sites. The cactus hedgerows on which the nests were attached, bordered either homogenous fields of crops or meadows. As such, variation was likely to be greater between sites, yet we found no effect of site on productivity. Individual quality, however, was quantified to some degree in our study. Mean body size and the proportion of the group with a clypeal mark both had a significant positive effect on group productivity. The importance of mean body size is perhaps

not surprising, given that larger *P. dominula* foundresses can carry heavier loads (Brown *et al.*, 2012).

What is somewhat surprising, however, is that the proportion of group members with a clypeal mark was important. Within the native European range of *P. dominula*, there is only limited evidence for a relationship between clypeal markings and dominance (Zanette and Field, 2009), a factor that might be linked to the quality of an individual. Furthermore, many nests lack any individuals with a clypeal mark (Zanette and Field, 2009). Studies on invading populations of *P. dominula* in the U.S., however, have shown that the 'brokenness' (a measure of spatial disruption; (Tibbetts, 2010) of the melanin pattern on the facial clypeus is a signal of status in dominance and competitive interactions (Tibbetts and Dale, 2004; Tibbetts and Lindsay, 2008; Tibbetts, 2010). Moreover, it has been shown to be an honest signal of RHP, with reliability being maintained through social costs (Tibbetts and Dale, 2004). In our study, clypeal marks could be signal of individual quality if for example, those individuals with marks have greater energy reserves (Tibbetts and Curtis, 2007). Further investigation would need to be carried out to confirm this, though our results suggests that the clypeal mark may be more important in European populations than previously thought.

Survival

We found no difference in nest survival between founding tactics, but larger groups had significantly higher survival (aim 3). Several studies have investigated whether survival differs between single and multiple foundress associations of *Polistes*, due to interest in why foundresses give up independent breeding. These studies are largely in agreement that multiple foundress nests have higher survival. (*P. dominula* - Höcherl and Tautz, 2015a; Leadbeater *et al.*, 2011; Queller *et al.*, 2000; Tibbetts and Reeve, 2003; Zanette and Field, 2011; but see Shreeves *et al.*, 2003; *Polistes annularis* - Strassmann, 1989; and *Polistes versicolor* – Oliveira *et al.*, 2010). It has been suggested that colony survival might increase in multiple foundress nests for several reasons: individual wasps have

higher survival in a multi foundress group (Nonacs and Reeve, 1995; Reeve, 1991; Tibbetts and Reeve, 2003), they may be better able to defend from usurpation than a solitary foundress (Gamboa *et al.*, 1978; Reeve, 1991), and multi foundress nests will rarely be left unattended, again leaving them less vulnerable to usurpation (Gamboa *et al.*, 1978, 1992; Reeve, 1991). Whether the survival benefits are limited to the increase from a lone foundress to more than one, appears to be contentious. Two studies in a Spanish population of *P. dominula* found that multiple foundress nests of different group sizes had similar failure rates (Shreeves *et al.*, 2003; Zanette and Field, 2011). These findings conflict with those of Leadbeater and colleagues (2011) from the same population, as well as Höcherl and Tautz (2015a) from a population in Germany. Our finding, that larger multi foundress groups benefit from increased colony survival in a Spanish population *P. dominula*, provides further supporting evidence. There is evidence for increased survival of individuals and / or colonies in larger groups across many taxa, including polistine wasps (Strassmann, 1989), termites (Miramontes and DeSouza, 1996), social spiders (Bilde *et al.*, 2007), birds (Brown *et al.*, 2003; Brown and Brown, 2004; Conner *et al.*, 2004; Khan and Walters, 2002; Serrano *et al.*, 2005; but see Brouwer *et al.*, 2006; Seddon *et al.*, 2003), fish (Balshine *et al.*, 2001; Brouwer *et al.*, 2005; Heg *et al.*, 2004; Heg *et al.*, 2005; Taborsky, Michael, 1984) and mammals (Clutton-Brock *et al.*, 1999; Courchamp *et al.*, 2002; Robinette *et al.*, 1995; Waterman, 2002; reviewed in Russell, 2004; but see Rogovin *et al.*, 2004). Overall, it seems that group size has an important influence over colony survival in *P. dominula*. Differences between studies may result from variation in ecological conditions between years, such as population density, food availability and predation pressure (Borries *et al.*, 2008; Gusset and Macdonald, 2010; Hass and Valenzuela, 2002; Hayes *et al.*, 2009).

Group size

Group founded nests have a larger group size than solitary founded nests, though the difference decreases prior to worker emergence (aim 4). The mean number of foundresses initiating group

founded nests was 2.5 (\pm s.e. = \pm 0.13). On day 30 after initiation, group and solitary founded nests differed by roughly 1 individual, with group nests having gained 2 joiners on average (group size = 4.6), and solitary founded nests having gained 2.5 (group size = 3.6). Thus, the difference in group size appears to primarily be a factor of group size at nest initiation. At day 50 after nest initiation, group size has dropped equally in both founding types, with group founded nests (group size = 3.6) still differing from solitary founded nests (group size = 2.7) by one member. At day 70, however, the discrepancy in group size appears to be reducing (0.5); group founded nests have lost an additional member (group size = 2.7), while solitary founded nests have lost only half a member on average (group size = 2.1). It is possible, if this reduced difference is real, that it comes about from reduced predation in solitarily founded nests. Group members are most vulnerable to predation when off the nest foraging (Nonacs and Reeve, 1995; Strassmann, 1981). As the worker phase approaches, all groups reduce the amount of time they spend foraging. It is possible that the reduction in foraging is relatively higher in nests with smaller group sizes. Nests with a single remaining member are at particular risk as the whole nest is likely to fail if the foundress dies when foraging. Thus, there should be strong selection for a single remaining foundress to remain on the nest when worker emergence is imminent, though this may slow development of those brood that still require food.

Given our findings, that group size differs between founding types and also affects productivity and survival, an important question is whether founding tactic is an active choice (i.e. cause), or the result of characteristics of the social environment leading up to nest founding (i.e. consequence). We will now consider how each of these scenarios might come about. Cause: ARTs have evolved in many animal societies in order to maximise fitness in different conditions (Taborsky *et al.*, 2008; Taborsky and Brockmann, 2010), including Hymenoptera (Foitzik *et al.*, 2010; Ratnieks *et al.*, 2006). Foundresses at the beginning of the founding stage may detect factors such as the availability of nesting sites (Gunnels, 2007), availability and quality of potential co-foundresses, as well as their own quality (e.g. RHP and / or fecundity; (Tibbetts, 2007). For example, the highest quality founding females may seek out potential co-foundresses, even if none are initially available in their

hibernacula, as they will likely gain greater fitness as a dominant in a group founded nest. A medium quality founding female might choose to found a nest alone, as by arriving first their chance of becoming the dominant increases. Consequence: Foundresses that hibernate in the same aggregation tend to found nests together in the spring (Dapporto *et al.*, 2004; Zanette and Field, 2011; but see Pratte, 1979). Different founding types may result from variation in the success of the natal nest, hibernation cluster size, and / or the number of those gynes ready to begin nest founding on a given day. Solitary founding may occur when an individual is from an unproductive natal nest, hibernated alone, was alone in surviving in that hibernacula, and / or was alone in exiting diapause. While group founding might occur when an individual is from a productive natal nest, where they hibernated as a part of a large group in which numerous others survived and / or exited diapause on the same date.

Founding females

Solitary founding wasps did not differ in size depending on whether they had a clypeal mark. In comparison, group founding females with a clypeal mark were larger, and those without a mark were smaller (aim 5). One explanation for this result is that the presence of two distinct classes of individual has been detected, perhaps related to rank, through the random selection of a single foundress from each group founded nest. Group founded nests are initiated by two or more foundresses, and as only one can become the dominant, the remainder/s must be subordinate/s or leave. It may be that, prior to beginning nest founding these groups had already started the process of forming a dominance hierarchy. This might have occurred in the hibernation cluster during the pre / post-hibernation stages (reviewed in Dapporto and Palagi, 2006), or alternatively, it may have occurred on the nesting substrate (e.g. cactus) just prior to nest initiation. In either case, randomly selecting a foundress from a group founded nest would ostensibly be the same as selecting a random individual in a solitary founded nest after it had gained its first joiner/s; sometimes the first-arriver / dominant would be selected, and sometimes the joiner / subordinate.

Thus, perhaps our results are explained by larger 'first-arrivers' with clypeal marks, and smaller joiners without them.

In Chapter 2, we found that solitary first-arrivers were larger than joiners, supporting the hypothesis that the difference in size between those group founding females with and without a clypeal mark may represent different categories (i.e. first-arriver and joiner). We did not find, however, any evidence that the presence or absence of a clypeal mark affected the likelihood of gaining the dominant position. Furthermore, the proportion of first-arrivers with a clypeal mark, did not differ from that of joiners. It is important to consider, however, that if solitary and group founding represent different ARTs, then foundresses employing each ART may exhibit different characteristics. For example, in this chapter we found that solitarily founding females with and without a clypeal mark do not differ in size. They are, however, slightly smaller than group founded females with clypeal marks. The size difference between solitary females and the larger group founding females might reflect the difference in fitness return between the dominant position in a group founded nest, compared to that of solitarily founded nest. In this chapter we have found that being the dominant in a group founded nest is likely to indirectly result in a higher fitness payoff, compared to a dominant in a solitary founded nest; as group founded nests are more productive and have greater survival, as a result of a larger group size (see above). Thus, the best quality founding females might be more likely to select the group founding tactic over the solitary founding tactic.

Summary

We found that only group size was directly affected by nest founding tactic. Solitary and group founded nests both gained roughly the same number of joiners on average, so group founded nests tended to have c.1 additional co-foundress than solitary founded nests. This difference has implications, however, as group size had a significant effect on both productivity and survival.

Larger groups had larger nests and were more likely to survive to produce workers. Within-group relatedness, on the other hand, was not impacted by either group size or nest founding tactic. Finally, there was some limited distinction in morphology between founding females employing the different reproductive tactics, consisting of an interaction between body size and clypeal mark. Solitary founding females did not differ in size based on whether they had a clypeal mark, but group founding females with clypeal marks were larger than those without. This may reflect the random sampling of groups formed prior to nest initiation, of larger 'first-arrivers' with clypeal marks, and smaller joiners without them.

Part II: *Ammophila pubescens*

Chapter 4: Intraspecific parasitism in the solitary digger wasp *Ammophila pubescens*

4.1 - Introduction

Many animals carry out parental care in order to increase the growth and/or survival of their offspring, and thus enhance their own fitness (Royle *et al.*, 2012). Parental care often comes at a cost to the parents' own survival and future reproduction, however, and to maximise their own lifetime reproductive success, parents must find an optimal balance between the benefits and costs of investment in individual offspring (Williams, 1966). Interspecific parasites attempt to minimise the costs of parental care by exploiting the investment of other species, and imposing those costs on the host (Davies, 2010). When this occurs between individuals of the same species it is known as intraspecific parasitism (IP; also known as: Intraspecific brood parasitism - McRae and Burke, 1996; egg dumping - Tallamy, 2004; or conspecific brood parasitism - Lyon and Eadie, 2008), as it too is thought to impose the cost of parental care on the host (Åhlund, 2001; Andersson, 1982; Craik and Titman, 2009; Eadie, J.M., 1989; Jaatinen *et al.*, 2009b; Lyon and Eadie, 2008). The cost imposed by IP may be the result of reduced survival of host brood, or nest desertion by the host (Åhlund, 2001; Andersson and Eriksson, 1982; Craik and Titman, 2009; Eadie, 1989; Jaatinen, *et al.*, 2009b; Waltho and Coulson, 2015). Despite these potential costs to the host, there can also be benefits, such as higher survival of host offspring in larger broods (Eadie and Lumsden, 1985; Öst *et al.*, 2008), and

reduced level of predation through dilution and perimeter effects (e.g. Lace bug *Gargaphia solani*; Loeb, 2003).

IP is an Alternative Reproductive Tactic (ART). Most documented ARTs are carried out by males (Alonzo, 2008; Taborsky and Brockmann, 2010), whereas IP involves females laying eggs in the nests of other females (or hosts) of the same species. This occurs in a wide range of taxa, including amphibians (Harris, 2008; Summers and Amos, 1997), birds (Lyon and Eadie, 2008; Yom-Tov, 2001), fish (Taborsky, 2008) and insects (Tallamy, 2004; Field, 1992). The majority of research effort has been focused on birds, which, amongst other advances that will be discussed below, has resulted in a shift in our understanding of the relationship between kin selection and IP; advances that have yet to be applied to IP in insects.

In this chapter we capitalise on the progress made with birds, to investigate IP in a subsocial species of digger wasp, *Ammophila pubescens*, in which IP is reasonably common (2012-13: 10% of nests; Field et al., in prep). Previously studied by Baerends (1941) and Tinbergen (1958), the species is locally abundant in the south of England, aggregating their nests in areas of bare ground within their dry heathland habitat (see Chapter 1: General Introduction). Recent evidence has shown that provisioning in this species is costly in terms of lifetime reproductive success (Field et al., 2007). In this study, we test whether genetic relatedness, distance between host and parasite nests, nest density and body size influence the occurrence of IP. We first discuss these factors in the light of past research.

Factors influencing IP

Relatedness

IP is particularly common in waterfowl, in which there is an unusually high level of female natal philopatry (Anderson et al., 1992; Andersson, 1982; Eadie et al., 1988). As such, local females are

often related (Andersson and Waldeck, 2007; Eadie and Lyon, 2011; Gong *et al.*, 2016; Nielsen *et al.*, 2006; Pöysä *et al.*, 2014b). Long before an attempt was made to validate the idea, Andersson (Andersson 1984) theorised that due to this clustering of relatives, so called parasitism within the species may be between close relatives, and therefore may not be costly to the host. There is now a growing body of evidence in waterfowl that many of the host- parasite pairs in IP are actually closely related (Andersson *et al.*, 2015; Andersson and Åhlund, 2000; Andersson and Waldeck, 2007; Gong *et al.*, 2016; Jaatinen *et al.*, 2009a, 2011a; McRae and Burke, 1996; Moore *et al.*, 2012; Nielsen *et al.*, 2006; Pöysä *et al.*, 2014a, 2014b; Tiedemann *et al.*, 2011; Waldeck *et al.*, 2008; but see Anderholm *et al.*, 2009; Pöysä, 2004; Semel and Sherman, 2001), and that in some cases kin recognition may be involved (Andersson *et al.*, 2015; Jaatinen *et al.*, 2011a; Pöysä *et al.*, 2014a). These findings have caused IP to be considered in a new context, that of kin selection (Hamilton, 1964). It seems that this spatial clustering of relatives may be the perfect context to facilitate the evolution of kin-selected female cooperation, which at first glance seems like parasitism (Andersson, 1984, 2001; Dickinson and Hatchwell, 2004; Zink, 2000; Zink and Lyon, 2015). Providing that the indirect benefit is sufficient to compensate for any costs, hosts will increase their fitness by accepting only the brood of closely related conspecifics (Andersson, 2001; Jaatinen *et al.*, 2011b; López-Sepulcre and Kokko, 2002).

IP has been described in a number of insects species (reviewed in Tallamy, 2004), yet to our knowledge, the relationship between relatedness and IP has only been investigated in one insect species, the Lace bug *Gargaphia solani*. Loeb *et al.* (2000) found that host-parasite pairs of *G. solani* were often related, and that parasites preferentially selected kin as hosts. *G. solani* hosts, on the other hand, do not discriminate between related and unrelated parasites, almost certainly because the host gains direct benefits from being parasitized, in this case through dilution and perimeter effects (Loeb, 2003). Parasites lay their eggs around the perimeter of the host's clutch, and these hatch into nymphs that feed on the host plant. The host guards the clutch by patrolling the perimeter from predators, which steal eggs / nymphs starting at the perimeter and moving in. Thus,

the parasite's brood provide a perimeter and dilution effect on the host's brood, raising her direct fitness. *G. solani* populations also exhibit spatial structuring of relatives, similar to the patterns found in waterfowl species through the effect of natal philopatry. In the case of *G. solani*, however, the spatial pattern is brought about by extremely short dispersal distances, with sisters inhabiting neighbouring host plants and often parasitizing neighbours. It has been argued that the IP behaviour displayed in solitary Hymenoptera, unlike *G. solani*, is unambiguously parasitic (Eickwort, 1981; Tallamy, 2004), as it imposes a large cost on the host. This is because, again unlike *G. solani*, it is typical for sphecids wasps such as *A. pubescens* to produce several nests, each with only a single offspring. When a parasitism event occurs, the egg is removed and replaced with that of the parasite. Thus, IP in such a system seemingly offers no compensation or benefit to the host, only cost.

In this chapter, we will test in *A. pubescens* whether 1) host-parasite pairs are closely related; and 2) there is spatial clustering of relatives within the aggregation.

Distance and density

In a population with spatial structuring of relatives, host-parasite relatedness could be high incidentally due to parasites choosing nearby nests (Anderholm *et al.*, 2009; Gong *et al.*, 2016; Jaatinen *et al.*, 2009a). The distance that individuals travel to parasitize conspecifics is interesting for other reasons too. By spending time at a nest providing parental care to their brood, the owner would be expected to gain more information about its surrounding area than elsewhere. This might include the location of other nests, the stage of development of the brood, whether the owner is currently at the nest, and perhaps whether they are a close relative. This information could be useful if the individual later chooses to parasitize a conspecific nest (Jaatinen *et al.*, 2009a).

In fact, it has been theorised in both birds (Andersson, 1984) and Hymenoptera (Eickwort, 1981), that aggregated nesting in species that do not interact socially has come about as it increases the

efficiency that individuals can search for opportunities to usurp nests and provisions from conspecifics. Those same individuals are presumably putting themselves at greater risk of predation, however, by choosing to nest close to others. Most gregarious nesting hymenopterans do seem to spend at least some time searching for the nests of conspecifics (Eickwort, 1981; Field, 1989a; Zobel and Paxton, 2007). There is no direct evidence supporting this proposition, however, though there is considerable evidence for a relationship between density and rate of IP in birds (Hill *et al.*, 1997; Lank *et al.*, 1990; Lyon and Everding, 1996; Saitou, 2001; Semel *et al.*, 1988; Vedder *et al.*, 2007; Waldeck *et al.*, 2004; - but see Moore *et al.*, 2012), megachilid bees (Eickwort, 1975) and *Publilia* treehoppers (Zink, 2003); however in some cases this seems to be due to limited nesting sites (e.g. cavity nesting birds; (Saitou, 2001; Semel *et al.*, 1988; Vedder *et al.*, 2007). Studies investigating this relationship have focused on its expression on a broad scale, comparing rates of IP and nest density between different populations, or between different years in the same population. On a finer scale, however, nests are not uniformly spaced within a population / aggregation. Nest A might have 2 other nests within a set radius, but nest B might have 10 within the same area. Thus, if nesting individuals are actively seeking, and gaining information about nearby nests aids this process, then an individual nesting in a relatively dense part of the aggregation may be more likely to parasitize. This same pattern could also be brought about by chance, however, if foreign wasps parasitize nests opportunistically (i.e. without seeking them out), the probability of coming across a conspecific's nest is also likely to increase with higher nest density. Differentiating between these two possible underlying mechanisms is beyond the scope of this study.

In this chapter, we will test in *A. pubescens* whether 3) the mean distance between a parasites nest and that of the host affects the likelihood of parasitism; and 4) the density of active nests around the hosts nest affects the likelihood of parasitism.

Body size

When investigating the characteristics of individuals involved in IP, both those that become parasites and those that become hosts are of interest. Focusing first on the characteristics that might explain which individuals become parasites, there are two competing theories. First, females are of low quality, and are parasitizing because they have limited alternative options. Second, they are of high quality, allowing them to make use of opportunities to parasitize in addition to their own nesting attempts. It was originally thought that the individuals that chose to parasitize others were likely to be of low quality. If females were limited in their ability to provide parental care (e.g. poor body condition or loss of nest), they might choose to parasitize another nest as the “best of a bad job” (Eadie *et al.*, 1988; Lyon, 1993; Yom-Tov, 1980). Later evidence in birds, however, suggests that by laying some eggs parasitically, individuals can increase their fitness (Åhlund and Andersson, 2001; Brown and Brown, 1998; Tallamy and Horton, 1990; Zink, 2003), and so perhaps IP may represent an opportunistic strategy for better quality females.

We can test these hypotheses by using measures of quality that correlate with body size. One measure of quality could be the ability to provision food for offspring, as this can have a large effect on resulting insect body size (Quezada-Euán *et al.*, 2011; Radmacher and Strohm, 2010; Sutcliffe and Plowright, 1990). In flying insects, such as in the nest building Hymenoptera, body size correlates positively with load carrying capacity (Asís *et al.*, 2011; Brown *et al.*, 2012; Coelho and Hoagland, 1995; Dillon and Dudley, 2004; but see Polidori *et al.*, 2009). Thus, if an individual's carrying capacity is sufficiently low that it limits her ability to provision her own brood, she may parasitize a conspecific as the “best of a bad job”. Similarly, parasitism might occur in birds if their own nesting attempt fails, or they are unable to find a suitable nesting site (Saitou, 2001; Semel *et al.*, 1988; Vedder *et al.*, 2007). Another important measure of female quality is fecundity, which has also commonly been found to correlate positively with body size in insects (Graziosi and Rieske, 2014; Honěk, 1993; Hood and Ott, 2017; Omkar and Afaq, 2013; Ong *et al.*, 2014; but see Bosch and Vicens, 2006; Moczek and Cochrane, 2006). In the context of IP, higher fecundity may enable an

individual to use spare eggs to parasitize others, in addition to laying eggs in their own nests, and so potentially increase their fitness. Their maximum possible fecundity may even exceed what they could provision for, and so parasitism enables their fecundity potential to be met.

When considering the characteristics of hosts, it is important to take into account that in species where IP is an established ART for females, available information on the nests of nearby conspecifics is likely to inform host selection in order to maximise a parasite's fitness gains (Pöysä *et al.*, 2014a). This has been well established in obligate parasites (Davies, 2010), and though selection pressures are likely to be greater in their case, there is evidence for potentially large fitness gains in at least some species that exhibit IP (Åhlund and Andersson, 2001). Thus, it is important to consider host characteristics in the context of parasite choice. As stated above, body size commonly correlates with load carrying capacity in flying insects, so a parasite might be unlikely to select a host which is limited in this respect, as this would affect the success of the parasite's offspring. There are two plausible ways in which a parasite may gain information about the body size a potential host. First, if the host visits their nest when the parasite is in the locality, so the parasite directly observes the individual. This is likely to be the most accurate source of host body size information, but will not always be available. Second, when the parasite opens the nest of the host, the size of the burrow may provide information about the size of owner. For example, burrow size often correlates with the body size of the owner (reviewed by Roulston and Cane, 2000). This cue to potential host body size will be readily available on entry to the nest, but is likely to be less accurate than a direct observation.

A second host character that could affect the fitness of a parasite is a host's acceptance threshold. A host typically benefits by recognising that it has been parasitized, and subsequently ejecting the parasite's brood. The acceptance threshold is the bar that relevant cues are compared against, and the decision of acceptance or rejection is made. Kin recognition, in which close kin may be preferentially aided over non-kin, for example by acceptance into nests or social groups, is one such context that has received considerable attention (Breed *et al.*, 2004; Butler and Free, 1951; Couvillon

et al., 2009, 2013; Gamboa *et al.*, 1996; Getz and Page, 1991; Hölldobler and Wilson, 1990; Jones *et al.*, 2012; Moore *et al.*, 1987; Reeve, 1989; Wilson, 1971). Recognition errors can occur, however, either by incorrect acceptance of an unrelated individual, or rejection of a relative. Both can be costly, but optimal acceptance threshold theory predicts that the discriminator will seek to minimize the net cost by behaving more or less permissively, depending on context (Couvillon *et al.*, 2009, 2013; Jones *et al.*, 2012; Reeve, 1989). The fecundity of an individual could conceivably influence this threshold. In the case of *P. dominula*, the availability of excess eggs (i.e. a larger, more fecund wasp) could reduce the cost of replacing an egg when parasitized. Thus, a more fecund individual might be willing to reject an egg with a lower level of certainty that it is foreign. A nest owner might also lower her permissiveness if she detects that she is in a high nest density area, due to the potentially higher risk of a parasitism event.

In our 2018 study, using data employed in this chapter, Field *et al.* (In prep.) showed that the same *A. pubescens* individuals acted as both parasite and host within the population. There is variation, however, in the ratio between the number of times wasps parasitize, and that they themselves are parasitized. The fitness that an individual gains will reflect that ratio, with wasps that parasitize more and host less likely having higher fitness.

In this chapter, we will test in *A. pubescens* whether whether absolute body size affects the proportion of time that wasps parasitize vs being parasitized (aim 5).

Aims

To reiterate, in this chapter we will investigate how the factors discussed above influence IP in *A. pubescens*. Specifically, we will be asking whether: 1) the host-parasite pairs have an elevated level of relatedness above the background level in the population; 2) there is relatedness structure in the population; 3) the mean distance between a parasites nest and that of the host affects the likelihood of parasitism; 4) the density of active nests around the hosts nest affects the likelihood of

parasitism; 5) the size of parasites affects the proportion of time they parasitize vs being parasitized.

4.2 - Methods

A. pubescens study system

A. pubescens digger wasps produce a series of L-shaped nests, aggregating them in areas of bare ground within their dry heathland habitat. Each nest contains a single offspring, which the mother provisions with prey in the form of paralysed lepidopteran caterpillars. The species is a progressive provisioner, with food being provided gradually as the offspring develops. Specifically, a newly dug nest is supplied with an initial prey item, to which the owner attaches her egg. She then returns some days later to assess the status of her offspring (Baerends, 1941). If acceptable, the mother will then provision the remainder of the required prey.

Between digging and the start of the main provisioning bout, an *A. pubescens* nest is particularly vulnerable to being opened and entered by conspecifics (see Chapter 1: General Introduction). After entering, a foreign wasp (hereafter, 'the parasite') typically removes the prey item bearing the owner's (host's) brood. She then typically brings a new prey item to the nest, upon which she lays her own egg, then re-sealing the burrow. Thus, when the host returns for her assessment visit, she discovers the parasite's brood on a different caterpillar. At this point the host may either accept the parasites brood and carry out the main provisioning bout, or reject it, and replace it with a new prey item and egg of her own (Field *et al.*, In prep).

Morphological data collection and unique identification

Having selected a study area, over the next 1 – 2 days *A. pubescens* (hereafter referred to as ‘wasps’) adults active in, or traversing, the nest aggregation were caught using a butterfly net. Each individual was checked to confirm the species, as *A. sabulosa*, a very similar looking species, nests in the same habitat. All wasps were then given a unique combination of three enamel paint spots on the thorax. Wing length was measured using callipers (RS Pro digital calliper) themselves accurate to 0.01 mm. Due to limitations of human accuracy, however, wing length was recorded to nearest 0.1 mm. Our measurement of wing length was used as a proximate measure of body size, as it is known to correlate with overall body size in wasps and bees (Sullivan and Strassmann, 1984).

Finally, before releasing each wasp, the distal half of an antenna was removed from each female and stored individually in alcohol. This DNA sample was used for determining relatedness using micro-satellite markers. A pilot study, in which the behaviour of wasps with and without antennae removed was compared, found no effect of the removal on behaviour, including the frequency of parasitism (Field *et al.*, In prep).

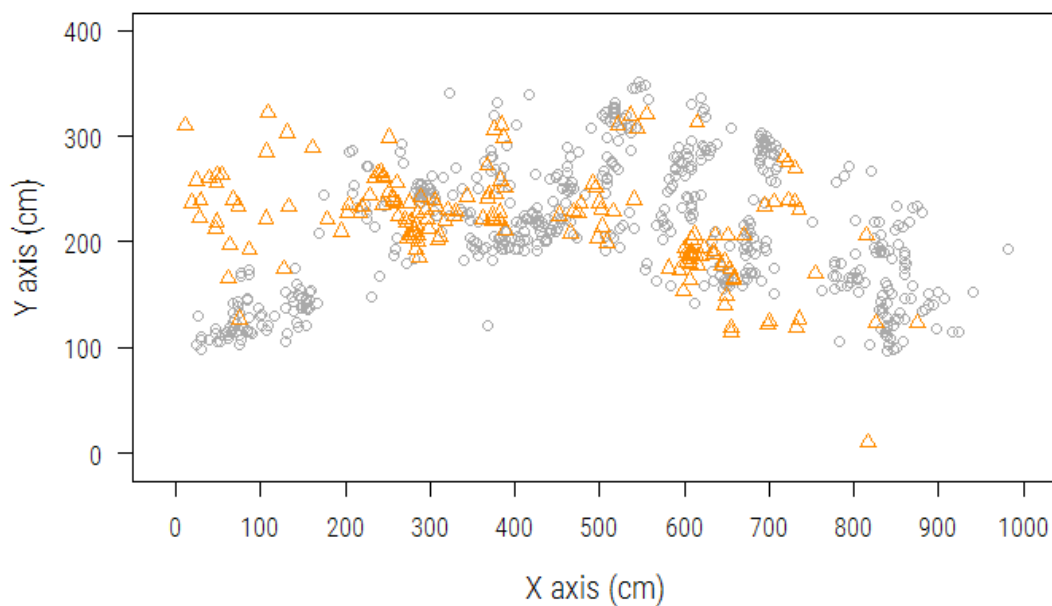


Figure 1 – Plot of *A. pubescens* nest locations at our study site. Orange triangles are nests from 2012 and grey circles are nests from 2013.

Behavioural observations

Once the majority of the wasps were marked, behavioural observation commenced, and were carried out throughout the active period of the wasps. This was roughly between 8am and 7pm every day, unless raining or particularly cold. Observations were carried out from beside the nest aggregation (c. 2m away), close enough to see behaviour by eye and / or using close focusing binoculars, but far enough to minimize disturbance. The location of each nest was marked using a small numbered flag and nail (see picture 3). All behaviours at nests were recorded (e.g. digging, entry, provisioning prey, laying egg, prey removal, fighting, closing etc.). For each recorded event, the date, time, wasp ID/s and nest number were also recorded. Observations covered the period of 23rd of July to 28th of August 2012, and 1st to 29th of July 2013. At the end of each field season the location of every nest was mapped, accurate to the nearest 1cm (figure 1).

Genotyping and relatedness

Genotyping was carried out and pairwise relatedness calculated by Field et al., (In prep), using 17 microsatellite loci. The genotyping work was not part of this thesis and so I will simply use the calculated relatedness values between each pair of individuals in the population provided by J.P. Field.

4.3 - Statistical analysis

All statistical analyses were carried out using R Studio version 1.0.136 (<https://www.rstudio.com>) and R version 3.2.3 (<http://www.r-project.org>).

Multiple comparisons

When setting a significance threshold (or alpha) of 0.05, for example, it is accepted that there is 5% chance the observed result may have come about even though the null hypothesis is true, otherwise known as a false positive result. Conducting multiple statistical tests, or comparisons, on a dataset can lead to an increase in false positive results (Forstmeier *et al.*, 2016). I take this into account in this chapter by moderating my conclusions on analyses where P values come just within the acceptance threshold.

Permutation method

All the analyses in this chapter share the same general permutation method. Thus, I will describe the first of these in greater detail, and in subsequent analyses just highlight the differences.

Relatedness

The aim of this analysis was to ascertain whether the pairwise relatedness (r) between the host and potential parasite affects the likelihood of a parasitism event occurring (aim 1). To that end, we compared the mean observed value of r , with those attained by randomly selecting (with replacement) host and parasite from the population.

A pool of candidates was initially constructed for each parasitism event, starting with the potential hosts. For a host nest to be included in the pool of a given event, the nest must have been active and the owner alive on the date (including the year) of that event. Then, a pool of potential parasites, each of which again must have been alive on that date, was compiled for each potential host. Thus, when a record was later randomly selected from this pool, both a random host and parasite was selected. The mean value of r was calculated for each set of 115 randomly selected samples (i.e. across both years), and the process of random selection and calculating the mean was repeated

10,000 times to produce a null distribution of mean r . A two-sided P value was then estimated based on the proportion of simulated (absolute) values, equal to or greater than the observed (absolute) value. Correlations were considered significant if $P \leq 0.05$.

Spatial structure in relatedness

The aim of this analysis was to ascertain whether there is clustering of relatives within *A. pubescens* aggregations (aim 2). To that end, we compared the mean observed value of r between the host and the owners of the 10 closest nests, with those attained by randomly selecting (with replacement) host and 10 'closest' nests from the population. We also compared the variance of the observed data against the variance calculated from the same randomly selected records.

Finally, we carried out a mantel test (Dray and Siberchicot, 2018) to test for a possible correlation between the pairwise relatedness and euclidian distance between the wasp's nest centroids. Wasps cluster their nests within the population and the centroid represents the centre of a wasp's nest locations. A wasp's nest centroid, consisting of an X and Y coordinate, is determined by calculating the mean of the X coordinates of all her nests, followed by the mean of the Y coordinates.

Distance from parasite's nest/s

The aim of this analysis was to ascertain whether distance between the host's nest and foreign wasp's active nests affects the likelihood of a parasitism event occurring (aim 3). To that end, we compared the mean observed distance between the host's nest and those active nests belonging to the parasite, with those values attained by randomly selecting (with replacement) host and parasite from the population. We also compared the variance of the observed data against the variance calculated from the same randomly selected individuals. Finally, to test whether the frequency distribution of the observed data differs from a uniform distribution, we performed a one-sample Kolmogorov-Smirnov test.

Nest density

The aim of this analysis was to ascertain whether the density of active nests around a host nest affects the likelihood of a parasitism event occurring (aim 4). To that end, we compared the mean observed density of nests around a host's nest, at 4 different spatial scales, with those values attained by randomly selecting (with replacement) the host from the population. We also compared the variance of the observed data against the variance calculated from the same randomly selected data.

Body size

The aim of this analysis was to ascertain whether the absolute body size affects the proportion of time spent engaged with parasitism versus being parasitized. To that end we carried out a Generalized Linear Model (GLM) with a binomial error distribution, where the response variable was the proportion of the time that an individual spent parasitizing vs being parasitized. The potential explanatory variables considered were: absolute body size and year. VIFs were very low (absolute body size = 1.003, year = 1.003), so all considered explanatory variables were initially included. Explanatory terms were considered significant if $P \leq 0.05$.

4.4 - Results

Morphological data were collected from c.95 wasps over 2 years (2012 = 36; 2013 = 59), of these, 88 wasps were observed to have an active nest in our aggregation during our observation period. In 2012, each wasp had on average 4.68 nests \pm 0.42 (mean \pm s.e.; figure 2 [grey]), which had a mean distance of 382.6cm \pm 33.3cm (mean \pm s.e.; figure 2 [orange]) between each other, and were active for 10.1 days \pm 0.61 (mean \pm s.e.), between being dug and the last visit by the owner. In 2013, each wasp had on average 9.8 nests \pm 0.75 (mean \pm s.e.), which had a mean distance of 463.7cm \pm 17.7cm (mean \pm s.e.) between each other, and were active for 4.3 days \pm 0.14 (mean

\pm s.e.), between being dug and the last visit by the owner. Pairwise relatedness within the population ranged between c. -0.3 and 0.8 (figures 3a for 2012; and 3b for 2013).

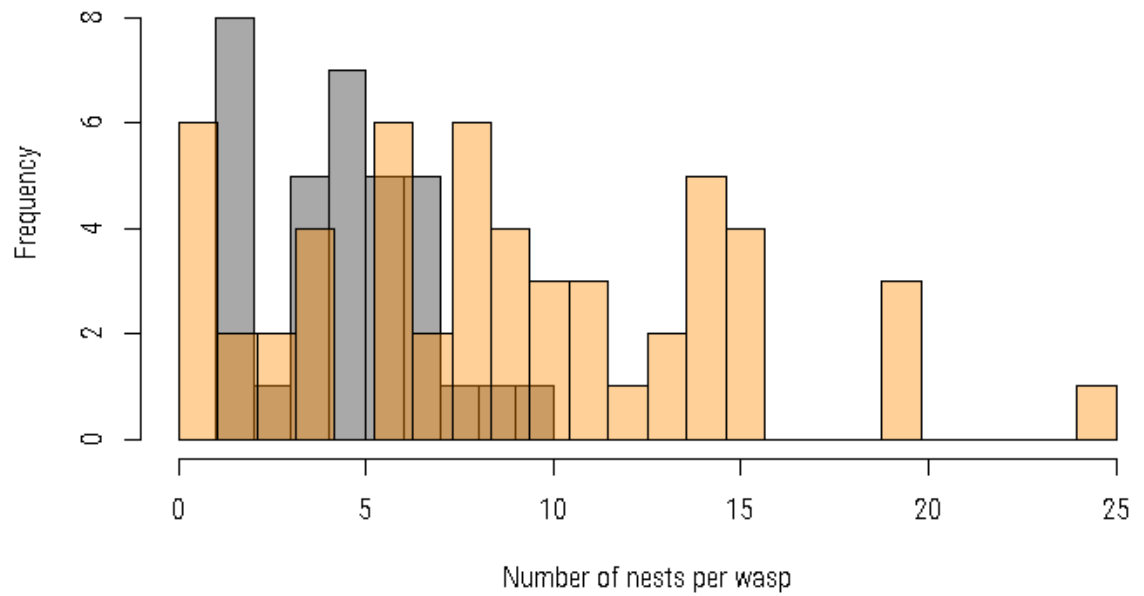


Figure 2 – Frequency distribution of number of nests per wasp for 2012 (grey) and 2013 (orange) populations.

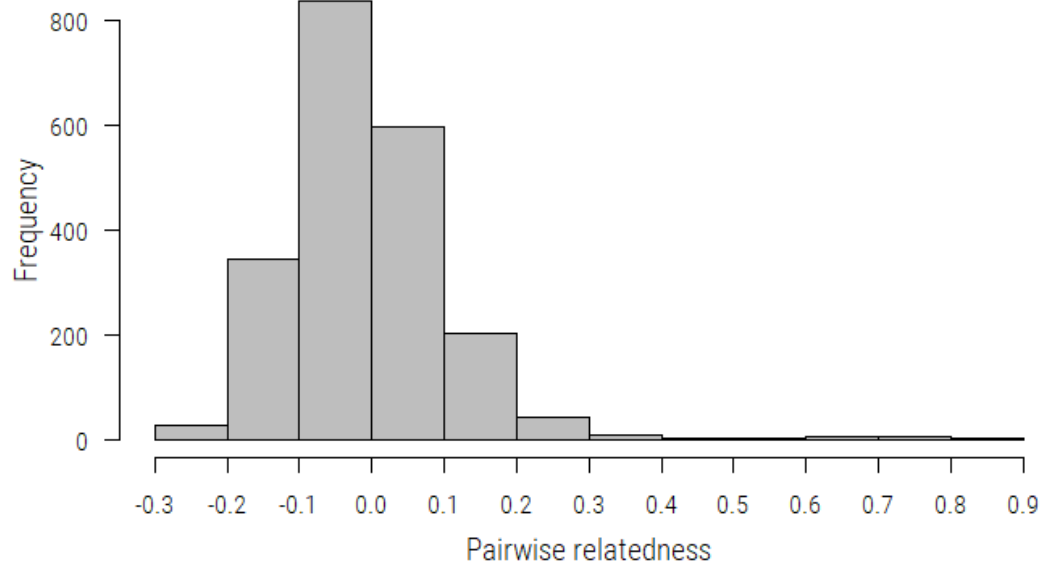


Figure 3a – Frequency distribution of observed pairwise relatedness for 2012 population.

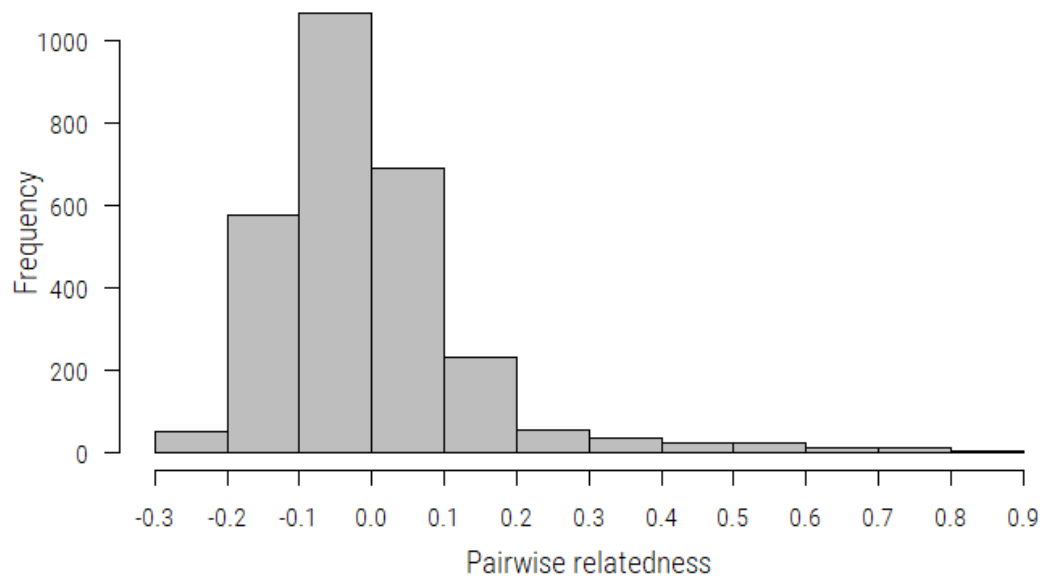


Figure 3b – Frequency distribution of observed pairwise relatedness for 2013 population.

Relatedness

Our result indicates that host-parasite pairs do not have an elevated level of relatedness above the background level in the population (aim 1). The mean pairwise relatedness between the observed hosts and their parasites was 0.0033 (mean; figure 4a). There was no significant difference between the observed value and that obtained based on randomly selected hosts and parasites from the population (mean = -0.0069, $n = 115$, $P = 0.45$; figure 4b).

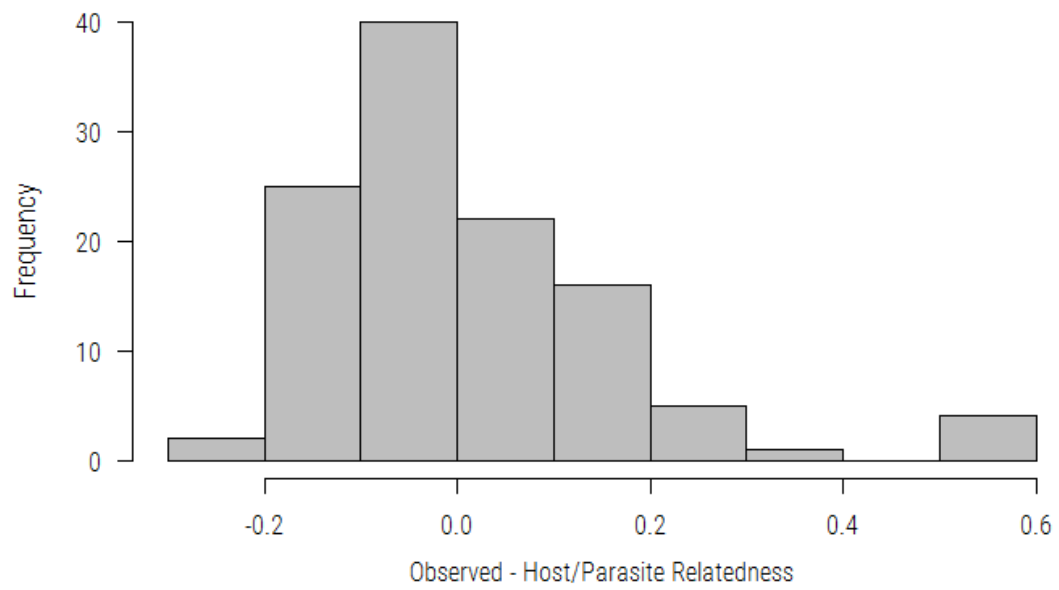


Figure 4a – Frequency distribution of observed host/parasite pairwise relatedness.

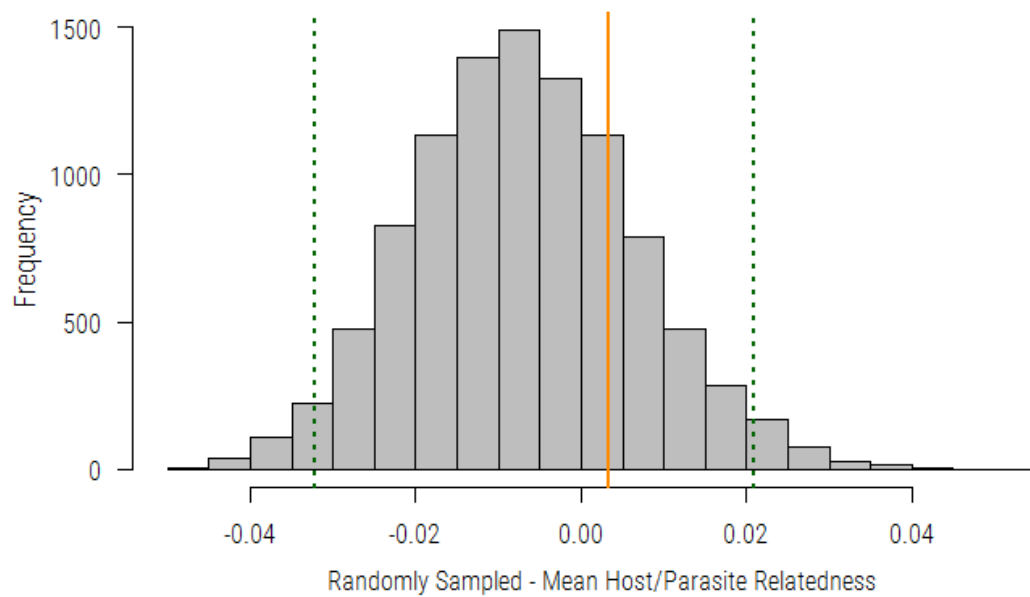


Figure 4b – Frequency distribution of mean pairwise relatedness for randomly sampled hosts and parasites.

The orange line shows the mean observed value. The dark green dotted lines show the upper and lower limits of the two-tailed permutation test. The test is significant when the orange line is above the upper limit, or below the lower limit.

Spatial structure in relatedness

Our result suggests that relatives do not cluster together within the aggregation, but that they may actually avoid each other (aim 2). The mean cluster relatedness between the observed hosts and the owners of the closest 10 nests was -0.026 (mean; figure 5a). There was a significant difference between the observed value and that obtained when the host nest and the 10 'closest' nests are randomly selected from the population (mean = -0.0079, $n = 115$, $P = 0.0022$; figure 5b). There was no difference, however, between the observed variance (variance = 0.0023), and that of the randomly selected samples (variance = 0.0026, $n = 115$, $P = 0.6$). The results from mantel tests for 2012 (correlation = -0.021, $n = 34$, $P = 0.67$) and 2013 (correlation = -0.053, $n = 55$, $P = 0.97$) find no relationship between pairwise relatedness and distance between conspecific's nest centroids.

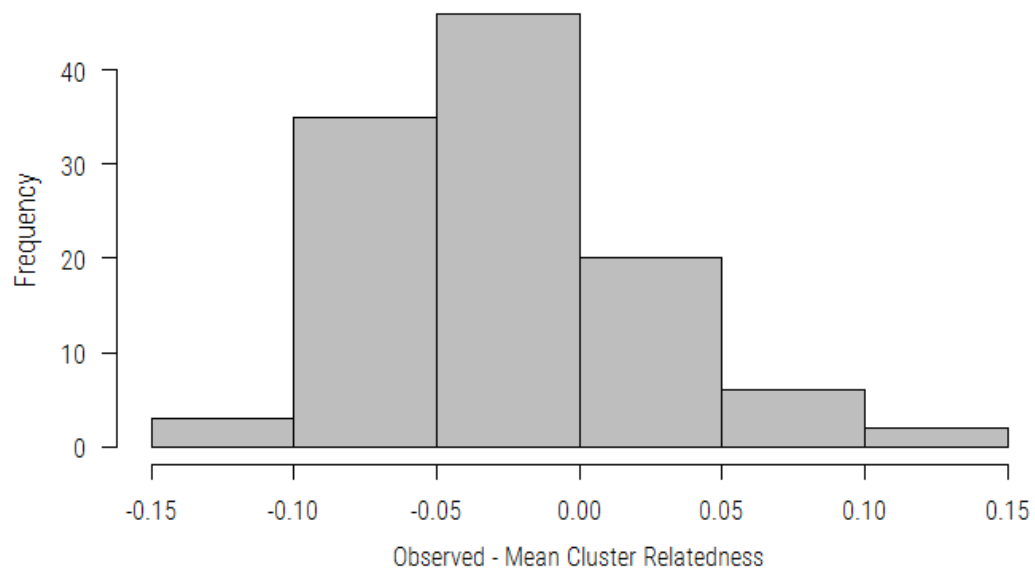


Figure 5a – Frequency distribution of observed mean cluster relatedness between host and owners of 10 closest nests.

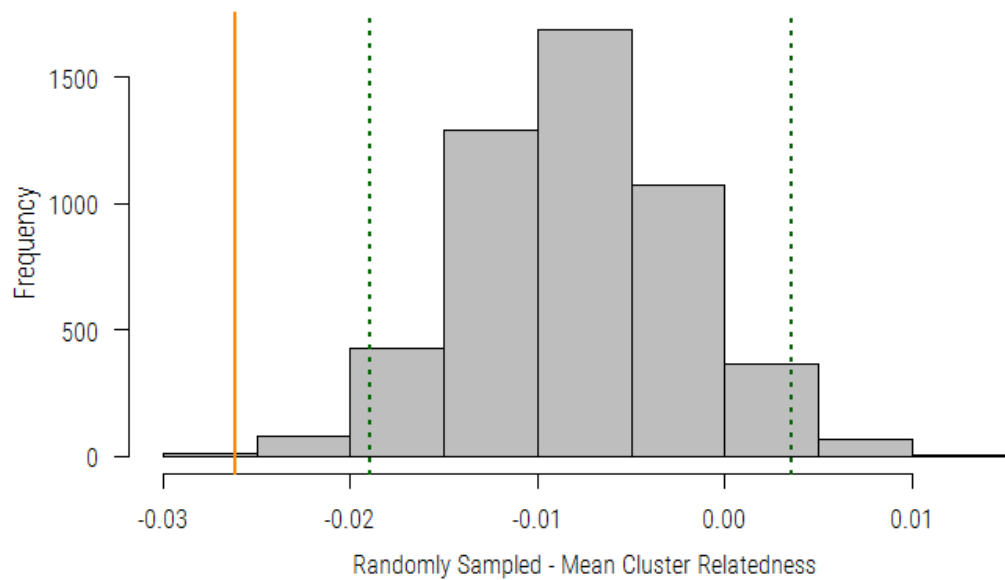


Figure 5b – Frequency distribution of mean cluster relatedness for randomly sampled hosts and randomly sampled 10 closest nests. The orange line shows the mean observed value. The dark green dotted lines show the upper and lower limits of the two-tailed permutation test. The test is significant when the orange line is above the upper limit, or below the lower limit.

Distance from parasite's nest/s

Our result suggests that the mean distance between a parasite's nests and that of the host does not affect the likelihood of a parasitism event occurring (aim 3). The mean observed distance between host nests and their parasites active nests was c. 506.4 cm (mean; figure 6). This did not differ from the values obtained based on randomly selected hosts and parasites from the population (mean = 502.3 cm, $n = 115$, $P = 0.86$). Variance, however, did differ between the observed value (variance = 70601) and that obtained by randomly selecting hosts and parasites from the population (variance = 47421, $n = 115$, $P < 0.01$). Furthermore, the frequency distribution of the observed data was not found to differ significantly from a uniform distribution (One-sample KS test, $D = 0.11$, $P = 0.12$).

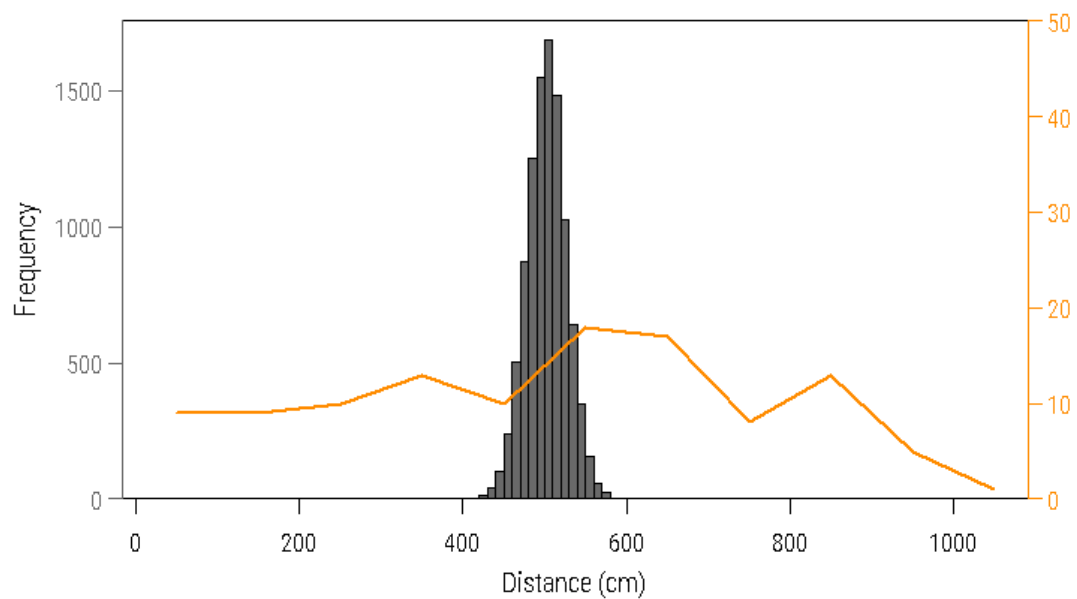


Figure 6 – This figure depicts a histogram and density plot which are scaled differently on their y-axes to allow for better comparison of the data. In grey, is the frequency distribution of the mean distance (cm) between the nests of randomly sampled hosts and their parasites, this is associated with the Y-axis on the left of the plot. In orange is a density plot of the frequency distribution of observed mean distance between hosts and parasite's active nests.

Nest density

Our results suggest that the density of active nests around a host's nest does not affect the likelihood of it being parasitized (aim 4). There was no significant difference between the mean density of active nests around observed parasitized nests, and the mean density around randomly selected host nests. This was the case at all 4 scales at which density was calculated: 1.5m² observed density (mean = 4.5; figure 7) vs random density (mean = 4.7, $n = 115$, $P = 0.85$); 3m² observed density (mean = 2.52; figure 8) vs random density (mean = 2.77, $n = 115$, $P = 0.68$); 6m² observed density (mean = 1.81; figure 9) vs random density (mean = 2, $n = 115$, $P = 0.52$); and 12m² observed density (mean = 1.31; figure 10) vs random density (mean = 1.46, $n = 115$, $P = 0.52$). There was also no difference in the observed variance and that of the randomly selected values. This was again true across all 4 scales used in this chapter: 1.5m² observed density (variance = 113) vs random density (variance = 109, $n = 115$, $P = 0.87$); 3m² observed density (variance = 26) vs random density (variance = 27, $n = 115$, $P = 0.94$); 6m² observed density (variance = 11) vs random density (variance = 11, $n = 115$, $P = 0.77$); 12m² observed density (variance = 5) vs random density (variance = 5, $n = 115$, $P = 0.86$).

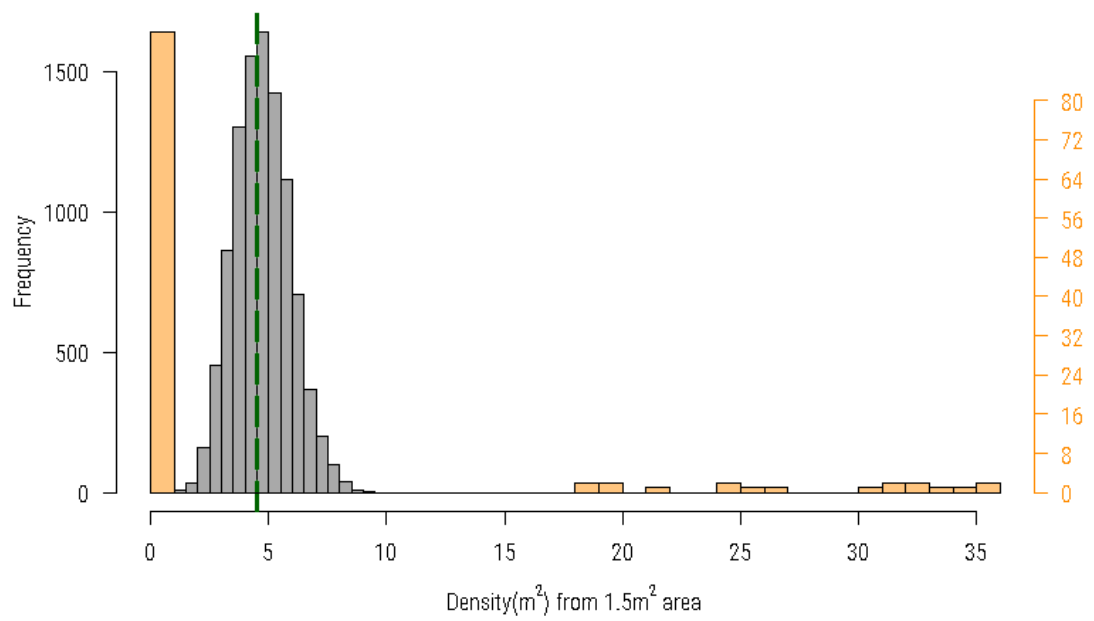


Figure 7 – This figure depicts 2 histograms which are scaled differently on their y-axes to allow for better comparison of the data. Nest density was calculated over an area of 1.5m² around host nests. In grey, is the frequency distribution of the mean density of active nests around randomly selected host nests, which is associated with the Y-axis on the left of the plot. In orange is the frequency distribution of density around observed host nests from the same area. The dashed green line shows the mean observed value.

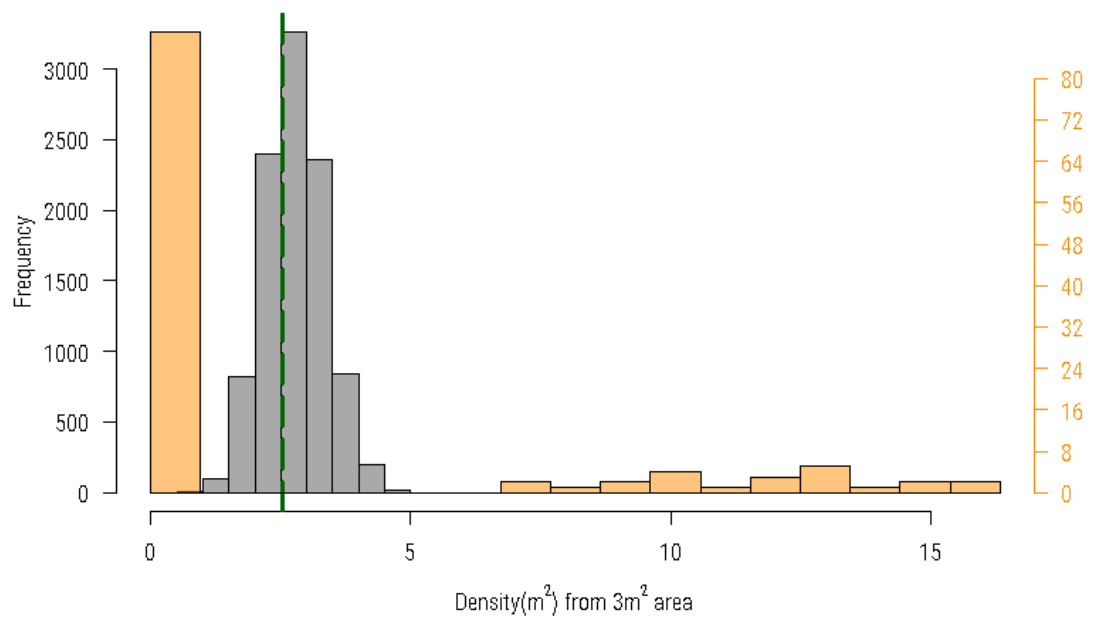


Figure 8 – This figure depicts two histograms which are scaled differently on their y-axes to allow for better comparison of the data. Nest density was calculated over an area of 3m² around host nest. In grey, is the frequency distribution of the mean density of active nests around randomly selected host nests, which is associated with the Y-axis on the left of the plot. In orange, is the frequency distribution of density around observed host nests from the same area. The dashed green line shows the mean observed value.

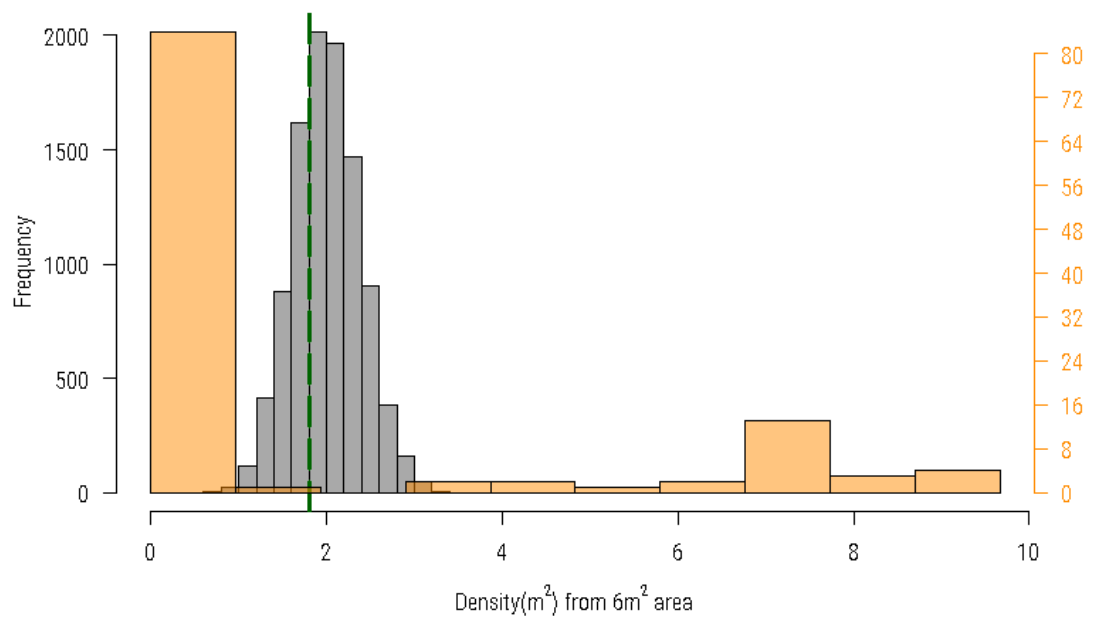


Figure 9 – This figure depicts two histograms which are scaled differently on their y-axes to allow for better comparison of the data. Nest density was calculated over an area of 6m² around host nest. In grey, is the frequency distribution of the mean density of active nests around randomly selected host nests, which is associated with the Y-axis on the left of the plot. In orange, is the frequency distribution of density around observed host nests from the same area. The dashed green line shows the mean observed value.

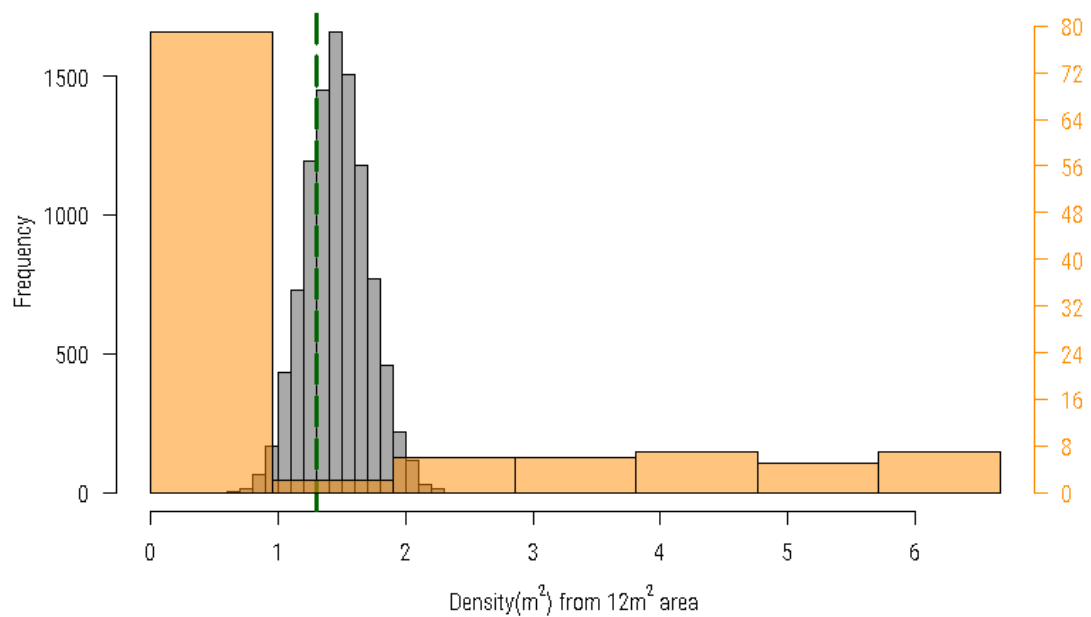


Figure 10 – This figure depicts two histograms which are scaled differently on their y-axes to allow for better comparison of the data. Nest density was calculated over an area of 12m² around host nest. In grey, is the frequency distribution of the mean density of active nests around randomly selected host nests, which is associated with the Y-axis on the left of the plot. In orange, is the frequency distribution of density around observed host nests from the same area. The dashed green line shows the mean observed value.

Body size

We found no effect of body size ($n = 58$, $\chi^2_1 = 0.8$, $P = 0.37$) or year ($n = 58$, $\chi^2_1 = 0.0074$, $P = 0.93$) on the proportion of time wasps spend parasitizing vs being parasitized. This result suggests that the size of an individual does not affect the proportion of time spent engaged with parasitism vs being parasitized.

4.5 - Discussion

Our main finding in this chapter is that parasites do not restrict their selection of host's nests to those in the locality of their own nests, but instead parasitize throughout the aggregation.

Relatedness

In our population of *A. pubescens* we found that the mean pairwise relatedness of host-parasite pairs did not differ from those values obtained by randomly selecting hosts and parasites from the population. Thus, our observed host-parasite pairs do not have an elevated level of relatedness above the background level in the population (aim 1). The relatedness dynamics of solitary species of insect exhibiting what appears to be IP, is of interest regarding the evolution of sociality. The potential for an evolutionary link between IP and cooperative care has been well established (Andersson, 2001; Vehrencamp and Quinn, 2004; Vehrencamp, 2000; Zink, 2000; Zink and Lyon, 2015), with each now considered to be two ends of a spectrum of parental care by a secondary female; IP being the extreme in which the secondary female provides no care (Andersson, 2001; Zink, 2000; Zink and Lyon, 2015). In order for cooperative care to develop, however, the inclusive fitness of both host and parasite must increase as a result of a 'parasitism' event: $C < rB$ (Hamilton's rule; (Hamilton, 1964). As our host-parasite pairs are unrelated, this cannot be the case in our population.

Only one prior study appears to have investigated the role of IP in a species of solitary insect (Lace bug *G. solani*; (Loeb *et al.*, 2000), and they found host-parasite pairs were often related. In *G. solani*, the mother provides care for a clutch of numerous offspring simultaneously, when parasitized, eggs are added to the perimeter of the clutch. In our system, however, where a nest contains one egg, and the parasite replaces the host's egg with their her own, the host incurs a direct cost, and the parasite incurs a direct benefit. Unlike in taxa such as *G. solani*, where parasitism dilutes the effect

of predation (reviewed in Tallamy, 2004), there is no opportunity for direct benefits to the host that can come from the addition of another brood. Furthermore, the host will not gain compensation with indirect fitness by providing care for the foreign offspring, and the parasite will not lose indirect fitness from killing the host's brood, as relatedness is zero between host-parasite pairs in our population of *A. pubescens*. It seems highly unlikely that cooperative care would evolve in such a system. Moreover, parasites would be expected to avoid relatives, due to the accompanying loss of indirect fitness. If the cost is sufficient, the selection pressure could drive relatives to nest progressively further away from each other, or even disperse to other aggregations.

Spatial structure in relatedness

Our results showed that mean relatedness within observed spatial clusters was significantly lower than the values obtained by randomly selecting the host and '10 closest nests' from the population. The results of the mantel test also indicated that relatives do not cluster, finding no relationship between pairwise relatedness and distance between conspecifics' nest centroids. Finally, there was no difference in variance between the observed relatedness within spatial clusters and those values by chance (aim 2). Aspects of a species behaviour, such as natal philopatry (Andersson and Waldeck, 2007; Eadie and Lyon, 2011; Pöysä *et al.*, 2014b) and short dispersal distance ((Loeb *et al.*, 2000), can bring about the clustering of relatives. Such clustering, by causing increased interactions with related conspecifics, could provide the right context for the evolution of kin-based female sociality (Andersson, 1984, 2001; Dickinson and Hatchwell, 2004; Zink, 2000; Zink and Lyon, 2015). If relatives are instead avoiding each other within the confines of the aggregation, as may be implied in the permutation analysis, and as was suggested due to the costs imposed on parasites when a host is related, then what behaviours might be creating such a pattern?

Perhaps the least complicated mechanism could be through long-distance dispersal, as it can make use of an existing behaviour. Population relatedness structure and long-distance dispersal are

typically considered together in the context of inbreeding avoidance (Szulkin *et al.*, 2013), due to the high costs that inbreeding can incur (Hatchwell, 2010). IP is another common context in which social interactions occur among kin. Dispersal reduces the probability of social interactions with kin, and thus inbreeding, in many species (black grouse, *Tetrao tetrix* - Lebigre *et al.*, 2010; collared flycatcher, *Ficedula albicollis* - Pärt, 1996; great tit, *Parus major* - Szulkin and Sheldon, 2008), and without the need to evolve more complex behaviours like mating bias. The outcome, reduced inbreeding, occurs regardless of whether the source of the selection pressure for dispersal was inbreeding avoidance, or other forces such as local resource availability or kin competition (Lehmann and Perrin, 2003; Pusey, 1987). Thus, in *A. pubescens*, long-distance dispersal could result in the outcome of relative avoidance in IP, though dispersal may have evolved for an entirely different reason.

Despite long-distance dispersal likely being the most parsimonious mechanism underlying our result, that relatives were distributed further away from each other than expected by chance, there is little evidence showing that long-distance dispersal occurs in *A. pubescens*. Instead, philopatry appears to be common in both ground and aerial-nesting species of Hymenoptera, and it has been suggested that the location of the nest an individual emerges from (a.k.a. emergence point) may be used as a cue by newly nesting females, as it implies the local microhabitat is favourable (reviewed in Polidori *et al.*, 2010). In the case of the digger wasp *Stizus continuus* (Crabronidae), however, though females rarely nest outside of their natal aggregation, they were not found to nest closer to their emergence point than expected by chance (Polidori *et al.*, 2010). In fact, evidence suggests that female digger wasps may be more attracted to nesting near other conspecific nests, rather than their emergence point (Asís *et al.*, 2014; Polidori *et al.*, 2008). There is one example of a digger wasp species that has been observed carrying out long-distance dispersal. *Sphex ichneumoneus* L. (Sphecidae) has in some limited cases been recorded dispersing between 8m and 300m away (Brockmann, 1979). Further investigation is clearly required within *A. pubescens* to understand how

factors such as dispersal, emergence point and location of nesting conspecifics, may be involved in the surprising distribution of relatives in our aggregation.

An alternative but more complex explanation for our result, that relatives were distributed further away from each other than expected by chance, could be that kin recognise, and are actively avoiding, each other. Kin recognition requires three components: first, production of kin-specific cue by the sender; second, perception of the given cue by the receiver; and third, a conditional action by the receiver based on the cue (Gamboa *et al.*, 1986a, 1986b; Reeve, 1989; Waldman, 1988). In this scenario, receivers perceiving kin would move to another area in the aggregation to dig a nest. Though extensively studied in eusocial insects, very few examples of kin recognition in solitary insects have been reported (Lihoreau *et al.*, 2007; Lihoreau and Rivault, 2009; Pfennig and Reeve, 1993, 1989), and investigations into kin recognition between siblings in solitary insects are extremely limited (Halpin and Hepper, 1991; Lizé *et al.*, 2010; Metzger *et al.*, 2010). A recent study by Field and colleagues (in prep) on *A. pubescens*, however, suggests that mothers are unable to distinguish between their own offspring and that of conspecifics, making sibling recognition unlikely.

There may be some doubt, however, about the biological significance of our result. Despite the difference between the observed (-0.026) and random (-0.0079) relatedness values being statistically significant, the difference was relatively small (0.0181). For example, this is 10 fold smaller than the relatedness value of cousins in the Hymenoptera ($r = 0.1875$), while between mother and daughters ($r = 0.5$), and full sisters ($r = 0.75$), the difference is even greater. Whether the difference in relatedness we found is sufficient to convince us that relatives were really distributed further away from each other than expected by chance (via kin recognition and avoidance, or dispersal), it certainly suggests that clustering of relatives is not occurring. This is a conclusion that is supported by the result of the mantel test, which finds no relationship between pairwise relatedness and distance between conspecifics' nest centroids.

As far as we have been able to determine, this is the first study exploring the relatedness structure of a solitary hymenopteran species that locate their nests in conspecific aggregations. There have, however, been studies of the genetic structure of advanced / primitively eusocial hymenopteran populations. Though these studies have been focused primarily on whether aggregated colonies are formed by budding (i.e. mother – daughter colonies), and statistical power is often limited due to sample size, results suggest that there is not clustering of closely-related colonies (Cameron *et al.*, 2004; Oldroyd *et al.*, 2000; Paar *et al.*, 2004; Wattanachaiyingcharoen *et al.*, 2008; but see Crozier *et al.*, 1987; Friedel *et al.*, 2017). These examples may have limited utility as a comparison with our solitary digger wasps, due to the large differences between their behaviour, including being advanced eusocial species and highly migratory. Migratory behaviour would be likely to reduce relatedness within an aggregation, while reproduction swarming, where a group of bees permanently departs the parent nest, might be expected to increase relatedness (unless the reproductive swarm then dispersed). Interestingly, the two species of bee that have the most similar ecology and behaviour to our system (*Lasioglossum zephyrum* - Crozier *et al.*, 1987; *Lasioglossum malachurum* - Friedel *et al.*, 2017), both show some evidence of fine-scale genetic structure within aggregations, unlike what we have found in *A. pubescens*. Sweat bees, such as these species, nest in the soil, produce annual colonies, and are primitively eusocial. As such, each individual maintains the ability to build a nest and lay eggs, their role / caste is primarily a function of their behaviour as opposed to their morphology as in advanced eusocial species like honeybees. The evidence of a fine-scale genetic structure is most convincing in *L. malachurum* (Friedel *et al.*, 2017). Crozier and his colleagues (1987) only found a weak spatial pattern of relatives within some of their aggregations of *L. zephyrum*. They suspected that where present, it may be due to recent colonisation – i.e. insufficient time to erode the relatedness structure through outbreeding etc.

Distance from parasite's nest/s

We found no difference between the observed mean distance between host nests and their parasite's active nests, and the values obtained based on randomly selected hosts and parasites from the population. However, we found that the observed variance was significantly higher than that calculated from the same random sampling. Furthermore, the observed frequency distribution did not differ significantly from a uniform distribution. Thus, our data suggest that the distance between the host and parasite's nests does not affect the likelihood of a parasitism event occurring (aim 3). A difference from the random mean value might have been expected for one of three main reasons, two of which are expected to produce an observed distance that is shorter than random. The first is that parasites may have more information (e.g. location and status) about conspecific's nests that are located around their own active nests, and thus would select from these. The second and third reasons are based on the assumption that clustering of relatives occurs. In this scenario, if the benefits outweigh the costs of parasitizing a relative, at least for the parasite, then parasites could be expected to select closer nests as hosts (i.e. those likely belonging to relatives). Alternatively, if the costs outweigh the benefits, then parasites might avoid relatives by selecting nests further away. Even if distance affected the chance of parasitism in *A. pubescens*, the last two of these potential hypotheses could be discounted because we found no evidence of clustering of relatives, and also no elevated or depressed relatedness in host-parasite pairs.

Our result, that there is no difference between the observed and random distance between host and parasite nests, suggests that parasites are not making use of information they have gathered from the locality of their own nests. It is still possible, however, that parasites use information they have gathered to select nests to parasitize. Instead of merely the locality of their nests, though, information may be gathered from the wider population. Our aggregation, which itself is on bare ground, is surrounded by heather plants (picture 2), the landscape in which *A. pubescens* hunts for its Lepidopteran prey. Thus, when a wasp successfully captures a prey item and then travels to its nest, it may arrive from any direction. This could allow a potential parasite to gain information about

the wider aggregation, especially as each wasp will typically have several active nests on a given date. It is unlikely that, if this were the case, the pattern would be picked up in our analysis of the mean distance between nests. Arguably our other findings, that the observed frequency distribution fits a uniform distribution and that the observed variance is significantly greater than that from the randomly sampled data, support this interpretation.

A last point to consider here, is the window of time during which parasitism normally occurs; between digging and the start of the main provisioning bout. It seems reasonable that a host is more likely to accept a foreign brood replaced in this window, as it will then be at a similar stage of development to its own and therefore harder to differentiate. Regardless of whether that is true, the narrow window means that many of the nests that are active on a given day are not suitable for parasitism. Thus, the likelihood of finding a nest at the correct stage would increase with either knowledge of, or the willingness to search, a wider area.

Nest density

We found no difference, at any of the scales we used, between the observed mean and /or variance of density of nests around the host's nest, and that found when randomly selecting hosts from the population. This suggests that a host's vulnerability to parasitism was not affected by the density of nests in its locality (aim 4). Given that we found in the previous analysis that parasites make use of most of the aggregation when choosing a host, this result seems unsurprising. The mean observed distance between hosts and their parasite's nests was 502.3 cm (range: 36 cm to 1,017 cm), suggesting that parasites are not restricting themselves to the locality of their nests (figure 1). Fine scale patterns in density might therefore not be expected to change risk of parasitism in this context. That is unless IP is typically opportunistic, though our result does not support it. Wasps may inadvertently come across host nests when searching for their own active nests, or for sites to dig new nests. It will be interesting to compare these results to future studies investigating fine

scale patterns in other taxa, especially where a broad scale relationship between nest density and rate of IP has been found.

Body size

We found that the size of an individual does not affect the proportion of time spent engaged in parasitism vs being parasitized (aim 5). The same *A. pubescens* individuals both parasitize and are parasitized themselves, with those that parasitize more, relative to being parasitized, likely gaining greater fitness. Surprisingly, our finding suggests that a wasp's body size does not influence this ratio and therefore has no impact on fitness through this mechanism.

With respect to how body size may influence likelihood of an individual parasitizing, many studies have produced results that suggest IP is an opportunistic tactic that high quality females use in addition to raising other brood themselves, to increase their fitness (Åhlund and Andersson, 2001; Brown and Brown, 1998; Tallamy and Horton, 1990; Zink, 2003). For example, the lifetime reproductive success of female cliff swallows (*Petrochelidon pyrrhonota*) that also parasitized, was 1.84 times higher than those that did not. This was explained by their higher quality, as reflected by their own higher survival, and that of their brood (Brown and Brown, 1998). Also, female goldeneye ducks (*Bucephala clangula*) that parasitized others in addition to their own nesting, laid 1.5 times as many eggs as non-parasites (Åhlund and Andersson, 2001). There are several studies, however, that have found parasites to be of similar quality to other females; neither lower or higher (birds - Jaatinen *et al.*, 2011b; Robertson, 1998; Waldeck *et al.*, 2004, 2011; Waldeck and Andersson, 2006; insects - Field, 1992; Moczek and Cochrane, 2006).

In the introduction to this chapter, we suggested that individuals of larger size may be more likely to parasitize due to their increased fecundity, but what evidence is there of this relationship? Prevailing dogma states that larger / heavier insects are more fecund than those that are smaller / lighter (reviewed in Blanckenhorn, 2000). There is considerable empirical support for this general

relationship (Gall Maker, *Dryocosmus kuriphilus* - Graziosi and Rieske, 2014; reviewed in Honěk, 1993; *Belonocnema treatae* - Hood and Ott, 2017; parthenium beetle, *Zygogramma bicolorata* - Omkar and Afaq, 2013; yellow lac scale, *Tachardina aurantiaca* - Ong *et al.*, 2014); however, there are exceptions (solitary bee, *Osmia cornuta* - Bosch and Vicens, 2006; dung beetle *Onthophagus taurus* - Moczek and Cochrane, 2006). Interestingly, an investigation of IP in the dung beetle *Onthophagus Taurus*, found no evidence of a relationship between body size and either fecundity, or the likelihood of engaging in parasitic behaviour (Moczek and Cochrane, 2006). Furthermore, research into the fecundity-body size relationship has predominantly been carried out under highly controlled laboratory conditions. As ectotherm physiology is heavily temperature dependent, the potential fecundity benefits of a large body size may not be met in all species, or in all years, due to the inconsistent nature of natural conditions (Berger *et al.*, 2008; Bosch and Vicens, 2006). Our understanding of IP in *A. pubescens* would clearly benefit from investigating the relationship between body size and fecundity in field conditions.

Our finding, that body size does not affect the proportion of time spent engaged in parasitism vs being parasitized, suggests that body size is also not as important as we predicted in determining whether an individual is parasitized. We hypothesised that parasites might select individuals that were more likely to accept a foreign egg (i.e. more permissive), as this would increase the chance of their brood surviving. An individual or group's permissiveness, or acceptance threshold, is known to vary depending on context, in order to minimise the net costs of incorrect acceptance and rejection. We proposed that a host's fecundity could influence their acceptance threshold. Specifically, that parasites might select smaller hosts as, due to their lower fecundity and relatively high costs associated with rejecting their own eggs, these hosts may be more permissive when assessing a possible parasitism; however, this does not appear to be supported by our result. There is evidence that *A. pubescens* host's can detect and reject foreign eggs (Field *et al.*, in prep). Our result, however, that body size does not affect the proportion of time spent engaged in parasitism vs being parasitized, suggests that variation in fecundity does not affect host acceptance threshold.

Summary

Our results shed light on the factors that influence IP in *A. pubescens*. It seems unlikely that IP is a precursor to cooperative care in this species, because host-parasite pairs are unrelated. In-fact, active avoidance of relatives might be occurring when parasites choose their hosts. Distance of the host from the parasite was also considered as a factor that might influence chances of IP; however, it appears that parasites do not preferentially choose host nests that are close-by, but instead choose hosts from across the whole population. Moreover, the density of nests surrounding a host did not affect its chances of parasitism. We also found that body size did not affect the the proportion of time spent engaged in parasitism vs being parasitized. The detailed dataset of *A. pubescens* behaviour we present has enabled us to investigate factors that influence IP, on a within-aggregation scale. At larger scales, such as between multiple aggregations, we would likely get quite different results. For example, relatedness of a given host-parasite pair within our aggregation would be higher if calculated using gene frequencies from multiple aggregations, compared to the value we gained by using the gene frequencies from within our aggregation.

Chapter 5: General discussion

5.1 - The characterisation of ARTs

In my general introduction I stated that a major objective of evolutionary biology as a whole, and the study of alternative reproductive tactics more specifically, is to understand how alternative phenotypes such as those described above, are maintained within populations (Taborsky *et al.*, 2008). In order to dissect the processes involved in maintaining such discrete morphological, physiological and behavioural morphs, however, we must first characterize the patterns they make (Taborsky *et al.*, 2008). A categorisation method inspired by the well-developed sex-allocation literature was suggested by Brockmann (2001), in which alternative reproductive tactics (ARTs) can be distinguished based on when and how tactic-specific phenotypic determination occurs (Engqvist and Taborsky, 2016; Taborsky *et al.*, 2008; Figure 1).

Fixed ARTs

ARTs can first be divided by whether they are fixed for life or flexible (a.k.a. plastic). In those species that employ fixed ARTs, the irreversible selection of the tactic typically occurs during early development (Buzatto *et al.*, 2014; Emlen, 1997; Moczek and Emlen, 2000; Rowland and Emlen, 2009; Stockley *et al.*, 1994; Tomkins and Brown, 2004). For example, males of the dung beetle *Onthophagus taurus* exhibit one of two potential ARTs, either large body size with a long horn, or small body size with almost no horn. Body size in this species is determined entirely by nutrition

during early development, and forms a normal distribution within the population. Horn size, on the other hand, forms a bimodal distribution, with individuals above a threshold body size exhibiting the large horn morph. These two phenotypes employ classic bourgeois and sneaker male tactics, in line with their horn sizes. Thus, large horned males defend tunnels dug by females and fight other males for mating access (bourgeois), while small hornless males attempt to sneak past large males (sneaker), either by use of existing tunnels or by digging their own tunnels to circumvent the guard. Large horns allow large males to win in combat, while very small horns allow small males to sneak more effectively. The morphological and behavioural phenotypes of these ARTs are fixed for life from that early developmental stage (reviewed in Nijhout, 2003).

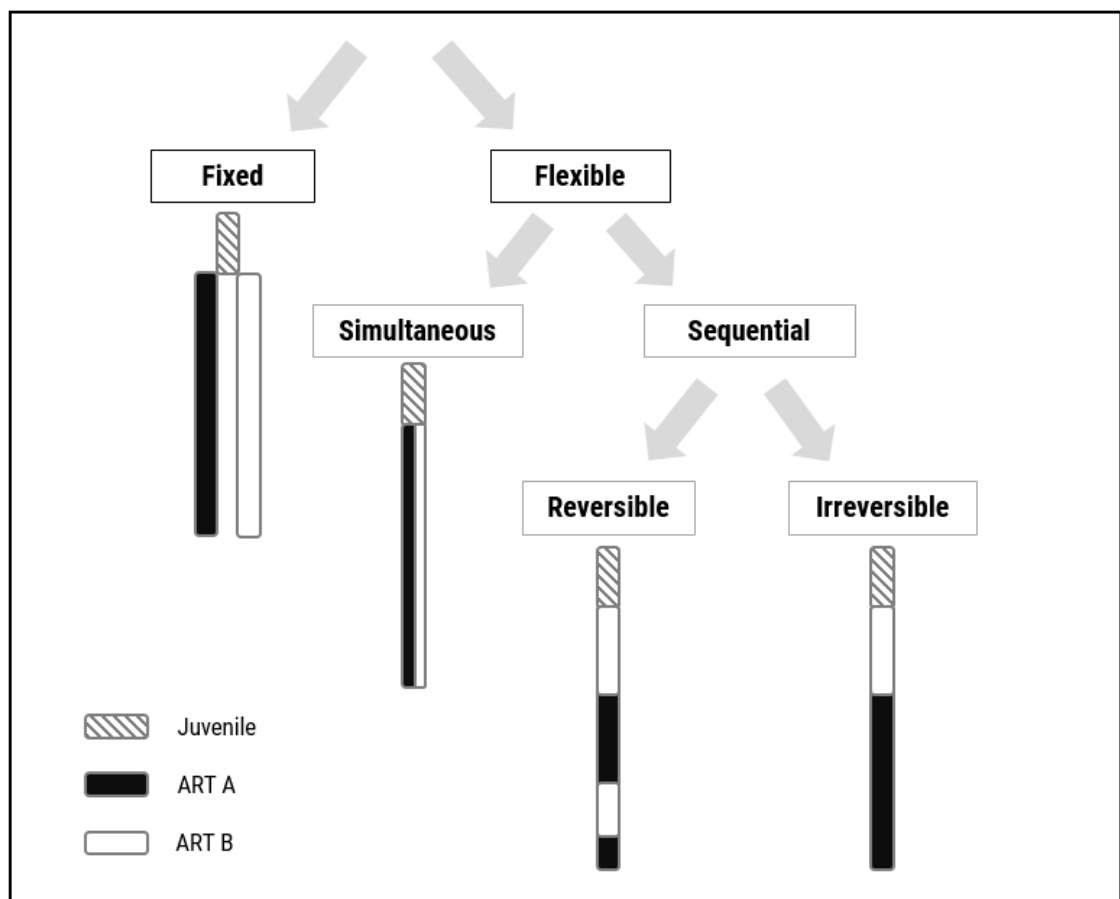


Figure 1 – Alternative reproductive tactics (ARTs) can be either fixed for life or flexible. Flexible ARTs can be further subdivided into simultaneous and sequential; sequential ARTs being either reversible or irreversible. The stage prior to exhibiting an ART is referred to as ‘Juvenile’. Figure adapted from Taborsky *et al.*, (2008).

As with the *O. taurus* example above, most studied ARTs come in the form of polyphenisms, whereby two or more phenotypes can be produced by the same genotype. Genetically determined ARTs are much less common (Buzatto *et al.*, 2014; Oliveira *et al.*, 2008). Females of the damselfly *Ischnura elegans*, one of the most common damselflies in Europe, exhibit three different fixed colour morphs that are genetically determined. First, the androchrome morph, which closely resembles the male in both colour (blue) and markings. There are also two gynochrome morphs, which are both brown in colour. The first of these gynochromes, infuscans, has three black stripes on the thorax, resembling the male pattern. The other gynochrome morph, infuscans-obsoleta, differs from the male's markings, in that it has only a single stripe on the thorax. All three morphs have similar survivorship, but differ in the proportion of the population they represent, and disproportionately differ in their mating frequencies (e.g. infuscans females represented 27% of females in the population but gained 40% of matings). The polymorphism resulting in these three damselfly morphs is caused by an autosomal locus with three alleles (Cordero *et al.*, 1998; Svensson *et al.*, 2005).

Flexible ARTs

Returning to Brockmann's (2001) categorization method, ARTs classified as flexible have two further levels of possible subdivision. First, flexible ARTs can be divided into simultaneous or sequential. Simultaneous ARTs, as the name suggests, involves two or more tactics being carried out concurrently. A good example of this is extra-pair copulation, in which promiscuous mating occurs in what appears to be a predominantly monogamous species. The superb fairy-wren (*Malurus cyaneus*) is well known for having high levels (61–76%) of extra-pair copulation (reviewed by Colombelli-Négrel, 2016). Superb fairy-wrens are socially monogamous with lifetime pair bonds, and males help to raise nestlings in their own nest. In addition to this, however, males will occasionally seek out the nests of nearby conspecifics and attempt to mate with the female. Thus,

these males can be said to be carrying out the bourgeois and sneaking ARTs simultaneously (Kruger, 2008; Taborsky, 2008).

In the case of sequential ARTs, however, an individual carries out two tactics one after the other, with a clear distinction between the first and the second tactic. For example, males of the peacock blenny (*Salaria pavo*), which inhabit coastal lagoons, may carry out one of two ARTs: nest-holding and parasitism. Males that initially employ the nest-holder ART will only ever exhibit this tactic. However, individuals that initially choose the parasite tactic, whereby they sneak fertilisations inside a conspecific's nest, will later become nest-holders themselves (Fagundes *et al.*, 2015).

The final level of categorisation within Brockmann's (2001) framework is the subdivision of sequential ARTs into irreversible and reversible. In the peacock blenny (*Salaria pavo*) example above, males adopt the two ARTs in an irreversible sequence. Those males that initially adopt the parasitic ART will later go on to be a nest-holder, and can never revert to the parasitic tactic. Those that become nest-holders initially will only ever exhibit that ART. These fixed series of ARTs are sometimes referred to as life history pathways and, in the peacock blenny, the pathway an individual adopts is dictated by when they are born. Those born at the start of the breeding season exhibit the nest-builder pathway, those born late in the season exhibit the parasitic to nest-builder pathway (Fagundes *et al.*, 2015).

In contrast to the previous example, individuals in species categorized as exhibiting reversible sequential ARTs may switch back and forth between available tactics. As a predominantly socially monogamous rodent, Prairie voles (*Microtus ochrogaster*) are a model system for the investigation of pair bonding and related behaviours. Males in this species may either form a monogamous bond with a female and together defend a territory (resident), or alternatively they may adopt a wanderer tactic. Wandering males do not hold a fixed territory, but instead roam through the territories of conspecifics, gaining paternity through extra-pair fertilisation with resident females (Vogel *et al.*, 2018). Observations in wild populations have shown, however, that individuals may switch between tactics in either direction. Resident males in social groups that have broken down can take up the

wanderer tactic, and wanderers have formed or joined a social group and established a territory (reviewed in McGuire and Getz, 2010).

5.2 - The characterisation of ARTs in

P. dominula and *A. pubescens*

In this thesis, I have investigated three examples of alternative reproductive tactics in two species of social wasp. In the remainder of this chapter, I will present a summary of my findings and then categorize them within the framework described above. I will then go on to consider future directions for research in each case.

Part I: summary of thesis findings

In Part I of this thesis (**Chapters 2 - 3**), I focused on ARTs in foundress associations of the primitively eusocial paper wasp *Polistes dominula*. In this species, small paper nests are founded in the spring by either lone mated females (foundress) or small groups of co-foundresses. Most nests then gain a number of additional co-foundresses after the initial founding (joiners). A dominance hierarchy is then formed in which the rank 1 female lays most or all of the eggs (Leadbeater *et al.*, 2011; Reeve, 1991). Thus, the choice of tactic employed at this stage has large fitness implications. Since the discovery of the dominance hierarchy in *P. dominula*, the intense research interest in the field has made considerable headway into understanding its complexities, but many questions have remained. In this thesis I have addressed some of those previously unanswered questions.

Chapter 2: What determines dominance in the paper wasp *Polistes dominula*?

In **Chapter 2** I used data from a Spanish field site to investigate the rules involved in establishing the dominance hierarchy in *P. dominula*. Specifically, how two potential ARTs, where a female either initiates a nest of her own, or joins an existing nest, affect reproductive status.

We found that initiating the nest (i.e. arriving first) appears to be the most important factor influencing the attainment of a rank 1 reproductive status within the *P. dominula* hierarchy. This result is in line with evidence in another temperate *Polistes* wasp, *Polistes carolina*, which showed that foundresses that initiate nest founding almost always became the dominant in their nest (Seppä *et al.*, 2002; but see Zanette and Field, 2009). Additionally, we showed that first-arrivers are, on average, larger than joiners, and that the specific rank attained by a first-arriver may also depend on her relative body size. Thus, though far less important than arriving first, there is some suggestion that body size may have more influence over the establishment of the dominance hierarchy than most research in *P. dominula* has previously suggested (Cant *et al.*, 2006; Leadbeater *et al.*, 2011; Zanette and Field, 2009; but see Cervo *et al.*, 2008; Tibbetts and Izzo, 2009). It is unknown, however, whether body size is an accurate reflection of an individual's agonistic ability. We suggested the use of an established approach to find the best morphological correlates of agonistic ability in *P. dominula*. Potential morphological traits would first be measured in a group of foundresses, after which a series of paired contests would be conducted to determine which trait/s best predicts agonistic ability. From this an index can be produced, whereby any future foundress can be given an agonistic score based on the measurement of only the most informative morphological predictor/s (Firth and Turner, 2012; Stuart-Fox *et al.*, 2006). This will enable a more accurate assessment of the relationship between agonistic ability and hierarchical rank in the future.

We found no evidence that presence or absence of a clypeal mark (a potential status symbol) had an effect on a first-arriver's rank. This result is largely in line with other studies in European based populations (Cervo *et al.*, 2008; Zanette and Field, 2009). Furthermore, given the low proportion of

wasps with clypeal marks in European populations, as well as the strong evidence for a climatic influence in its expression (Green *et al.*, 2012), it seems likely that clypeal markings do not have an important influence on the dominance hierarchy in our study population.

We also carried out the first field-based investigation of mechanisms underlying order of arrival. We found that smaller first-arrivers tend to start founding their nests earlier, but that presence or absence of a clypeal mark had no effect on founding date. First-arrivers that initiated nest founding earlier were also more likely to become the dominant in the group. Possible explanations for these unexpected results were discussed, such as competition over preferential locations within the hibernation cluster.

Our findings in this chapter now make it possible to determine, with a reasonable degree of accuracy, which individual will become the dominant in a *P. dominula* nest. This a priori knowledge will enable previously impracticable questions to be addressed in the future. Of particular interest will be the question of whether wasps that become the dominant are intrinsically different from subordinates.

Chapter 3: The effects of solitary versus group nest initiation on co-foundress associations

P. dominula nests can be initiated by one foundresses or by a group, which may or may not later gain additional joiners. Previous work in this area has exclusively focused on comparing solitary nesting females (i.e. initiated alone with no joiner) with those nesting in a group. This was of particular interest as it begged the question of why an individual would join a group as a subordinate, and so give up the opportunity for direct reproduction (Field and Leadbeater, 2016; Leadbeater *et al.*, 2011; Queller, 1996; Reeve, 1991; Tibbetts and Reeve, 2003). Yet the question of how the method of nest initiation, solitarily or by a group of foundresses, subsequently affects group characteristics (e.g. productivity and survival) and fitness outcomes remained unexplored until now. This may be an important question as differences in group composition, such as whether

the male helper in a cooperatively breeding group remained on its natal nest or joined from elsewhere, have been shown to correlate with differences in behaviours such as mobbing (Maklakov, 2002), vigilance (Griesser, 2003; Valdez, 2010) and foraging effort (Magrath and Whittingham, 1997; Magrath and Yezerinac, 1997). Thus, method of nest initiation employed could have important fitness implications.

In light of this, I went on to use field data and molecular genotyping to compare characteristics of multi-female nests (and their founders), that were originally initiated by either a solitary foundress or a group of foundresses. Specifically, in **Chapter 3** we asked how solitary and group founded multi-female groups differed in characteristics such as relatedness, productivity and survival. We also investigated whether there were morphological differences between those foundresses initiating each ART.

We found that founding tactic (i.e. solitary or group founded) had a significant effect on eventual group size, with group founded nests having larger groups on average. Solitary and group founded nests both gained roughly the same number of joiners on average, so that group founded nests tended to have c.1 additional co-foundress than solitary founded nests, reflecting the difference in group size at initiation.

Through its influence on eventual group size, founding tactic affected both nest survival and productivity (Leadbeater *et al.*, 2011; Tibbetts and Reeve, 2003; but see Höcherl and Tautz, 2015a). The larger, group founded nests having greater survival and producing more cells than the smaller, solitary founded nests. After controlling for eventual group size, however, founding tactic had no effect on productivity or survival, suggesting that founding tactic did not affect the behaviour of the group members, such as their foraging effort.

Within-group relatedness, on the other hand, did not depend on group size or nest founding tactic. We might have expected group founded nests to have higher genetic relatedness than solitary founded nests if co-founders tended to be close relatives (maximising indirect fitness: Hamilton,

1964) while and joiners tended to be more distantly related. This appears not to be the case, however, consistent with previous work by Zanette and Field (2011), who found that within-group relatedness was unaffected by late arriving joiners.

Finally, there was some limited distinction in morphology between foundresses employing the different reproductive tactics, in the form of an interaction between body size and clypeal mark. Solitarily founding females did not differ in size based on whether they had a clypeal mark, but group founding females with clypeal marks were larger than those without marks. In group founded nests we randomly selected one of the initiating foundresses to mark. Given this, what our findings might indicate is that in newly initiated solitarily founded nests we are seeing one category of individual (i.e. founder / first-arriver), and in the group founded nests we were seeing two categories (i.e. founder and joiner). This might be the case if, unlike solitarily founded nests, members of group founded nests began the process of group formation prior to initiation of the nest, perhaps during the poste-hibernation stage. In that case one individual might still have the characteristics of a founder or group initiator, while those forming a group with that founder could have the characteristics of a joiner.

Categorization of ARTs in *P. dominula* foundresses

P. dominula foundresses exhibit a range of nesting behaviours during the spring nest initiation phase. This behavioural range has typically been divided into four distinct ARTs: i) initiating a nest alone; ii) initiating a nest in a group, iii) joining a nest / group; and iv) sit-and-wait to usurp / adopt a nest (Reeve, 1991). However, this division does not appear to accurately reflect all the choices available to foundresses. Specifically, ART i (initiating a nest alone) would perhaps be more accurately described as two separate tactics: i) initiating a nest alone and remaining alone; and ii) initiating a nest alone and gaining a joiner/s. A foundress that initiates a nest alone may or may not gain a joiner/s; if not she may stay on her nest alone, but will gain lower fitness than a subordinate

in a group nest (Leadbeater *et al.*, 2011). Alternatively, if the solitary initiating female does gain a joiner/s, she (the founder) is most likely to gain the dominant position in the resulting hierarchy, especially if she is large relative to the joiner/s (**Chapter 2**). Due to the lower group size that results from initiating a nest alone (as opposed to group founding), however, the nest is likely to be less productive and have a lower chance of survival than a nest initiated by a group of foundresses (**Chapter 3**). Interestingly, foundresses that choose to initiate a nest alone, whether or not they gain a joiner, do so even though they may have relatives on another nest (Grinstead and Field, 2017; Leadbeater *et al.*, 2011). This suggests that females select this tactic despite other, seemingly better, options available, not as the ‘best-of-a-bad-job’.

Based on the above information, it appears that the initial choices open to foundress at the beginning of the nest founding stage could be more accurately divided into five, rather than four, ARTs: i) initiating a nest alone and remaining alone; ii) initiating a nest alone and gaining a joiner/s; iii) initiating a nest in a group, iv) joining a nest / group; and v) sit-and-wait to usurp / adopt a nest. Original assumptions about the tactics available to foundresses have clearly been oversimplified, and we have attempted to move towards a solution with this re-categorisation. However, it could be argued that the distinction we have drawn, between solitary founders that do (i) or do not (ii) gain a joiner, would be better described as two outcomes to one tactic (i.e. initiating a nest alone). Further work is required to clarify this distinction, however, for the remainder of this thesis I will assume that the range of nesting behaviours exhibited by *P. dominula* foundresses are best categorised by the five ARTs I have described.

I will now consider how these five ARTs might be characterised within the framework proposed by Brockmann (2001). If having selected an initial tactic, an individual was locked into that behaviour for the remainder of their life, this would be categorised as a fixed ART. This is not true, however, in the case of *P. dominula* foundresses, as some individuals are observed carrying out multiple tactics within their lifetime (reviewed in Starks, 2001). Therefore, ARTs in *P. dominula* can be considered

plastic / flexible. For example, a wasp that founds a nest alone, but gains no joiner, may choose to abandon their attempt and join another nest (personal observation).

The next level of categorisation to consider is whether these ARTs are carried out simultaneously or sequentially, a distinction that is perhaps not so easily made. For example, tactics i) initiating a nest alone and remaining alone, and ii) initiating a nest alone and gaining a joiner/s, could be considered simultaneous until either a joiner is gained, or the likelihood of gaining a joiner has become very low. That said, an individual could not initiate a nest alone and simultaneously do so as part of a group. Thus, the categorisation of the ARTs available to *P. dominula* foundresses at this stage is more complex than in many cases (Oliveira *et al.*, 2008).

With the information we have available at this time, the initial ART choice on starting the founding phase might be described as follows: initially the ART is undecided (similar to the juvenile stage prior to selecting a fixed tactic); then, an individual can make a choice between three initial pathways: a) initiate a nest alone, b) initiate a nest in a group, c) wait. An individual following pathway a) will typically await either a joiner, or the end of the joining period before selecting a specific ART from the available options. Those options are to remain alone, to gain a joiner and form a group nest, or to abandon the nest and choose an alternative pathway. An individual selecting pathway b) has more limited options: remain a part of the group nest, or to abandon the nest and choose an alternative pathway. Finally, pathway c) leaves all possible ARTs available to a given individual, at least until the season progresses far enough to make some ARTs no longer viable. For example, initiating a nest at the end of the summer / beginning of autumn would be unlikely to result in reproductive offspring before the end of the season.

The complexity of the tactic choices available to *P. dominula* foundresses make the process of fitting them within Brockmann's (2001) framework very difficult. However, it is clear at least that in many cases tactics are exhibited sequentially. As such it seems appropriate to consider the final level by which we can categorise ARTs, i.e. whether the sequence is reversible or irreversible. In some species (see peacock blenny example above) individuals progress through an irreversible

sequence of ARTs with size or age, but this does not seem to be true in our *Polistes* foundresses. Females that adopt abandoned nests (ART v), for example, may have previously been part of a multi-foundress association (ARTs iii or v), but later decided to leave. Alternatively, they may have lost their nest due to predation, or bad weather conditions such as strong wind or heavy rain (reviewed in Starks, 2001). Additionally, a wasp that initiates a nest alone but gains no joiner may join another nest as a subordinate. Multi-foundress associations that lose their nests to predation / other damage might also initiate a new nest together in a group (personal observations). Thus, clearly at least some ARTs continue to be available after the initial selection, though presumably there are some constraints, such as how far the season has progressed. As such, these ARTs can be categorised as a reversible sequence, though some ARTs at some points could be considered to be carried out simultaneously (see above).

Future directions

In *P. dominula* foundresses, the interplay of two key factors, rank in the dominance hierarchy and which ART is adopted, can be of major significance for the fitness that individuals attain. For example, a dominant individual in a singly founded but eventually multi-female nest, will likely gain lower fitness than a dominant in a group founded nest (**Chapter 3**). Few studies have determined the fitness of different ARTs in *P. dominula* (e.g. Leadbeater *et al.*, 2011), and so the full implications of the relationship between rank in the dominance hierarchy and which ART is adopted remains unclear. To explore this important relationship, a large scale study is required.

The aim of this study would be to explore the fitness outcomes of all the tactic choices within one year. This would include detailed behavioural observations, in which the movements, tactic choices and fitness outcomes of the majority of individuals within a population are assessed from the beginning of the founding stage to the end of the reproductive stage. Such a project would be of great value for several reasons. First, in this discussion I have characterized the ARTs available to

P. dominula foundresses based on available evidence and personal observation, but obvious holes in this knowledge still exist. We know, for example that wasps initiating a nest alone may sometimes abandon the attempt and join another group, but it is unclear whether this occurs when a window of opportunity for gaining a joiner closes. Second, we would gain detailed information about how the ranks that wasps attain are affected by current and previous ART choices, as well as by other factors, such as order of arrival and morphological characteristics like body size. We could then determine how fitness varies as a function of these different pathways and factors. Beyond the benefit that researchers using *P. dominula* as a study system would gain from this new understanding, female ARTs in general are known to be understudied. This could be a particularly useful addition to the female ART literature because the ART system is so complex in *P. dominula*. Moreover, there is a broad base of associated knowledge of *P. dominula* and the genus *Polistes* as a whole because it is a model system in several fields (Chapter 1: Introduction).

Part II: summary of thesis findings

In Part II of this thesis (**Chapter 4**), I used field data and molecular genotyping to investigate ARTs in the solitarily nesting subsocial digger wasp *Ammophila pubescens*. The species nests in open sand within heather-dominated heathland, and females produce a sequence of L-shaped nests or burrows alongside those of conspecifics in multi female aggregations. Each nest contains a single brood, which the mother provisions with prey in the form of paralysed lepidopteran caterpillars, typically carried to the nest in flight. *A. pubescens* is a progressive provisioner, which means that food is provided gradually as the offspring develops, as opposed to being provided in a single mass. As such, the owner makes repeated visits to the nest during development of her offspring; therefore, if the nest has been parasitized by a conspecific, she will encounter the brood of that foreign wasp (Field *et al.*, 2007).

Typically, a wasp digs the nest and provisions a single prey on which she lays an egg, returning c.2 days later to assess its status (i.e. assessment visit) before provisioning the remainder of the prey required (i.e. main provisioning bout). Between digging and the start of the main provisioning bout, an *A. pubescens* nest is particularly vulnerable to being opened and entered by conspecifics. After entering, a foreign wasp (parasite) removes the prey item bearing the owner's (host's) brood. She then typically brings a new prey item to the nest, upon which she lays her own egg, then re-sealing the burrow. Thus, when the host returns for her assessment visit, she discovers the parasite's brood on a different caterpillar. At this point the host may either accept the parasite's brood and carry out the main provisioning bout, or reject it, and replace it with a new prey item and egg of her own (Field, 1992; Chapter 4).

Chapter 4: Intraspecific parasitism in the solitary digger wasp *Ammophila pubescens*

In Chapter 4 I investigated the factors influencing the occurrence of intraspecific parasitism (IP). IP occurs in a wide range of taxa (Harris, 2008; Summers and Amos, 1997), birds (Lyon and Eadie, 2008; Yom-Tov, 2001), fish (Taborsky, 2008) and insects (Tallamy, 2004; Field, 1992), but has been best studied in birds. Advances in this field have only begun to be applied in insects (reviewed in Tallamy, 2004), and the detailed nature of our field data allowed us to investigate it at an unusually fine scale. Specifically, I asked whether factors such as host-parasite relatedness, nest density and parasite body size affected the likelihood of a parasitism event occurring.

We found that host-parasite pairs are unrelated. As host-parasite relatedness is thought to be a prerequisite for the evolution of cooperative care, it seems unlikely that IP in *A. pubescens* is a potential precursor of sociality (Zink and Lyon, 2015). Furthermore, we find no evidence of fine scale natal philopatry. In fact, wasps might be actively avoiding relatives when deciding where to nest.

The mean distance between host and the parasite's existing nests was also considered as a factor that might influence the occurrence of IP; however, it appears that parasites do not preferentially choose host nests that are close-by. In fact, we found that parasites appear to select nests from throughout the aggregation, over a greater range than expected by chance. The frequency distribution of the observed distances between nests not differing from that of a uniform distribution. Moreover, we also found that the density of nests surrounding a host's nest did not affect its chances of parasitism. Taken together these findings suggest that parasites are not solely making use of information they have gathered from the locality of their own nests to select hosts, and so may not select a nesting location to maximise information and likelihood of successfully parasitism. Instead, wasps may be making use of information gathered as they travel between their nests and the surrounding heather where they hunt for prey.

Finally, we found that the size of an individual does not affect the proportion of time they spend engaged in parasitism vs being parasitized. This was a surprising result and one that would benefit from future work to enable a more developed interpretation. For example, work into the relationship between body size and fecundity in this species would help to better place these findings in the context of whether IP is an opportunistic strategy employed by high quality individuals.

The detailed *A. pubescens* dataset we present has enabled us to investigate factors that influence IP, on a within-aggregation scale. At larger scales, such as between multiple aggregations, we would likely get quite different results. For example, relatedness of a given host-parasite pair within our aggregation would very probably be higher if calculated using gene frequencies obtained by pooling multiple aggregations.

Categorization of ARTs in *A. pubescens* females

The process of characterizing ARTs in *A. pubescens* females within the framework proposed by Brockmann (2001) should be much more straightforward than in the case of *P. dominula*, due partly

to the limited number of tactics available. In *A. pubescens*, females appear to have two ARTs to choose between: i) digging their own nests and provisioning the offspring; and ii) parasitizing the effort of a conspecific female.

To begin the process of categorisation, we must first consider whether the tactics are fixed or flexible. During our observation we observed individuals carrying out both ARTs, so clearly in this species the ARTs can be considered as flexible. The next level by which we can categorise ARTs in this species is simultaneous or sequential, and this distinction can be made more easily than in the case of the *P. dominula* ARTs. *A. pubescens* females carry out their ARTs simultaneously, similarly to the example of extra-pair copulations (EPC in the superb fairy-wren, *Malurus cyaneus*). When an *A. pubescens* female parasitizes a conspecific, she does not give up her ongoing nesting efforts, which from burrow digging completion can take over 2 weeks to complete. In fact, she is likely to have several nests at various stages of completion when she carries out parasitism. Simultaneous ARTs, such as in this case, are not further sub categorised by Brockmann's (Brockmann, H. J., 2001) framework, so the process of placing the tactics of *A. pubescens* females into Brockmann's (2001) framework is complete.

Future directions

Unlike *P. dominula*, the ARTs employed by *A. pubescens* females are limited to two, and are easy to characterise using Brockmann's (2001) framework (see above). **Chapter 4** left some outstanding questions, however, and it is two of those that I will address here. The first, are parasites selecting hosts in an opportunistic fashion or making use of information they have gathered from the wider aggregation? We found no evidence that a parasitism event was likely to occur in high density areas within the aggregation, or that a host's nest was close to its respective parasite. This does not necessarily mean, however, that parasites do not use information about conspecific's nest in their selection of potential host's nests. Nests at the right stage (between egg laying and the start of the

main provisioning bout) may be relatively rare, and wasps returning from the surrounding foraging habitat may gather information on potential hosts as they travel to their own nests with prey. Investigation of this question may require, in addition to data on the developmental stage of nests and records of parasitism events, detailed data on the movement of wasps as they travel through the aggregation (e.g. when provisioning their own nests), perhaps making use of advances in automated monitoring (Kennedy *et al.*, 2017).

The second is regarding the surprising result whereby body size was found not to affect the proportion of time spent engaged in parasitism vs being parasitized. We hypothesised that larger individuals would tend to parasitize more frequently due to a positive correlation between body size and maximum fecundity. This relationship between body size and fecundity remains unstudied in *A. pubescens* or related species, and despite empirical support for the general relationship (Gall Maker, *Dryocosmus kuriphilus* - Graziosi and Rieske, 2014; reviewed in Honěk, 1993; *Belonocnema treatae* - Hood and Ott, 2017; parthenium beetle, *Zygogramma bicolorata* - Omkar and Afaq, 2013; yellow lac scale, *Tachardina aurantiaca* - Ong *et al.*, 2014); however, there are exceptions (solitary bee, *Osmia cornuta* - Bosch and Vicens, 2006; dung beetle *Onthophagus taurus* - Moczek and Cochrane, 2006). A highly detailed observation conducted in field conditions over multiple years would be of particular value, as ectotherm physiology is strongly temperature dependent, and the potential fecundity benefits of a large body size may not be met in all species, or in all years. It would be of particular interest if larger individuals were found to have higher fecundity in field conditions on average, given our finding that body size does not affect the the proportion of time spent engaged in parasitism vs being parasitized.

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