The effects of succession on aggression and foraging dynamics in

Polistes dominula

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THESIS SUMMARY

Eusocial insects have been the subject of a great deal of attention from bioscientists since at least as early as the mid-1960s, and the social structure of some were researched even before the term "eusocial" had been introduced. *Polistes dominula* is an incredibly well researched species, the first invertebrate to have its dominance hierarchy documented, but there are still unanswered questions regarding how and why their linear hierarchies persist and flourish when unrelated helpers have the option of founding a nest singly. Investigating the dynamics surrounding these altruistic aggregations may contribute a wealth of knowledge to the current understanding behind sociality and dominance as a concept. This thesis investigates some of the important aspects of *Polistes dominula* hierarchical societies, focusing namely on aggressive interactions and helping effort. The specific aim of this study is to identify the consequences of new leadership on aggression and foraging efficiency, particularly around the point of succession.

In Chapter 1 a large sample of relevant studies are critically reviewed to provide a summary of the current understanding of *Polistes dominula* societies. This chapter highlights the biggest questions not yet conclusively answered about social evolution, and this species as a model of linear dominance hierarchies. This is for the purposes of introducing relevant discoveries, indicating which gaps this research is aiming to fill, and which questions future researchers should be trying to answer. Following this review is an outline of the general methods that apply to this study as a whole, including reasoning behind the study species, study site, and methods of data collection (Chapter 2). In Chapter 3 the results of a dominant removal experiment, aiming to test the role

of aggression in hierarchy establishment and maintenance among foundresses, are reported. No evidence was found that suggested that aggression was used by a successor to establish herself as the new dominant. Similarly, there was no evidence found that suggested aggression displayed by a successor was influenced by the aggression displayed by the original dominant. In Chapter 4 another dominant removal experiment tested whether foraging effort of the nest collectively changed during succession. The results of this experiment suggested that foraging effort decreased from the day a foundress was removed, regardless of her rank, to the end of the sample period. This pattern was seen whether the foundress removed was the dominant or a low-ranking individual. Therefore, there was no evidence that foraging effort decreases specifically during periods of succession, but rather when any other foundress disappears. Foraging effort did not differ significantly between control and treatment groups but did significantly decrease from the day of removal once a foundress had been taken from the nest. This is possibly because remaining wasps became more vigilant following the disappearance of a nest-mate, or because the disappearance of any foundress causes instability in the social hierarchy, driving the rest of the nest to commit more time to dominance contests. These findings are put into context and their contributions towards the field are described in Chapter 5. A description of how this research contributes towards the synthesis of a broader understanding of *Polistes dominula* is provided, along with suggestions for further research that builds on these findings and those before it.

AUTHOR'S DECLARATION

All chapters in this thesis were written by Alexander T. Brown with comments provided by Professor Michael Cant, who provided guidance throughout. Additional comments and guidance on Chapter 1 were provided by Anthony Walker-Cook. Discussion and comments regarding data analysis methods in Chapters 3 and 4 were provided by Faye Thompson and Owen Greenwood. Data collection was performed by Alexander Brown, Feargus Cooney, Emma Inzani, and Sarah Ball. Video scoring was undertaken by Alexander Brown and Sarah Ball.

All research was approved by the Ethical Review Committee of the University of Exeter.

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GLOSSARY AND ABBREVIATIONS

Aposematic colouration: distinct markings and contrasting colours, providing a
conspicuous warning signal to potential predators that the species is
unpalatable or otherwise disagreeable (Leimar, Enquist, & Sillen-Tullberg,
1986).

Assured fitness returns: fitness benefits gained by investing in brood-rearing,
even if the individual dies before the offspring reach independence, through
the efforts of surviving nest-mates (Lucas & Field, 2011)

Clypeal mark: highly variable black facial spot or pattern on the portion of an
arthropod's face referred to as the clypeus (Tibbetts & Dale, 2004).

Clypeus: the portion of an arthropod's chitinous exoskeleton which covers the
lower part of its face.

Confirmation bias: interpreting evidence in a way that supports one's previous
expectations, even if other interpretations are equally plausible (Nickerson,
1998).

Cuticular signature: the unique long-chained hydrocarbon composition of an
insect's cuticle (Sledge, Boscaro, & Turillazzi, 2001).

Diapause: a physiological state of rest or dormancy (Andrewartha, 1950).

Eclose: to emerge from an egg or pupa casing.

Gamergate: mated, egg-laying workers; typically alternative reproductive
individuals to a queen in queenless ant colonies (Peeters & Tsuji, 1993).

Gyne: reproductive females that mate in preparation for founding new nests the
following year (Zanette & Field, 2009).

Inclusive fitness: the assured inheritance of one's genes achieved through the successful reproduction of a relative with replicas of the same gene, giving rise to altruistic behaviour (Hamilton, 1964).

Interdemic selection: a type of selection that acts on subsets within a population, or demes, and the reaction of the deme, rather than the individual, to an allele (Wright, 1959).

Lek: an aggregation, typically of males, to display to potential mates and defend territory from competitors (Kimsey, 1980).

Mesoscutum: the chitinous dorsal plate covering a large portion of an insect's thorax.

Reproductive dominance: a dominance hierarchy in which a ranking exists based on reproduction, with high ranking animals producing a greater number of offspring than those of low rank (Moritz & Hillesheim, 1985).

Reproductive potential: the relative capacity for an individual to reproduce under optimum conditions.

Reproductive skew: the partitioning of reproduction among communally breeding animal groups, whereby societies in which breeding is monopolised by one or a few individuals have high skew and those with more equitably distributed breeding have low skew (Johnstone, 2000).

Social dominance: a dominance hierarchy in which a ranking exists based on behaviour, often in the form of aggressive interactions, with the submissive animals passively accepting the imposing actions of those dominant to it (Rowell, 1974).

CHAPTER 1: THE DELICATE BALANCE OF HIERARCHY

<u>What is a dominance hierarchy?</u>

A dominance hierarchy is an organisation of individuals in a social group that coexist with a stable dynamic. A definition of dominance in a dyad was given by Drews (1993):

"Dominance is an attribute of the pattern of repeated, agonistic interactions between two individuals, characterized by a consistent outcome in favour of the same dyad member and a default yielding response of its opponent rather than escalation. The status of the consistent winner is dominant and that of the loser subordinate"

Dominance in animal societies is therefore identified as a relationship in which one individual consistently submits to the other. With enough of these pairs or "dyads" in one group, the result is a social structure in which every individual has a place and a standing relative to all other individuals. These dominance hierarchies vary in linearity. Linear dominance hierarchies, which are of particular relevance to this study, are defined as follows by de Vries (1998):

"In a linear hierarchy the dominance relation is transitive. This means that for every three individuals A, B and C in the group the following holds: if A dominates B and B dominates C then A also dominates C."

Thus, a linear hierarchy is a social structure where all members are ranked one after another in terms of status, and abide by those positions behaviourally. Dominance hierarchies can be observed across taxa and are often associated with benefits being afforded to those of higher rank. These are often a combination of benefits such as reduced demand to work, increased safety, and a better portion of resources. An example of this is reproductive skew in

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queenless ants (Ito & Higashi, 1991), where the gamergate is the only individual in colonies of *Pachycondyla krugeri* ants.

Hierarchical communities are abundant throughout the animal kingdom, which is a testament to how effective a hierarchical structure is at maintaining the stability of an aggregation. One of many examples of a linear hierarchy occurring in nature is in fluvial red-spotted salmon (*Oncorhynchus masou ishikawai*). This species are organised into a linear hierarchy based on their relative sizes, with unequal resource partitioning in favour of the territorial dominant individuals over the non-territorial subordinates (Nakano, 1995). This resource skew is evidence of a tangible benefit of being dominant in a hierarchy. Female chimpanzees from the Taï National Park also displayed a linear dominance hierarchy based on greeting behaviour and success in contests over food (Wittig & Boesch, 2003). Both of the previous examples have been linear dominance hierarchies, but other hierarchical structures exist across taxa. Banded mongoose (*Mungos mungo*) hierarchies contain between one and five dominant breeders of each sex, with subordinates that breed occasionally. Reproductive skew is enforced by the dominants through infanticide, which has led to synchronised oestrus and birthing events for all females in an attempt to disguise any subordinate's pups among dominant ones (Mitchell *et al.*, 2018). Tebbich, Taborsky, & Winkler (1996) undertook an interesting study on keas which also revealed a non-linear hierarchy that gave rise to interesting triadic and group interactions. While dyads of these keas resulted in one individual being coerced into operating an apparatus for the dominant to access food, in triads and groups often all individuals would get access to the food as they would each be dominant over at least one other. A different, non-linear hierarchy is adopted by the crayfish *Procambarus clarkia*, one that is referred to

as a superdominant hierarchy. This is where one superdominant emerges over the rest, all of whom are subordinate to the superdominant (Herberholz, McCurdy, & Edwards, 2007). These superdominants appear to be initially determined through aggressive means, particularly if the individual was the first to approach and attack others, but the largest individuals seem to take the superdominant status after some time (Issa, Adamson, & Edwards, 1999). Finally, Meese & Ewbank (1973) demonstrated that dominance hierarchies also occur in domesticated animals, and not just as a result of their captive conditions. Domestic pigs seem to form a hierarchy through aggressive interactions which is fixed within 48 hours of individuals first meeting one another. In this hierarchy males appear to be dominant over females, and display linearity at least among young females. One can see even from this small sample of species that hierarchical communities of varying degrees of linearity permeate a huge number of taxa in the animal kingdom. Of particular relevance to this study, it is evident that many of these examples establish their hierarchies through a series of aggressive contests and interactions, allowing each individual to determine their own rank or position in the complex community.

Research on Hymenopterans has played an important role in illuminating the behavioural mechanisms by which dominance hierarchies are formed and maintained. Hymenopterans are renowned for their large, eusocial, altruistic societies and the amazing feats that are accomplished when measures are in place to prevent conflict within vast nests. These societies often contain morphologically diverse castes, many of which simply cannot exist without the other castes. Many species achieve this complex cooperation through the sterility of subordinates, whether this means they are born sterile or are

mechanically sterilised (Hamilton, 1972). An example of the latter system can be observed in *Dinoponera quadriceps*, where workers are permanently prevented from mating when the gamergate bites off their innervated thoracic appendages (Monnin & Peeters, 1998). Sterilised subordinates have no potential reproductive opportunities, and so can only further the success of their genes by helping a relative's offspring reach adulthood, a process commonly referred to as kin selection (Gardner, West, & Wild, 2011). Thus, subordinates put the needs of the nest before their own in the interest of receiving inclusive fitness benefits. This is a fascinating adaptation, but requires little behavioural regulation once the option of reproducing is forcibly taken from potential rivals, leaving them with no option but to assist their fertile relative.

There are also many eusocial societies in which helpers are not sterile or incapable of reproduction. These systems raise questions as to why subordinates help reproductive individuals when they themselves have the potential to reproduce. One example, and the system used in this research, is that of *Polistes dominula*: a primitively eusocial paper wasp that can form nests singly or in an aggregation of foundresses. Within these nests all foundresses and workers are, or can become, reproductively viable. In systems such as this the society requires more complex dynamics to be stable, as every individual should furiously pursue their own reproductive opportunities and not assist others without very good reason. There is a wealth of primary literature on systems with reproductively viable helpers that contribute towards understanding how such conflicts of interests are regulated, but the discoveries proposed are still hotly debated among researchers.

<u>Why form or join a dominance hierarchy?</u>

There are a number of benefits and costs to living in a hierarchical community that have been researched regarding *P. dominula* colonies. The following critical analysis and comparison of this existing literature provides insight into which specific rules of a typical dominance hierarchy are relevant to this particular species.

One obvious benefit of living in a group is greater reproductive opportunities. In a dominance hierarchy, however, reproduction is often skewed in favour of the dominant individual. In some social wasp species the dominant foundresses are willing to share reproduction to an extent (Sledge, Boscaro, & Turillazzi, 2001; Seppä, Queller, & Strassmann, 2002; Cant *et al.*, 2006), and perhaps even preserve the offspring of deceased individuals, such as in *Microstigmus nigrophthalmus* (Lucas & Field, 2011). Shreeves *et al.* (2003) suggest that *P. dominula* also rear any offspring of helper foundresses after their deaths, giving foundresses that join existing nests the advantage assured fitness returns over foundresses that choose to nest singly. However, this can only serve as an incentive to join a dominance hierarchy to helpers that will have reproductive investments of their own to be preserved. A great deal of primary literature on *P. dominula* reveals that dominants of this species actually enforce very high reproductive skew, almost totally monopolising egg laying (West-Eberhard, 1969; Röseler *et al.*, 1984; Sledge, Boscaro, & Turillazzi, 2001). Queller *et al.* (2000) found that, even early on in the establishment of the dominance hierarchy, the dominant foundress laid 93.9% of eggs, which increased to 99.6% later in the season. Additionally, a comprehensive study by Liebert & Starks (2006) found no evidence for the reproduction sharing that one would expect to be offered to unrelated foundresses as an incentive for them to stay

and help. It is therefore unclear how an argument can be made for assured

fitness returns, given that only 0.2-2.6% of the brood could be attributed to the

average subordinate foundress. It is probable that the extra offspring reared by

the nest following a helper's death in the study Shreeves *et al.* were actually of

the dominant and not the deceased helper; thus, the subordinate would have

negligible assured fitness returns at best. Even if the deceased helper had laid

the full 2.6% of the total number of eggs, unless she did so towards the very

end of the season the offspring would emerge as workers and likely never

reproduce. There does seem to be conflicting evidence between those studies

suggesting that only dominant *P. dominula* have ovaries developed enough to

lay viable eggs (Sledge, Boscaro, & Turillazzi, 2001) and those that have

identified developed eggs in the ovaries of a far more substantial proportion of

the population (Cant *et al.,* 2006; Cant & English, 2006). While the analysis of

ovarian development undertaken by Cant *et al.* (2006) occurred after a removal

experiment, and could have feasibly been influenced by that, the examination of

ovarian development undertaken by Cant & English (2006) followed no such

manipulation. Therefore, it is likely that the variation in ovarian development of

the *P. dominula* population around Conil de la Frontera is best represented by

the findings of the study by Cant & English. Studies on the extent of

reproductive skew in this species, however, still suggest that subordinates are

afforded very little reproduction (West-Eberhard, 1969; Röseler *et al.*, 1984;

Sledge, Boscaro, & Turillazzi, 2001; Queller *et al.* 2000). It is therefore unlikely

that *P. dominula* subordinates join a dominance hierarchy for a share of the

nest's reproduction, at least not immediately. It should be acknowledged,

however, that a study by Leadbeater *et al.* (2011) found that, contrary to the

aforementioned evidence, an individual can achieve greater direct fitness as a

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subordinate than as a solitary foundress. This offers the alternative explanation that gregarious nesting still offers enough reproductive prospects, relative to those of a single foundress, to act as an incentive to join a hierarchy. Immediate reproductive opportunities are not the only means of achieving fitness; non-reproductive individuals often further the inheritance of their genes through indirect fitness. This is where an individual gains fitness benefits through the lifetime reproductive success of their relatives (Hamilton, 1964; Nonacs & Reeve, 1995; Field, Shreeves, & Sumner, 1999; Seppä, Queller, & Strassmann, 2002; Cant & English, 2006; Zanette & Field, 2009). Sterile worker castes in eusocial groups often work in this way, assisting reproductive relatives to ensure that the genes they both share have a high chance of being inherited (Sledge, Boscaro, & Turillazzi, 2001). Indirect fitness benefits can only be accrued by helping a relative; the higher the relatedness the more profitable helping them is to the individual. This is the most compelling reason that indirect fitness is unlikely to be the main reason for *P. dominula* helpers to join a multiple foundress dominance hierarchy. A lot of evidence suggests that unrelated *P. dominula* foundresses nest together frequently, and that there is often a large proportion of subordinate foundresses that are unrelated to the dominant (Queller *et al.*, 2000; Cant, Llop, & Field, 2006). In an Italian population studied by Queller *et al.* (2000), this proportion was approximately 35%. Zanette & Field (2009) only found closely related foundresses on 26 of 53 nests, and discovered a negative correlation between rank and proportion of full sisters on the nest. They do suggest that opportunities for indirect fitness benefits may be capitalised on by nesting with relatives that are soon to inherit, though this is unlikely given the aforementioned negative correlation, and raises a logical question of why an individual would nest with rank 2 or 3 relatives, but

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not dominant relatives, in the pursuit of indirect fitness benefits. Contrary to these findings, Cant *et al.* (2006) found that ranks 1 and 2 were full sisters in 90% of their experimental nests. The same numbers of microsatellite loci were used in this study as in the one by Queller *et al.* (2000), so it is difficult to identify a reason for this discrepancy. One possible reason is that Cant *et al.* focused on relatedness between ranks 1 and 2, which does not necessarily account for helpers of lower ranks. This therefore provides evidence of frequent relatedness between the top two ranks, but is not evidence of low ranking subordinates joining groups for indirect fitness through a dominant, reproductive sister. Indeed, the paper makes no such suggestion. A study by Zanette & Field (2008), using nests from the same rough geographical location as Cant *et al.* (2006), investigated within-group relatedness and found an average relatedness of <0.5. This suggests that, even with high relatedness between rank 1 and 2 individuals, indirect fitness is unlikely to be the main or only reason for helpers to join an existing group of foundresses. However, Dapporto, Pansolli, & Turillaziz (2004) discovered that winter hibernacula consisting of individuals from differing parentage often result in a mixing of CHC profiles. This would confound attempts to identify one's kin through chemical cues. Hence there is a possibility that foundresses intending to nest with relatives end up nesting with unrelated foundresses due to mistakes in recognition of chemical profiles. This would explain the vast amount of seemingly contrary evidence around relatedness in *P. dominula* hierarchies.

Despite the high reproductive skew enforced by dominant foundresses, there may be substantial future fitness benefits for subordinates if they stand a chance of inheriting the position of breeder in future. This possibility seems to be a strong incentive for helper foundresses to join an existing group (Pardi,

1948; Field, Shreeves, & Sumner, 1999; Seppä, Queller, & Strassmann, 2002; Zanette & Field, 2009). This theory of delayed reproduction and future fitness driving altruism in *P. dominula* is widely accepted and there is much evidence to support it (Queller *et al.*, 2000; Cant & Field, 2001; Cant & English, 2006), particularly earlier in the season when the eventuality of inheriting the nest is plausible even for those of lower rank (Nonacs & Reeve, 1995; Cant, Llop, & Field, 2006). A more recent study by Leadbeater *et al.* (2011) found that subordinates that do inherit nests have greater direct fitness benefits than lone foundresses. However, it also found that indirect fitness benefits of subordination often surpass direct fitness benefits if the individual is a relative of the dominant foundress. This provides some insight as to why high percentages of unrelated foundresses aggregate and complements the possible role of mistakes in kin recognition on nesting decisions. While other factors, discussed later in this chapter, may influence an individual's decision to join a hierarchy, the current evidence suggests that the main driving force behind gregarious nesting of *P. dominula* is a combination of direct fitness benefits as a result of nest inheritance, indirect fitness benefits through relatives, and failure to differentiate between kin and unrelated individuals.

Unrelated helpers appear to benefit from delayed reproductive opportunities more than indirect fitness benefits, but factors such as group size and nest size may determine how big of an incentive nest inheritance is. One would expect that a large nest is more enticing to inherit on the grounds that it can support more offspring (Shreeves *et al.*, 2003), but that a shorter queue would improve an individual's chance of reaching the dominant position. One might therefore suspect that a trade-off exists between nest size and group size, with the nest size to group size ratio determining how enticing the prospect of joining a

hierarchy is. This is consistent with the observed tendency of the dominant evict other foundresses once the nest is well developed and workers have begun eclosing (Shreeves *et al.*, 2003). However, Field, Shreeves, & Sumner (1999) found that experimentally reducing group size did not increase frequency of joiners in colonies of eusocial hover wasp *Liostenogaster flavolineata*. This suggests that, at least in eusocial hover wasps, group size is not a consideration of potential joiners. However, these wasps are tropical and not polistine, so direct comparisons with *P. dominula* should be made with caution; previously discussed factors may play a greater role in different wasp species than in *P. dominula*. In addition, whilst proportions of wasps joining experimentally reduced groups and control groups did not significantly change after removals, a negative relationship between group size and number of joiners was identified in un-manipulated nests and between removal treatment nests after removals. The fact that joiners appeared to preferentially choose removal nests, even before removals took place, suggests the possibility of sampling bias whereby a disproportionate number of favourable nests ended up in the removal treatment. An explanation offered for this unexpected set of results by Field, Shreeves, & Sumner (1999) was in fact the presence of another variable affecting decisions, such as nest size. An alternative outlook can be inferred from the discovery by Cant *et al.* (2006) that a promoted rank 2 is more likely to defend its rank from a returning dominant when group size, not nest size, is larger. Whilst not explicitly about joiners, this does emphasise that a large work force is valued by the dominant. It is possible then that the benefits of a large work force and capacity for more offspring are cancelled out to an extent by the drawback of a long queue for inheritance, and hence effects are only observed when the long waiting queue is no longer a consideration.

The previous paragraphs have focused on the benefits of joining an existing dominance hierarchy, but the difficulties associated with nesting alone should also be considered when assessing the incentives behind an individual's nesting strategy. For example, usurpation seems relatively rare in multiple-foundress nests (Klahn, 1988; Queller *et al.*, 2000), which is no incentive for a subordinate since they will not be the target of the usurper. The significantly higher threat of usurpation of single-foundress nests (Klahn, 1988; Nonacs & Reeve, 1995), however, is far more relevant: a helper contributes to building, brood care, and foraging and has a chance of inheriting the nest and capitalising on all the investments made by all helpers, whereas a usurped single foundress has undertaken all of the building, foraging, and brood care alone with no guarantee that her reproductive investments will be preserved by a usurper. In fact, Nonacs & Reeve (1995) observed usurpers destroying the eggs and early instar larvae of previous foundresses. Shreeves *et al.* (2003) found a similar trend where a high proportion of lone foundresses suffered nest failures before their adult offspring emerged, whereas multiple-foundress associations were more robust to nest failure regardless of the size of the group. Therefore, an unrelated helper has a low chance of gains but also stand to lose far less, making a subordinate role a "low risk, low gains" strategy. It is not unheard of that late usurpation, leaving the usurped foundress with few options, might drive an individual to seek out and join a nest for whatever slim chances at fitness returns it might provide, as has been observed in *Polistes biglumis bimaculatus* (Lorenzi & Cervo, 1994). Contrary to the findings that single-foundress *P. dominula* colonies have higher risk of usurpation, Gamboa, Greig, & Thom (2002) witnessed no usurpation attempts by *P. dominula* foundresses, nor any successful usurpations against them. A plausible reason

for this discrepancy is that usurpation is a high-risk strategy itself, adopted primarily by displaced single-foundresses (Nonacs & Reeve, 1995). The study by Gamboa, Greig, & Thom emphasises that their study population of *P. dominula* in America are faring incredibly well and replacing the native *Polistes* wasps via exploitative competition. The lack of parasitism, desynchronised activity with predators, and more striking aposematism clearly offers a survival advantage, which is possibly responsible for the lower number of displaced foundresses that must then resort to usurpation.

A critical review of the literature suggests that the primary incentive for non-relatives to join a hierarchy in *P. dominula* nests is actually a complex combination of indirect fitness benefits, failure to accurately recognise kin, and potential inheritance of a multiple-foundress nest robust to usurpation. Naturally, with a large proportion of the population opting for this low risk but low gain strategy, there is a competitive advantage that comes with successfully founding a successful nest alone. Assuming the aforementioned hurdles are overcome, a solitary foundress gains 100% of the reproductive shares on a nest without having to queue and need not share any nutrition with a more dominant, which is the case on multiple-foundress nests (Pardi, 1948). Furthermore, by the time workers begin to eclose the dominant often attempts to drive away remaining auxiliary foundresses who have served their purpose (Pardi, 1948; Nonacs & Reeve, 1995). By this stage in the cycle, assuming the nest does not fall to late season usurpation, the solitary foundress is granted returns on her investments; even if she dies one of her worker offspring is capable of ascending to dominance and producing reproductives for the next year (Pardi, 1948; West-Eberhard, 1969; Queller *et al.*, 2000). The contrast of these two strategies should feasibly allow both to persist in the population: the helper

strategy as low risk strategy with slim opportunities for gains, and the solitary foundress strategy with a high risk of usurpation but great rewards for those that succeed. Usurpation ties into this balance as a contingency strategy for those that take the high risk but lose their nest before it can yield returns.

Maintaining a dominance hierarchy

The relative benefits or drawbacks of being part of a dominance hierarchy are often dependent on rank; total reproductive dominance is only a negative if you are not the dominant, for example. With such a high skew of benefits and resources, mechanisms must exist that prevent this linear hierarchy descending into chaotic scramble competition. Some possible explanatory factors have been analysed with somewhat conflicting results.

Though there is evidence, as analysed above, that difficulties in determining relatedness to the dominant means it is probably not an essential factor in a wasp's decision to join a nest, relatedness is a factor one might expect to influence ranking within a hierarchy. There are two structures one might expect to see if relatedness is a factor that influences ranking. One eventuality that might be expected is the formation of kin-coalitions by relatives (Widdig *et al.*, 2006), in order to secure higher ranks so one may become dominant and the others receive inclusive fitness benefits. Alternatively, individuals all related to the dominant may be expected to be of consistently lower rank as a concession by the dominant to unrelated individuals as an incentive to help (Reeve & Keller, 2001). However, in many studies it seems that relatedness does not appear to determine rank in *P. dominula* at all; related and unrelated subordinates seem indiscriminately organised across the hierarchy (Queller *et al.*, 2000; Cant & English, 2006; Cant, Llop, & Field, 2006; Leadbeater *et al.*, 2010). Zanette &

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Field (2009) did find rank negatively correlated with the number of full sisters an individual has on the nest, consistent with the concession model, but no significant trend was found between relatedness of helpers to the dominant and their ranks. This says more about which individuals nest together than how rank is predicted, and thus cannot be taken as evidence of relatedness determining rank. Cant *et al.* (2006) also shows that relatedness does not significantly sway the decision to escalate conflict over a rank. Whilst not direct evidence that relatedness does not determine rank, the logical inference from this would be that individuals are no more or less likely to challenge their relatives. Furthermore, when considering the findings by Dapporto, Pansolli, & Turillaziz (2004), it is plausible that the mixing of CHC profiles, both during winter hibernation and while sharing a nest, makes differentiating kin from unrelated individuals extremely difficult. Together, these suggest relatedness is an improbable predictor of rank.

Perhaps the first factor one might consider is order of arrival onto the nest. It would make sense that a new helper, who has invested very little in the nest, should be pushed to the bottom of the queue. This trend was observed in *P. fuscatus* by West-Eberhard (1969). In a study by Seppä, Queller, & Strassmann (2002) on *Polistes carolina* this also seemed to be the case with regards to who achieved the rank 1 position. It is important, however, to consider that this study was not on *P. dominula*. Seppä, Queller, & Strassmann also note that aggressive competition continued throughout the season, which suggests that arbitrary order of arrival may be less important than general fitness or condition that may be correlated with early emergence from winter refuges. An experiment by Zanette & Field (2009) investigated order of arrival in *P. dominula* specifically. They used groups of the same average size as Seppä,

Queller, & Strassmann (2002), but they observed inheritance queue instead of social dominance hierarchy, and analysed the effect of order of arrival up to rank 5, not just on the dominant position. Zanette & Field (2009) found that order of arrival did not correlate with rank for ranks 1-5. However, a correlation emerged when limited to the top 3 ranks and became significant between the top 2. This is consistent with the finding by Seppä, Queller, & Strassmann (2002) that order of arrival can predict who will adopt the dominant position. Zanette & Field note that this correlation only meant that the dominant was present before the rank 2, not before all other nest-mates. Again, it was suggested that this limited importance of arrival order may indicate that it is a reflection of another, less obvious factor such as body condition or fecundity. Alternatively, this could indicate greater determination of higher ranks to hold onto their position in the hierarchy than low ranking wasps, who are not as close to inheriting the nest. The results of a study by Pratte & Gervet (1992) provide further indirect evidence that order of arrival determines rank with regards to the dominant position, whereby dominant wasps win competitions more frequently on their own nest, but again this cannot be extrapolated to lower ranks. As many of these trends leave the rankings beneath the top two unexplained, it is reasonable to suggest that something other than order of arrival must be involved, even if order of arrival does determine the dominant position.

The suggestion by Zanette & Field (2009) that order of arrival may be reflecting fecundity raises the question of whether other signals of ovarian activity explain dominance rank. Röseler *et al.* (1984) investigated the effects of ecdysteroid and juvenile hormone, hormones correlated with terminal oocyte length and corpora allata volume respectively; they found that wasps experimentally injected with these hormones stood a greater chance of becoming dominant.

Corpora allata and ovaries begin development based on environmental cues such as heat and light (Zanette & Field, 2009). Hence, it is possible that order of arrival reflects early emergence, which coincides with greater development of sex organs, higher levels of corresponding hormones, and thus a greater probability of acquiring dominance. Proportions of cuticular hydrocarbons are also seemingly associated with ovarian activity, as well as distinguishing workers from subordinate foundresses. However, differences between cuticular signatures of foundresses are not significant until later in the season (Sledge, Boscaro, & Turillazzi, 2001). Izzo *et al.* (2010) confirm the link between cuticular hydrocarbons and ovarian activity, but also provide evidence that fertility does not determine dominance in early stages of nest development. This is consistent with indistinguishable cuticular signature in early stages, and suggests that any significance shown by order of arrival cannot reflect ovarian development; however, it is possible and plausible that cuticular signature and hormonal cues are involved in maintaining a linear hierarchy later in the season. Chemical signalling may still only be half of the struggle to retain total dominance on a nest, as suggested by the lack of evidence for its role in establishing initial dominance, and by findings by Downing & Jeanne (1985) that show a need for aggression as well as chemical cues.

If ovarian activity, chemical signals, and order of arrival do not organise rankings beyond the dominant position then it is reasonable to suggest that contests may be the determinant. The role of aggression has already been highlighted by Downing & Jeanne (1985) in *P. fuscatus* at least; this may well be the case in *P. dominula* also. As a rule across taxa, body size is often correlated with fighting ability and contest success (Rowland, 1989). If this correlation is true, and conflict can be avoided using an honest, low risk visual

cue, it is probable that body size could predict rank. This is what is suggested

by findings and observations in studies such as Cervo *et al.* (2008) and

Turillazzi & Pardi (1977), and assumed in some others (Nonacs & Reeve, 1995;

Tibbetts & Dale, 2004). However, this trend was not observed in other studies

(Cant & Field, 2001; Zanette & Field, 2009; Röseler *et al.*, 1984), when applied

to *P. dominula*. Cant *et al.* (2006) also found that body size does not seem to

predict whether wasps will escalate conflict or not, providing evidence against

this use of a visual cue to avoid costly fighting. This inconsistency suggests

limited importance. As reasoned by Zanette & Field (2009), a morphological trait

such as body size being able to put newcomers far above existing wasps in the

ranking could destabilise hierarchies, and one would expect far more usurping

using this method, which was not observed by Nonacs & Reeve (1995). A

possible reason behind this discrepancy in theories could be due to the

assumption that body size is indicative of fighting prowess, and also that it is a

determinant and not a result of dominance. As Cervo *et al.* (2008) measured

body size of gynes in autumn, it is possible that the trend they discovered was

how size reflects condition and thus chance of surviving overwintering, but that

among those large enough to survive until spring it is not necessarily a deciding

factor. Nonacs & Reeve (1995) assumed that body size was indicative of

dominance, and used reduced nest development in those with less size

variation as justification; in reality reduced development in these nests does not

explicitly prove it is a factor and it is possible that a third unidentified variable is

biasing this trend. On the other hand, they did specifically find trends regarding

size relative to nest-mates, not absolute size, and this may also be a reason for

the differing finds. Turillazzi & Pardi (1977) found that dominant wasps were

often bigger, but that size differences were not always huge and a smaller wasp

could still come onto the nest and ascend to dominant. Given the growth in

ovaries associated with becoming the dominant, growth in body size to

accommodate growing ovaries, following forceful acquisition of dominance,

could be the reality of the situation. This would make body size a result of

dominance, rather than a determining factor. This concept of increased body

size to account for increasing ovarian size could be consistent with the study by

Röseler *et al.* (1984) and may be worth greater consideration in future.

On the same logic of body size as a visual cue comes one of the most hotly

debated, inconsistently supported suggestions regarding rank in *P. dominula*:

the presence of a clypeal mark as an honest signal of quality. This discovery by

Tibbetts & Dale (2004) puts forward the theory that specific patterns of clypeal

marks, relating to the brokenness of the black marks, are correlated with body

size and are used by conspecifics to assess the quality and fighting ability of

another, thus resulting in higher chance of dominance. Zanette & Field (2009)

also found a significant correlation between clypeal mark and rank, though they

noted that the number of foundresses with clypeal marks on any one nest was

small. Cervo *et al.* (2008), on the other hand, found no significance between

rank and clypeal mark when using experimental methods that used naturally

established hierarchies instead of induced dominance interactions. They also

critiqued a number of aspects of the Tibbetts & Dale (2004) study that may have

contributed to these inconsistent findings. These critiques revolved around the

different experimental designs between the two studies, and that a different

isolated population was used. The difference in population may well be an

important contributor when it one considers the study by Zanette & Field (2009)

on yet another population, however a more recent study by Green *et al.* (2013)

on a population from southern Spain found no evidence of clypeal marks

correlating with competitive ability or quality. The remarks on experimental design and ambiguity of results are reasonable, with particular focus on the time taken to establish a stable hierarchy compared to the time allotted for dominance interactions, and the underlying physiological determinants that are suggested to be in effect at this time. However, the fact that a similar trend between rank and clypeal mark has since been found to be significant in a different population by different researchers through a more natural and representative experimental method lends credibility to the setup used by Tibbetts & Dale. Furthermore, the findings, though ambiguous and with weak correlations in the case of body size and facial markings, were significant. Ordinarily a significant result would be considered exactly that, even if a weak correlation suggested something else might be at work. Thus, it is difficult to say that either set of findings is entirely incorrect. Given the loose evidence in favour of the badge-of-status hypothesis for clypeal marks, it is unlikely that clypeal marks convey information on competitive ability. However, considering the instance of significant trends, there could be some truth to the suggestion by Zanette & Field that clypeal marks are used as a visual cue to an extent but are not essential cues for the establishment of dominance. Alternatively, clypeal marks may only become important in specific circumstances such as when associated with the "sit-and-wait" strategy. Future researchers might also consider factors such as density of badge-bearing individuals in their study population; perhaps reliance on clypeal marks to resolve contests has a density dependent aspect which is responsible for these contrasting findings from isolated populations.

With inconsistent evidence for visual cues, chemical cues, or other determinants like order of arrival or relatedness, one would expect that

aggressive subjugation is likely to emerge as the method by which ranks are decided. It is hard to ascertain if aggression determines rank or is a result of it, as remarked by Zanette & Field (2009). There is evidence that shows aggressively enforcement of subordination (Downing & Jeanne, 1985; Sledge, Boscaro, & Turillazzi, 2001) and, conversely, dominance being challenged through aggressive means, so it is possible that aggression determines rank. Furthermore, the majority of aggression from an individual of a given rank is directed towards nest-mates of adjacent ranks (Pardi, 1948; Cant, Llop, & Field, 2006). This would be expected if moving up in rankings involved exerting aggressive superiority over the rank above. Aggressive behaviour seems to be more typical of higher ranks closer to inheritance (Cant, Llop, & Field, 2006), which would only be a worthwhile risk if it was a means of ascending rank. West-Eberhard (1969) also observed aggression being used by dominants to stimulate foraging, a job typically associated with lower ranking wasps. Though indirect, this is further evidence that dominants may use aggression to induce subordinate behaviour in nest-mates. There are conflicting views regarding whether aggression increases with time (Cant, Llop, & Field, 2006) or decreases with time (Sledge, Boscaro, & Turillazzi, 2001). It does not seem that one trend has more evidence than the other, but one possible cause for these seemingly opposing arguments could be whether spikes of aggression due to succession, attempted usurpation, and late season eviction of auxiliary foundresses are included in one general trend or analysed separately. Considering literature regarding chemical cues, a gradual decrease in aggression would be expected throughout the founding period, interspaced with aggressive peaks where hierarchy must be re-established by a new dominant or between helpers, and then heightened aggression once more when workers

have eclosed and subordinate foundresses are no longer required (Pardi, 1948). None of the evidence analysed appears to conflict with this, and many are consistent with this proposed pattern. Tibbetts & Dale (2004) found that wasps with experimentally altered clypeal marks received more aggression from dominants. If the findings regarding clypeal marks in this study are accepted, this is further evidence that aggression may be being used to subdue a potential rival. However, as recently observed in other paper wasp species by Sheehan & Tibbetts (2011), facial recognition is used by *Polistes* wasps to differentiate nest-mates from intruders. It therefore cannot be ignored that wasps in the study by Tibbetts & Dale (2004) may be reacting violently due to the strikingly unfamiliar facial pattern displayed by challengers. This explanation is supported by what Cervo *et al.* (2008) found, where wasps with facial patterns experimentally altered to simulate high and low aggression all received heightened aggressive responses.

It seems that, generally speaking, aggression or dominance behaviour is used as an indicator of rank, as initiated by Pardi (1948), so there is a good chance that it is fundamental in establishing, and possibly maintaining, dominance rank. Initial aggression seems to be replaced with chemical cues once ovarian regression in subordinates has made their cuticular signatures significantly different. Clypeal marks, body size, and order of arrival may be additional determinants in which wasp becomes the dominant, but without further evidence they should be considered with caution. Jandt, Tibbetts, & Toth (2014) compiled a helpful and comprehensive analysis of possible factors involved in determining rank and dominance that is consistent with the previous critical analysis.

<u>Factors driving helping effort</u>

Whilst the dominant is laying eggs the subordinate foundresses are left to build the nest, forage for food, and care for the brood. There is a balance of cost and potential returns that individuals must consider when deciding on how much effort to put into helping: on the one hand a subordinate's investments are going into a nest being used to rear the dominant's offspring, on the other hand one of the benefits of joining a nest is the possibility of inheriting it later on. Each subordinate should tactically adjust this helping effort to ensure they are not losing more than they will realistically gain. Each subordinate's situation will be slightly different depending on a number of variables.

The first point of consideration is whether certain individuals are under greater selection to help than others. This has been noted above with regards to relatedness. Relatives of the dominant should work harder, as they have a vested interest in the success of the nest (Hamilton, 1964; Zanette & Field, 2008). That is the logical assumption, but it is not always the case; certain social animals such as cooperative mongooses do not appear to factor relatedness into their helping effort (Clutton-Brock *et al.*, 2000). With regards to *P. dominula* there is remarkably little clear evidence that relatedness does, or does not, affect foraging effort. A weak correlation between highly variable foraging effort and relatedness was seen by Queller *et al.* (2000), but this appears to be taken cautiously within the field as it is far from a clear-cut result. More recently, a study by Leadbeater *et al.* (2010) found that relatedness had no significant effect on a number of behaviours including foraging, nest defence, and aggression. Given that relatedness also does not appear to determine rank or decision to join in the first place, this finding suggests that foundresses generally do not discriminate between relatives and non-relatives, probably

because there are mechanistic constraints in the ability to discern degrees of relatedness within foundress associations. This is likely as a result of the difficulties with discerning relatives from unrelated individuals (Dapporto, Pansolli, & Turillaziz, 2004).

Accepting that there is no initial bias regarding helping effort between relatives and non-relatives, one might expect that helping effort may vary with rank instead. The rationale behind this is that higher ranking wasps, who are closer to inheriting the dominant breeding position, work less intensively in the interest of saving their energy to invest more in future reproduction and achieve maximum benefits at minimal cost. It has already been noted previously that the dominant takes almost all shares of reproduction for herself, leaving the work to lower ranks, so it is clear that rank has some effect of distribution of helping effort, if only with regards to the dominant herself. A well referenced and respected study by Cant & Field (2001) indicates that *P. dominula* helpers of higher rank exert less effort helping than lower ranking subordinates. In a great deal of primarily literature this find is accepted, and it has been supported by consistent findings in other studies (Cant & Field, 2005; Field & Cant, 2006), however it should be considered that much of the empirical evidence supporting this is from the same two authors, and the possibility of confirmation bias would be lower given more evidence from other researchers. This cautious observation aside, there appears to be strong evidence for rank predicting helping effort and little against it. There is a question of why, then, low ranks would continue to work at an elevated rate to other subordinates but, as Queller *et al.* (2000) suggested with regards to non-relatives, perhaps high helping effort is the price demanded of low ranking wasps for them to be permitted to stay on the nest.

Cant & Field (2001) also found that help effort decreased with group size, which was consistent with their predictions since it increases the value of the nest and thus individuals should be less willing to take risks with such a large future benefit at stake. This is again supported by other works by Cant and Field. However, group size lengthens queue to inherit (Field, Shreeves, & Sumner, 1999), and so it is possible that this effect of increased payoff of inheritance is actually reflective of relative position in the queue, rather than absolute rank, affecting helping effort. This is plausible if rank is considered to predict helping effort, as there are a greater number of subordinates below a given rank on a larger nest to pick up the slack left by reduced helping effort. Cant & Field (2001) also assume energetic costs do not vary with group size, despite possibility that larger group size may entail less individual demand for fanning or abdominal waggling, or greater insulation of heat. However, there is no evidence that including a difference in energetic costs would change the results, and the close fit between what was found and the model suggests any difference in energetic costs would not have a large impact on the results of this study. No evidence could be found opposing this finding that individual help effort for a given rank decreases as group size increases, but future studies into how help effort varies with relative position in the inheritance queue would perhaps shed light on the specific reasoning behind this.

Thus, though there is relatively little research into helping effort over establishment of dominance or rank determination, the findings by Cant and Field are widely accepted and supported, with little conflicting evidence. Helping effort on *P. dominula* nests appears to be influenced largely by rank and group size; whether these are the only factors determining helping effort is yet to be seen.

Inheritance of dominance

Finally, relevant to this research, there are the consequences of dominance passing from one individual to another to consider. Unfortunately there is a paucity of critical material on this as on the mechanisms behind the hierarchy itself, and consequently there is little in the way of supporting or conflicting evidence behind these findings.

Pardi (1948) made a note that the successor next to inherit the nest reacts almost instantly to the absence of the dominant, but what this reaction consisted of and its implications on the nest have since been elaborated further. Cant, Llop, & Field (2006) mention increased aggression by the new dominant upon succession, which is consistent with the reaction observed by Pardi. Given the evidence around formation and assertion of rankings in the dominance hierarchy, this is what one would expect of a new dominant asserting her position over subordinates, and essentially reaffirming the hierarchy. Strassmann *et al.* (2004) further confirm this reasoning with their detailed study on the impacts of succession. They found that many of the more common aggressive behaviours did not increase, but there was an increase in "ritualistic" behaviours, such as mounting, which are specifically associated with asserting dominance rather than explicitly attacking. Pardi (1948) and Strassmann *et al.* (2004) also observed a gradual physiological change in new dominants that allowed them to fill the role of dominant breeder, with the new dominant's ovaries developing for the duration of the month following her inheritance. A study by Sledge, Boscaro, & Turillazzi (2001) identified another similarly gradual physiological development in cuticular signatures, whereby the proportions of cuticular hydrocarbons being presented by a new foundress gradually turned from one characteristic of a subordinate to that of a dominant a little while after

succession. As Izzo *et al.* (2010) showed, cuticular signature and ovarian activity are correlated and so this alteration in the new dominant's cuticular hydrocarbon proportions is quite likely to be linked to the gradual ovarian development observed by Pardi (1948) and Strassmann *et al.* (2004). Monnin *et al.* (2009) took these observations one step further and observed not only that new dominants become reproductively active and viable breeders, but also that they quickly adopt the behavioural oophagy of the previous dominant's eggs; this is typical of a dominant and lends credibility to this rapid shift into the role of a dominant.

Further to the effects on an individual undergoing the transition to dominant, there may be implications on the nest itself. Indeed, part of the premise of this study was to test the hypothesis that reduced nest productivity following succession acts as a stabilising factor to discourage usurpation by subordinates. Strassmann *et al.* (2004) undertook a comprehensive study and found no decline in nest foraging efficiency during the succession period. This is rather unexpected, particularly when considering that the absence of a dominant will both reduce the aggregation size and bring all subordinates up one rank at least, both of which have previously been identified as factors that reduce help effort. Also, given that the new dominant's ovaries are still developing soon after succession, coupled with the oophagy, there are fewer offspring to forage for. Strassman *et al.* did find that rate of nest growth diminished immediately following succession, which is to be expected on the same basis as with foraging, but this raises further questions on why one act of helping is reduced but another is not. It is possible that consistent foraging rates are forced; West-Eberhard (1969) witnessed aggression being used to stimulate

foraging and it would be in the interest of the new dominant to exert this kind of control.

What current literature predicts during a period of succession is a spike in dominant behaviour, including some aggression and oophagy, followed by a gradual change in the new dominant's chemical signature. Meanwhile, foraging rate remains unchanged but nest growth rate decreases. The subtle suggestions of these findings are that relative position in the inheritance queue, rather than absolute rank, is what determines help effort, and that there is an increased focus on foraging for food rather than wood pulp for nest construction. This may be due to the energetic demands of ovarian development that the new dominant is undergoing, paired with a reduction in the number of eggs present on the nest, but future research should endeavour to provide further evidence to confirm or deny these suggestions.

Conclusion

The sources reviewed in this chapter provide an idea of what the scientific community agree to be accurate of *P. dominula* societies. Collectively, these discoveries give researchers the following foundations to build their study on. It appears that the driving force behind unrelated helpers, and possibly related helpers, to join an existing nest and dominance hierarchy is a combination of potential inheritance of the dominant position, indirect fitness benefits, and mistakes in kin recognition. This seems to satisfy a great deal of the evidence, and few studies provide a compelling alternative. The threat of usurpation in a single-foundress nest and the size of group also appears to have some effect, though from the inconsistent findings it is unlikely that either of these are the primary reason for joining.

Within a dominance hierarchy, a combination of factors seems to determine ranking. Between the top two ranks order of arrival seems to predict which individual becomes dominant over the other, though whether this was because of the order or a hidden correlated factor is still up for debate. Below rank 2, aggressive behaviour or dominance displays are apparently utilised to determine ranks, with each individual testing the rank above and displaying to the rank below. A little while after these ranks have been established, chemical cues through cuticular signature and hormone activity replace aggression in the maintenance of this linear ranking. There were inconsistent findings regarding whether facial marks and body size influenced rank, so once again we have to assume they are not what is primarily used to determine rankings.

Amongst auxiliary or helper foundresses helping effort did vary. The findings of the literature showed that helping effort reduced with increasing rank and aggregation size. However, the trend regarding aggregation size may be reflective of relative place in the queue for any given rank. Thus, what can be confidently suggested is that helping effort decreases with increasing rank, and that there may be variation between group sizes as a result of either the greater payoff that a large group offers, or the increased length of the inheritance queue that comes with more members.

When a dominant is replaced, the successor is seen to reaction aggressively or "ritualistically" almost instantly following the dominant's absence to assert herself as the new alpha. This aggression is coupled with oophagy of the previous dominant's eggs and, after a while, the new dominant's ovaries develop enough for her to assume complete reproductive dominance. Meanwhile, nest growth is stunted but mysteriously foraging rates do not change. This final point is something this research will be investigating.

P. dominula have been a species of great interest because of their peculiar tendency to aggregate under such strict and total reproductive skew with non-relatives. This interest has sparked a wealth of studies and primary literature aiming to disentangle the underlying mechanisms behind their extremely altruistic societal structure which, whilst conflicting at times, provides future researchers with the luxury of plentiful empirical evidence and methods on which to design an experiment. The intention behind this research is to contribute to the ever-expanding understanding behind this species and, consequently, the formation of stable dominance hierarchies and altruism as a concept.

CHAPTER 2: GENERAL METHODS

Research objectives

The aim of this study is to investigate the consequences of succession of the dominant foundress on nests of *Polistes dominula*, and detail the dynamics that are associated with this transition period. The specific objectives of this research are:

1. To test the role of social aggression in the establishment of dominance status in co-foundress associations.

2. To test whether replacement of a dominance foundress results in a reduction of foraging effort from the other foundresses.

The predictions made regarding these objectives are largely informed by the wealth of primary literature already available on *Polistes dominula*, which have been critically reviewed in Chapter 1. With regards to the trend of aggression displayed by the succession, this study predicts that aggressive interactions with other foundresses will show a sharp peak immediately after the dominant is removed, followed by a gradual return to the previous level of aggression observed before removal. This is consistent with the majority of existing discoveries. Meanwhile, this study predicts that rate of foraging will show a sharp decrease that almost mirrors the peak in aggression, and returns to its previous rate as aggression stabilises. This is contrary to the findings by Strassmann *et al.* (2004), and is intended to test these results based on the expectation that helping effort should decrease with group size and increasing rank, both of which would be a factor if the dominant is removed. Foraging effort is also being investigated in the interest of whether helping effort decreases

during periods of succession as the other foundresses commit more time to defending their own rank and position in the inheritance queue.

The study species

Polistine wasps are species within the subfamily Polistinae. Of particular relevance to this study is the genus *Polistes*, composed of species of social paper wasps (West-Eberhard, 1969). Many polistine wasps, particularly within *Polistes*, share similar ecological characteristics. From the literature, we can infer typical colony cycles and nest dynamics for these wasps.

In temperate climates, such as the one this study was based in, polistine wasps generally undergo overwinter diapause (Zanette & Field, 2009). Mated females enter a torpor-like state during the coldest months and re-emerge when conditions are more favourable for the founding period of their cycle. During this phase nests are founded on surfaces in sheltered locations; thorough searching is therefore required to find them for research purposes, as was the case with this study. On the other hand, nests sheltered thusly are afforded a level of protection from predation and weather (Cervo, Zacchi, & Turillazzi, 2000), which is beneficial for both the wasps and researchers studying them. Nests can be founded singly, or by multiple foundresses, in many polistine species. The reasons behind these two strategies have been studied and various suggestions, such as congregating in response to the threat of usurpation, have been put forward as contributors to the decision (Klahn, 1988). Many of these possible reasons have been critically reviewed in Chapter 1.

On multiple-foundress nests the reproductive potential of all present foundresses gives rise to an interesting conflict of interests, whereby each wasp would ideally aim to take as great a share of the reproduction on the nest as

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possible. Consequently, linear dominance hierarchies form to regulate this, with a dominant foundress emerging above subordinate or auxiliary foundresses. These can arguably be separated into social dominance and reproductive dominance, though in reality the resulting hierarchy is often a combination of the two. An impressive number of studies have investigated the mechanics that structure and stabilise such pecking orders. Dominance has been noted to correlate with a number of variables, such as order of arrival (Seppä, Queller, & Strassmann, 2002), but the full reality is likely a complex interaction of numerous deciding factors, some of which have been discussed in Chapter 1. Depending on the species, queenship in polistine wasps is not always absolute; reproduction is shared to a degree in some species (Sledge, Boscaro, & Turillazzi, 2001; Seppä, Queller, & Strassmann, 2002). However, other polistine species show very high reproductive skew towards queen or dominant foundress (West-Eberhard, 1969).

The founding period ends when workers begin to emerge and by the end of summer, in temperate climates at least, gynes and males have normally eclosed. The males often lek away from the nest at sites that may be attractive to the gynes, who leave the nest to mate (Strassmann, 2001; Zanette & Field, 2009). As is the case with monopolising egg-laying, aggression may be employed to prevent any remaining subordinates mating (Sledge, Boscaro, & Turillazzi, 2001). An interesting characteristic of polistine wasps is that there are very few instances of multiple mating, and hence next to no chance of half-siblings (Strassmann, 2001). Once mated, the gynes seek a refuge in which to overwinter, ready for the nest season.

Possibly the best studied *Polistes* wasp is *Polistes dominula*, formally undistinguished from *Polistes gallicus*; a highly abundant polistine wasp with an

Old World distribution (Cervo, Zacchi, & Turillazzi, 2000). *P. dominula* have colony cycles that closely match the typical *Polistes* life cycle described previously. The founding period takes place from late winter to the end of spring, with nests being founded by around 1 - 10 mated foundresses. Whilst remaining nests from the previous year are seldom reused, many make use of the shelter they provide to overwinter safely. This tendency to take shelter behind discarded nests means foundresses tend to emerge and settle near their own natal nests. The result is high nesting site fidelity, with dense congregations of nests of the same species, or even sometimes different sympatric species all clustered together (Cervo, Zacchi, & Turillazzi, 2000). With regard to research, these high densities of conspecific nests allow comparisons to be made with very few environmental extraneous effects biasing the data. The dominance hierarchies on *P. dominula* nests seem to be formed primarily through aggressive means, and later maintained by chemical signalling when subordinate ovaries have regressed and the cuticular signatures of the dominant and subordinates is more distinguished (Sledge, Boscaro, & Turillazzi, 2001; Zanette & Field, 2009). This switch from aggression assertion of dominance to chemical enforcement is not unique to *P. dominula*. *Polistes fuscatus*, for example, also relies on a combination of aggressive displays and chemical cues to maintain both reproductive and social dominance (Downing & Jeanne, 1985). Once a dominance hierarchy has been established, work is distributed according to rank. Lower ranks must undertake the most exhausting jobs such as foraging, building, and brood care, while the dominant monopolises the egg-laying (Pardi, 1948; Zanette & Field, 2009). However, should the dominant die or disappear, the subordinate next in the dominance queue will inherit the nest and all the benefits that come with dominance (Pardi,

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1948; Cant & Field, 2001). Hence, there is a chance that being an auxiliary foundress may have a considerable payoff by the end of the season. This queue does not seem to be determined by relatedness or kin-coalitions (Cant, Llop, & Field, 2006; Zanette & Field, 2009). Rather, it appears that the inheritance queue is maintained, and possibly established initially, through a balance of dominance displays from the rank above and tests of strength from the rank below (Cant, Llop, & Field, 2006). This seems to stabilise once an individual reaches dominant rank and maintains it long enough for chemical cues to take over, though a consistent level of day-to-day aggression seems to suggest that these *P. dominula* hierarchies are always subject to change if an individual cannot hold their position.

Throughout the founding period foundresses may choose to switch, adopt, usurp, or found their own nests. More specifically, foundresses appear to found, switch, and join nests early on, and turn to adopting and usurping nests later on in the season (Nonacs & Reeve, 1995). The varied use of these strategies across the season suggests that the relative gains of each strategy may potentially fluctuate over the season, adding another interesting dynamic to the already complex life history of this species. One particular factor that may contribute to which strategy is adopted is the fact that a high proportion of lone foundresses suffer nest failures before their adult offspring are produced (Shreeves *et al.*, 2003). Nests may also be usurped by social parasites such as *Polistes semenowi*, or other foundresses looking to increase their chances of reproducing. These factors mean there are considerable costs that could come with singly founding a nest, even if this would ensure monopolising reproduction, and also leaves foundresses without nests or nests without foundresses. With this in mind, there is logic behind why displaced foundresses

may seek to join another nest, or why a foundress might decide to leave a hierarchy to adopt a premade nest. Particularly later in the season, nest joining may be employed as a contingency in cases where usurping has left them with little chance of founding another successful nest (Queller *et al.*, 2000). That said, there is evidence suggesting that, relative to other *Polistes* species, *P. dominula* displays low conspecific pressures and robustness to usurpation attempts (Queller *et al.*, 2000; Gamboa, Greig, & Thom, 2002). As a study species, this makes *P. dominula* nests less prone to failure and hence slightly more reliable. It is difficult to say whether these low conspecific pressures are evidence of effective strategies in repelling usurpers, or if usurping is simply not as great a risk as suggested in some literature. Given how well studied this species is, the former seems most likely.

The first brood of *P. dominula* workers eclose in early summer (Zanette & Field, 2009), after which point the dominant typically attempts to drive away any remaining auxiliary foundresses who have already served their purpose (Pardi, 1948). Whilst workers can reproduce, they seldom do unless all foundresses are absent from the nest (Queller *et al.*, 2000). Rather, they benefit from inclusive fitness, assuming their dominant relative is the one to produce the gynes for the next year. Broods continue to eclose throughout summer, with a gradually increasing proportion of males produced, and eventually gynes by mid to late summer. When the reproductive period ends in early autumn, colonies begin to dissolve and gynes take to their winter refuges (Zanette & Field, 2009).

P. dominula was the first invertebrate species in which dominance hierarchies were described (Pardi, 1948; Queller *et al.*, 2000). It is hence a species with which the science community is familiar, and has a wealth of primary literature on which this research was built. As far as *Polistes* wasps go, they are a robust

study species; they are capable of competitively excluding other sympatric species (Cervo, Zacchi, & Turillazzi, 2000) and display relatively high queen survivorship (Gamboa, Greig, & Thom, 2002) (but see Shreeves *et al.*, 2003), both of which provide reduced risk of nest failure. Their aposematic colouration also discourages predation to an extent, as well as making them fairly easy to spot on vegetation (Cervo, Zacchi, & Turillazzi, 2000).

Relevant to this study specifically, which focuses on the aggression and foraging consequences of succession, dominant *P. dominula* foundresses monopolise reproduction despite subordinates being fertile (Cant *et al.*, 2006). This gives auxiliary foundresses the means and the incentive to succeed or even usurp the dominant. Furthermore, successors appear to react to dominant absence almost instantly, meaning there is next to no lag to account for before measuring dominance displays by the new dominant (Pardi, 1948). As for the foraging and productivity aspect of the study, *P. dominula* nests appear to have high per capita foraging rates, giving this study lots to measure regarding foraging behaviour (Gamboa, Greig, & Thom, 2002). Thus, for the reasons detailed here, *P. dominula* serves as an ideal study species on which to investigate the effects of succession on a linear hierarchy.

The study sites

Data used for this study was collected from Cadiz province of Andalusia, Spain. Sites consisting of a pasture or field with hedges of prickly pear cactus (*Opuntia*), on which *Polistes dominula* nests occur, were surveyed to determine their suitability. Three sites were selected from which to gather data. Two of these sites were in Conil de la Frontera; data was collected from these between March and May 2016 before worker emergence. The other site was located

near Medina-Sidonia, and was used for the same time period in 2017 to gather a more substantial sample size. The shift in location between the 2016 and 2017 data collections was a consequence of the damage caused to the cacti in the previous sites by mealybug infestations. Relocation was necessary to ensure the results of the study were not biased by cactus condition. In the interest of consistency and comparability, only nests affixed to prickly pear cacti were used for this study.

At each site red electrical tape markers were placed along the cactus rows at 5 metre intervals from a pre-determined starting point. This provided a standard measure of distance in each site, and could be used to describe the location of recorded nests. Temperature probes were also set up at each site, though occasional failure of these probes meant online records of the general area had to be used instead.

General Methodology

The hedges of prickly pear cactus were thoroughly searched for *Polistes dominula* nests. Due to the contrasting green colour of a healthy cactus, and the distinctive shape of the nests, it was possible to find all but a few nests by eye. Nests were marked with yellow electrical tape to avoid confusion with the red interval markers. Nests containing 4 - 10 foundresses were also assigned a unique identification number and recorded, along with their relative location, the number of foundresses present, the number of cells, and the size and number of any larvae present. Any nests with fewer than 4 foundresses present were deemed unsuitable for the experiment at that time; a minimum of 4 foundresses presented the likelihood that there would still be enough foundresses remaining on a nest after the removal of the target wasp for it to be considered a social

environment. These nests were marked with a small piece of yellow electrical tape without an identification number as a reminder of their presence. Nests containing over 10 wasps were not marked or recorded, as they would have been practically impossible to census.

Suitable nests were then prepared for censusing. All individuals from a nest were carefully collected in the early hours of the morning, approximately between 07:00 and 08:00, before they were active. The wasps were collected using forceps and sieves, placed into plastic sample tubes, and kept chilled in a cool box for transportation. Each wasp was given a unique 4-colour combination, which was painted on its thorax using a pin and enamel paint, as has been successfully used in pre-existing experiments (Field, Shreeves, & Sumner, 1999). More specifically, the paint was applied to the mesoscutum in order to ensure the wasp's joints and wings were not impaired. Each site had an assigned colour; the final colour in every wasp's unique combination corresponded with the assigned colour for the site their nest was from. In this way it was possible to monitor if any wasps travelled between study sites. The unique identifying colour combinations for every wasp were recorded, along with each wasp's wing length measured with digital calipers, and the presence of any clypeal marks. Clear photographs were taken of the "face" of any wasp displaying a clypeal mark, followed by a photograph of its painted thorax for identification purposes. The wasps were processed and released as soon as possible to minimise the risk of an opportunist usurping the unguarded nest. All specimens were handled with latex gloves and forceps; care was taken not to damage the animals. Whilst not being handled, all wasps remained chilled in a fridge or ice box to prevent them escaping or exhausting themselves out of panic.

Once all the individuals on a nest had been marked and released, censusing could commence. For each census the wasps present on the nest at the time of the visit were ticked on the census sheet. The wasps that spent the most time on the nest, and were hence present for the most censuses, were considered to be of the highest ranks (Zanette & Field, 2009). Nests were ideally censused on sunny days with little wind between 12:00 and 17:00 when the day was hottest and foraging activity was at its peak (Cant & Field, 2001; Ortolani & Cervo, 2009). They were visited no more frequently than once every 30 minutes to allow time for any disturbed residents to return to the nest before the next census (Zanette & Field, 2009). A nest was censused a minimum of 15 times, and continued to be censused until a clear dominant and rank 2 emerged (Cant & Field, 2001). A foundress' apparent position in the linear hierarchy was considered to be accurate if it was on the nest for at least 15% more censuses than the next most regularly present wasp. Where a dominant or rank 2 never emerged as distinguishable by at least 15% of the censuses, a note was made to determine their position in the linear hierarchy later on in the experiment from dominance interactions captured on video. Occasionally, during a census, a new, unmarked foundress would be spotted on a marked nest. In these instances the unmarked wasp was collected the next morning, painted and processed in the same way as the other foundresses, and returned to the nest. A minimum of 15 more censuses were undertaken for the nest in question after the new foundress had been marked to assess its position in the hierarchy in accordance with the criterion.

Nests deemed too small for use were regularly visited to determine whether they had recruited enough foundresses to become suitable since their discovery. In instances where a nest did become suitable it was assigned an

identity number and processed in the same fashion as any other suitable nest. In the occasional instance where a suitable nest recruited over 10 foundresses part-way into using them a decision was made by one of the researchers; the nest was removed from the sample pool if and when it was deemed impossible to census. Nests were not removed from the records the instant an 11th foundress was identified to account for the possibility that one or more of the original foundresses may have disappeared, and the true number of active nest-mates may have been lower than 10.

Once a dominant and rank 2 could be identified videos of the nest could be taken. For each group of nests ready to be videoed half of them were randomly assigned to the top rank removal treatment and the remainder assigned to the control treatment. A pre-removal video was then recorded for each nest. The purpose of these videos was to give an indication of the typical behaviour for each nest, to which the behaviour displayed in post-removal videos could be compared. Videos were recorded with unmanned camcorders mounted on tripods between 12:00 and 16:30 in order to capture the hours when the wasps were most active with minimal disturbance. Cardboard sun-guards were affixed to tripods to prevent cameras from overheating, and were checked every 30 minutes to an hour to ensure the video cameras were still positioned correctly and unobstructed. The date, time, nest number, and weather conditions were announced at the beginning of each recording to account for the possibility that the dates and times of the video cameras were not calibrated. For the purposes of analysis, pre-removal videos were categorised as Day -1 videos.

The same procedure was followed for post-removal videos. In the case of a manipulated nest, the dominant foundress was removed in the same manner as those being collected for marking. For control nests the foundress removed was

the lowest rank present at the time of removal. These removals ideally took place the day after the pre-removal videos were taken, though in some cases poor weather conditions demanded that it be a few days later. The first post-removal videos, categorised as Day 0 videos, were taken on the same day the target wasp was removed. Where possible, another two post-removal videos were taken: one the day after removal, and the other 2 days after removal. These were categorised as Day 1 and Day 2 videos respectively. Where weather conditions made videos of successive days impossible the unsuitable days were not recorded, nor were they replaced with later days. In this way all post-removal videos were taken at standard intervals following the removal of the target wasp; no nests were given longer to adjust to the removal than others. Once all the videos for a nest were taken, the removed wasp was released next to its nest. Videos were filed every evening and named according to the nest and date, ready for scoring. For the main site in Conil de la Frontera 2016 (WK), the earliest video was taken on 6th April 2016 and the latest was taken on May 4th 2016. For the auxiliary site in Conil de la Frontera 2016 (BF), the earliest video was taken on 13th April 2016 and the latest was taken on 17th April 2016. For the 2017 Medina-Sidonia site (AP), the earliest video was taken on 1st April 2017 and the latest was taken on 27th April 2017.

CHAPTER 3: THE ROLE OF AGGRESSION IN THE ESTABLISHMENT OF DOMINANCE

Abstract

Insect societies function in such a way that the altruism they rely on is stable and sustainable. Often this involves a means of determining rank that enforces the prestige of one individual over another. Previous studies into *Polistes dominula* and similar species have identified aggression as a means of maintaining linear hierarchy in foundress aggregations (see Chapter 1). The objective of this study was to determine whether the aggression of a rank 2 foundress changed following the disappearance of the dominant. The prediction was that rank 2 aggression would sharply increase, indicating a successor preparing to become the new dominant, followed by a gradual decrease over the subsequent days until the new dominant displayed similar dominance behaviour to the old one. Videos of *Polistes dominula* nests were scored for aggressive behaviours on the day before and days after the removal of the dominant foundress. This analysis of this data revealed no significant difference in rank 2 aggression rates between nests under control or manipulated treatments. Rank 2 aggression also did not significantly differ between days. This suggests that foundresses succeeding an absent dominant do not increase aggression to assert themselves, nor do they react significantly to the disappearance of a dominant foundress, or any foundress absence for that matter. The findings of this experiment seem contrary to many findings of other researchers. This may be because most of the studies critically evaluated in Chapter 1 focused on dyadic interactions, rather than interactions with all nest-mates, or could be the result of a limitation of this study. If, as this experiment

has found, aggression is not the means by which dominance is established within a hierarchy, then it may have broader implications on how dominance is established in other species across taxa.

Introduction

Dominance hierarchies, by definition, entail an unequal distribution of resources among members of a group. If one considers that animals should all act in their own best interest, it becomes evident that individuals should not choose a subordinate position unless they have no option. In some instances, as mentioned in Chapter 1, an individual may be sterile and consequently accept a subordinate position to help their fertile relatives reproduce, and thus ensure the propagation to subsequent generations of genes, which they share through kinship. However, when an individual is not sterile, other measures must be acting to enforce this less than optimal rank upon the individual, such is the case with *P. dominula*.

Throughout the animal kingdom aggression is used to various ends, and forms an important aspect of an animal's ecology. Aggression has evolved as a strategy to deal with adverse circumstances or threats (Archer, 2001), but is costly enough that what is achieved through aggressive means must be considerably beneficial to the individual. Considering that being a subordinate with almost total reproductive skew in favour of the dominant is an adverse situation, it should be in the subordinate *P. dominula* foundress' best interest to attempt to aggressively supersede the individual ranking above it. Likewise, the risk of being aggressively overtaken should be incentive enough for a more dominant foundress to aggressively subdue the foundress below it. This pattern was observed in a study by Cant, Llop, & Field (2006) which found increased

rates of aggressive contests in dyads higher up the hierarchy, suggesting not only that aggressive contests are a means of determining rank, but also that the frequency of these contests increases closer to the breeder position. As discussed in Chapter 1, this balance of aggressive challenges and dominant displays seems to play a vital role in stabilising the linear hierarchy between *P. dominula* foundresses. There is less evidence, however, on how the immediate changes to the regular dynamics between nest-mates in the event of succession. Cant, Llop, & Field (2006) investigated the changes between dyadic relationships, but this mainly accounts for interactions involving contesting and defending one's rank. This study, on the other hand, encompasses interactions between the new dominant and all other foundresses to investigate how, if at all, a new dominant subjugates the rest of the group and maintains the order of the hierarchy. While some species with similar hierarchical societies, such as the naked mole-rat, display heightened aggression during periods of succession (Clarke & Faulkes, 1997), other social animals, such as cichlids, respond to disruption diplomatically by trying to firmly establish their place in the group through cooperation (Bergmüller & Taborsky, 2005). Although this "pay-to-stay" strategy is typically one adopted by subordinates, this in itself should create an interesting comparison between removals that do not impact the linear hierarchy and those that result in a new dominant. Looking at dominance displays and aggressive interactions during succession of the dominant position may provide insight into how these dominance hierarchies are initially established, and contribute towards current knowledge on the trade-off of employing an aggressive approach.

As seen in Chapter 1, the patchwork picture formed from a large sample of existing literature on this species suggests that linear hierarchies in *P. dominula*

are originally formed through aggressive means, possibly governed by chemical cues after they have been established, and have spikes in aggressive activity when the hierarchy must be re-established. Thus, the expectation is that a rank 2 ascending to the dominant position will undergo an aggressive peak as they assert themselves over the rest of the nest, such as in naked mole-rats (Clarke & Faulkes, 1997). Therefore, the hypothesis for this chapter is that aggression from the rank 2 will significantly increase following the removal of the dominant foundress.

Aggression and consequences of challenging higher ranks is a fundamental aspect of dominance hierarchies, and the research undertaken in this chapter addresses a crucial piece of the puzzle that is stable dominance hierarchies among fertile *P. dominula* foundresses.

Methods

The objective of this chapter is to identify the trend that the aggression of a rank 2 wasp takes before, during, and after its succession, and to identify any factors with which this varies.

After the sample nests had been censused to determine the order of the hierarchy, as described in Chapter 2, the nests were divided into treatment and control groups. Half of the nests were randomly assigned to the experimental treatment, where the top-ranking foundress was to be removed after a day of videoing, and the remainder assigned to the control treatment, where a low-ranking foundress was removed instead. A pre-removal video was recorded for each nest, where no removals of any foundresses occurred. The purpose of these videos was to set a baseline of the typical behaviour for each nest, to which the behaviour displayed in post-removal videos could be compared.

These videos were recorded with unmanned camcorders mounted on tripods between 12:00 and 16:30 in order to capture the hours when the wasps were most active with minimal disturbance. The camcorders were checked every 30 minutes to an hour to ensure they were still positioned correctly and unobstructed. For the purposes of analysis, pre-removal videos were categorised as Day -1 videos.

The same procedure was followed for post-removal videos. In the case of a nest subjected to the experimental treatment, the dominant foundress was removed in the same manner as those being collected for marking. For control nests the foundress removed was the lowest rank present at the time of removal. These removals ideally took place the day after the pre-removal videos were taken. The first post-removal videos, categorised as Day 0 videos, were taken on the same day the target wasp was removed. Where possible, another two post-removal videos were taken: one the day after removal, and the other 2 days after removal. These were categorised as Day 1 and Day 2 videos respectively. Where weather conditions made videos of successive days impossible the unsuitable days were not recorded, nor were they replaced with later days. In this way all post-removal videos were taken at standard intervals following the removal of the target wasp; no nests were given longer to adjust to the removal than others. Once all the videos for a nest were taken, the removed wasp was released next to its nest.

For the purposes of scoring, each nest was randomly assigned four 10-minute sample intervals using a random number generator to decide how many minutes after 13:00 to begin the interval. These intervals had to fall between 13:00 and 16:00 if possible, in order to capture a representative trend of the most active time of the day. Before behaviour scoring commenced, videos of

each nest were briefly checked to ensure that the focal wasp(s) was still present on that nest, and quickly assess the accuracy of the predicted dominance hierarchy. Notes were made of which foundress on each nest was on the nest the most, receiving the least aggression, and displaying submissive behaviour. Assuming these blind observations did not reveal that the predicted dominant consistently submitted to another foundress, the recorded hierarchy was assumed correct and scoring commenced. In instances where a dominant did consistently act submissively towards another foundress the nest was not included in the analyses, as this could have implications on the true rank of the focal wasp. Where the rank 2 and 3 foundresses, whom were observed on the nest for similar durations during the censuses, displayed consistent dominance interactions that implied that the rank 3 was in fact the more dominant of the two, adjustments were made to the hierarchy.

On the first day recorded, classified as Day -1 or the pre-removal day, aggression was scored for the rank 1 and rank 2. For all subsequent days, regardless of whether the wasp removed was the dominant or not, only the aggressive behaviour of the original rank 2 wasp was recorded. This allowed comparison between wasps that remained at rank 2 and those who moved from rank 2 up to a rank 1 position. Each aggressive act from a focal wasp was recorded on a spreadsheet, along with the identity of the recipient, the number of wasps on the nest, and the treatment group and nest it was from, all of which were important for classifying the behaviour. Actions were taken to be aggressive acts if they met the descriptions of aggressive behaviour used in other studies and by fellow researchers also investigating aggression in this study population. Each category of aggression had a name, an abbreviation, and a specific description of what each one was, as shown in Table 1. Displays

of aggression or dominance within 10 seconds of each other, directed at the same recipient, were taken as one aggressive display, as often this coincided with a single submissive response by the recipient. Also recorded were periods of time during which the rank 2 wasp was not present on the nest, and these periods were accounted for when determining the rank 2's aggression per minute. As was the case with recording "exits" for foraging behaviour analysis, a period of absence by the rank 2 was only recorded if they did not return for at least 30 seconds. This was to differentiate foraging trips from airborne nest defence, during which time the rest of the nest could feasibly still be subjected to dominance by the rank 2, and is based on the criteria for what was considered a departure in a study by Gamboa, Greig, and Thom (2002). Overall 23 nests were scored in this fashion; 15 were from the main Conil de la Frontera site used in 2016 (WK), 2 were from the auxiliary 2016 site in the same region (BF), and 6 were from the Medina-Sidonia site used in 2017 (AP). 11 were used as control nests; 10 from WK and 1 from AP. The other 12 nests were subjected to the experimental treatment; 5 from WK, 2 from BF, and 5 from AP. To avoid overparameterising, the individual differences in conditions between these nests is accounted for by including nest ID as a random factor in the analyses.

Table 1. Categories of aggressive, and other relevant, behaviours. The descriptions of these behaviours were adhered to strictly in order to ensure video scoring was as standardised as possible.

Name of behaviour	Abbreviation	Description
Antennation of body	AB	Drumming one's antennae along the body of another.
Bite	B	Biting or prolonged chewing on recipient's leg, wings, or mandibles.
Chase	CH	Rapidly pursuing a fleeing individual around or off the nest.
Dart	D	A quick, directional movement with no contact towards another individual.
Grapple	G	Grasping recipient, often around the head, and pushing them backwards.
Initiating antennation	IA	Clashing of antennae initiated by the dominant, often received with akinesis.
Lunge	L	A quick, directional movement with contact against another individual.
Mount	M	Climbing and staying atop another, forcing recipient into akinesis.
Not on nest	NON	The rank 2 wasp is absent from the nest for a minimum of 30 seconds.

Data Analysis

The statistical analyses were performed in R 3.1.1. (R Development Core Team, 2014). The data were fit to a linear mixed model (LMM). As in a study by Thompson *et al*. (2014), aggressive interactions from the rank 2 were tallied as a count, with each type of aggressive behaviour given equal weight. The rank 2 aggression count was converted into a per capita rate of aggression by dividing the aggression count by the number of minutes that the rank 2 was filmed on the nest that day, and then divided again by the average number of foundresses on the nest during the recorded period. The average number of foundresses on each nest was determined using the following calculation:

$$\frac{\sum(n \times t)}{s}$$

Where:

n = number of foundresses present on the nest, excluding the focal foundress

t = number of minutes without a foundress arriving or departing the nest

s = the sample period of time for that day; typically 10 minutes

This per capita aggression data was subjected to a square root transformation to improve the fit of the data to the assumptions of normal errors and homoscedasticity. Darts were excluded from the analysis since it proved too difficult to accurately differentiate darts from rapid changing of direction, and to account for the possibility that darts are not aggressive behaviours, as has recently been suggested (Sumana & Starks, 2004). Aggressive interactions with unmarked wasps that joined after recording had begun were also removed; this ensured that the aggression rates reflected the nest occupancy value factored into the analyses, taken from the initial censuses. The main fixed effect in this LMM was an interaction between treatment and day, testing the significance of any differences in aggression between manipulated and control nests, and how aggression differed before, during, and after the removal of a nest-mate. The aggression rate of the rank 1 on the day before removal of a foundress was also included as a fixed effect, since it was predicted that this would set a standard of aggression that the new dominant would eventually settle at. Nest ID was included as a random effect to account for inherent structure in the data linked to the location, developmental stage, or conditions surrounding a nest. All 23 of the nests scored were analysed in this way.

The R package 'lme4' (Bates *et al.*, 2015) was used during these analyses. The data were fit to a LMM using the 'lmer' function. A series of likelihood ratio tests were used to compare the maximal model to the model without each fixed

effect. Non-significant interactions were removed to allow for the testing of the main effects.

Results

The interaction between treatment and day had no significant effect on the aggression of the rank 2 (LMM$_1$, X^2_3 = 2.71, P = 0.438, Table 2), as displayed in Figure 1. The results also indicated no significant difference in rank 2 aggression between manipulated and control nests (LMM$_1$, X^2_1 = 0.51, P = 0.476; β = 0.033 ± 0.05). Furthermore, there were no significant differences in rank 2 aggression between any of the days before, during, and after removal of a foundress (LMM$_1$, X^2_3 = 2.98, P = 0.395, Table 2). Finally, the effect of rank 1 aggression on rank 2 aggression was also non-significant (LMM$_1$, X^2_1 = 1.75, P = 0.186, β = 0.146 ± 0.116). The results for this analysis are presented in Table 2.

There was no evidence that any of the fixed effects or interactions significantly affected rank 2 aggression, so no post-hoc tests were necessary for this investigation.

Table 2. Linear mixed model of rank 2 aggression towards nest-mates (LMM$_1$). An interaction between two fixed effects is shown using an asterisk (*) between the relevant variables. Significant p-values are indicated by bold font.

| Fixed effect		β	SE	df	X^2	P
Intercept	with interaction	0.250829	0.054902			
	without interaction	0.24858	0.04939			
Treatment*Day	Manipulated on Day 0	0.053629	0.078198			
	Manipulated on Day 1	0.024776	0.078198	3	2.7113	0.4383
	Manipulated on Day 2	-0.077573	0.086174			
Treatment		0.03347	0.04975	1	0.50862	0.4757
Day	Day 0	-0.06439	0.03869			
	Day 1	-0.0406	0.03869	3	2.98115	0.3945
	Day 2	-0.04377	0.04266			
Rank 1 aggression rate		0.14622	0.11634	1	1.75047	0.1858
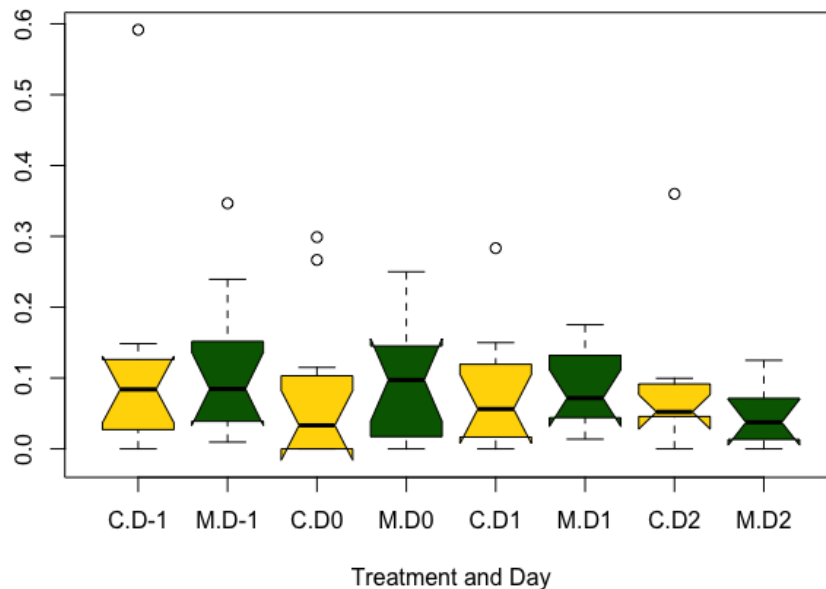
Figure 1. Per capita aggression rates of rank 2 foundresses on each day of the sample period for both treatment (M) and control (C) nests. Days referred to as "D-1" are days prior to removals. Days referred to as "D0" are days on which removals took place; in these instances the removals were undertaken the morning before the video was recorded.

Discussion

The purpose of this experiment was to identify what factors, if any, influenced aggressive behaviour of a rank 2 wasp towards the rest of the foundresses during a period of succession, with the hypothesis that removing the dominant wasp from a nest would provoke an aggressive response in the rank 2 as they assert themselves. The distinction between this study and that by Cant, Llop, & Field (2006) is that this study focused on the aggression of the rank 2 towards all other foundresses, not just dyadic interactions. The results show that treatment did not have a significant effect on the aggression of the rank 2, neither individually nor included in an interaction with day, so we must accept the null hypothesis that succession itself does not alter the amount of dominant behaviour displayed by a prospective new dominant to the rest of the foundresses collectively. This may be because the rank 2 had not considered the dominant's absence to be permanent until after the sample period, though according to Pardi (1948) subordinates react almost instantly to a dominant's absence, so this is unlikely. It could also be that the disturbance caused by the presence of researchers, as well as invasive activities such as collecting and marking the wasps, may have provoked a response that masked the natural change in behaviour caused by succession. This is not completely implausible, though other studies using these methods have made reliable observations on behaviour changes that this study did not.

Day also had no significant effect on rank 2 aggression, neither in an interaction with treatment nor individually, suggesting that all days of nests in both treatment groups displayed similar levels of rank 2 aggression. This was again an unexpected result, as one might predict that a nest might undergo upregulated aggression after a foundress is abducted and not returned,

regardless of rank, in response to the invasive nature of the removal. One possible explanation for why no significant change in aggression was observed may be that a different behaviour, perhaps of a more cooperative nature like the cichlids in the study by Bergmüller & Taborsky (2005), was being upregulated in response to the disappearance of a foundress or the presence of the researchers. Repeating an experiment similar to this one where all behaviours, rather than just aggressive interactions, are scored would be an interesting and worthwhile continuation of this study that could put these findings into context. The aggression rate of the rank 1 prior to removal of a foundress appeared to have no significant effect on the aggression rate of the rank 2. While it was predicted that the previous dominant's rate of aggression would set a standard that the new dominant would eventually meet, this would have only been relevant if the aggression of the rank 2 significantly changed during succession, which it did not. It is also possible that the aggression rate of the rank 1 prior to the sample days may have influenced the baseline levels of aggression of the rest of the foundresses on the nest, but since nest ID was incorporated as a random effect this variation may not have been attributed to rank 1 aggression. The study had certain limitations that, if it were to be repeated, should be considered and accounted for. One limitation, which has been mentioned previously, is the effect of disturbance associated with collecting, marking, censusing, and removing wasps. It is possible that the disturbance associated with this methodology is responsible for the lack of reaction of the foundresses to the disappearance of a nest-mate or even the dominant. If this was indeed due to the disturbance it is possible that a more targeted response to removals that avoid disturbing the entirety of the nest would yield clearer results. One should consider, however, that similar methods have been used in previous

studies that have observed aggressive reactions to removal experiments, and therefore there may be another systematic limitation specific to this study that is responsible for the lack of aggression. The calculation of per capita aggression rates was also logistically limited. The calculation used to determine aggression rate involved dividing counts of aggressive interactions by the number of minutes the focal wasp was present on the nest and dividing this number by the average number of other foundresses present on the nest. This, however, suggests that opportunities for dominance interactions increases linearly with group size, which is not realistically the case. Increasing group size is correlated with a number of other factors, such as nest size, which means that the relationship between group size and opportunities for interactions is not truly linear, as it was taken to be for the purposes of analysis. Finally, while a few factors could not be included due to considerably incomplete data or practical issues in recording them, the developmental stage of the nest is one factor that almost certainly would have explained a great deal of the variation in the data. From observations during data collection, and from trends identified in the literature reviewed in Chapter 1, it is clear that levels of aggression naturally fluctuate throughout the wasps' annual cycle; developmental stage of the nest means variability in how substantial the incentive to defend the nest is. Research into the aggression of *P. dominula* should ideally account for some measure of development such as cell count or number of larvae present on each nest, which is something that this study was unable to accurately include for the majority of nests. On the other hand, the videos scored for this study were all taken from between 6th April and 4th May 2016, or between 1st April and 27th April 2017. These time periods are almost equal to those used by Cant,

Llop, & Field (2006) in their study, and thus aggressive interactions should not be substantially less common as a result of this sampling period.

Given that the results of this experiment vary greatly from those of many other similar studies, making adjustments for the limitations discussed may have provided a clearer and more enlightening insight into precisely what behavioural changes occur during succession. This should be considered by researchers intending to investigate success in *P. dominula* in the future. Also of great use to this study would have been a detailed assay of dominance behaviours. This would have allowed behaviours to be weighted according to the level of aggression or dominance associated with each one. An analogous study to the one by Monnin and Peeters (1999) on *Dinoponera quadriceps*, where an assay of dominance behaviours clearly justifies weighting certain interactions over others, would therefore be worthwhile undertaking in the future as it would provide valuable insight for further studies on aggression in this species.

CHAPTER 4: HOW IS THE FORAGING EFFORT OF A NEST AFFECTED BY SUCCESSION OF A DOMINANT FOUNDRESS?

Abstract

The formation of a linear hierarchy requires mechanisms that stabilise social rank. In circumstances where there are large incentives to seizing the dominant position, such as under high reproductive skew or when there are limited inclusive fitness benefits, one would expect high levels of queue jumping. Such activity threatens to destabilise the delicate structure of a linear hierarchy. This is applicable in *Polistes dominula*, which has high reproductive skew, a fairly high proportion of non-relative helpers, and nest inheritance as a large incentive to join the hierarchy. One might therefore expect to see foundresses dedicating more time to defending their current position in the queue when there is a risk that their social status will be challenged, such as when a new dominant has just inherited the breeding position. While previous studies have investigated changes in behaviour of dominant foundresses during periods of succession, there has been little exploration of how the behaviour of lower ranking foundresses changes during these periods. This study tested the hypothesis that succession reduces foraging effort from the rest of the foundresses on the nest because low ranking foundresses dedicate more time to establishing their place in the queue. Dominant foundresses were removed from half of the sample of *P. dominula* nests, and low-ranking foundresses from the remainder. The number of returns to the nest, and whether each return was productive, were recorded through scoring of videos taken in the field. There was no evidence to suggest that foraging effort decreased during periods of succession specifically, but productive returns per foundress did significantly decrease from

the day of removal of a foundress to two days after removal regardless of treatment. This suggests that the disappearance of a nest-mate, rather than succession of the dominant position, causes a reduction in foraging effort of the entire nest. This finding not only contributes to the puzzle of succession in *P. dominula*, but may also have broader implications regarding how individual self-preservation influences the dynamics within a group during times of disruption and instability.

Introduction

Linear dominance hierarchies in animal societies consist of a queue of individuals whereby each individual is dominant over those below their position and subordinate to those above their position; in a perfect linear hierarchy no two individuals are equal in rank. In *Polistes dominula,* each time a new individual joins the hierarchy they become part of the queue; their position is determined by a number of factors addressed in Chapter 1, including but not limited to relatedness, time of joining, and competitive ability. As a rule, the disappearance or death of the top-ranking individual means that each below it shifts up one rank, with the rank 2 becoming the new dominant, the rank 3 becoming the new rank 2, and so on. Since natural selection should lead to individuals ultimately acting in self-interest, an individual should be selected to enforce its social rank and defend it against those trying to achieve a higher rank. As explored in previous chapters, these aggressive contests, particularly between dyads, are used in *P. dominula* as a mechanic behind the stable linear hierarchy between fertile foundresses (Cant, Llop, & Field, 2006).

In a stable linear hierarchy of *P. dominula,* lower ranking individuals perform riskier jobs such as foraging for food, while those at the top of the hierarchy

spend more time on the nest, laying eggs and provisioning larvae. In *P. dominula*, where all the foundresses are fertile, lower ranking females maximise their inclusive fitness by investing in foraging to find and deliver food for the larvae of the rank 1 foundress. The behaviour of the foundresses that have a higher probability of inheriting the nest is different, and reflects the growing importance of maintaining high social rank and the potential future fitness benefits it brings. Cant, Llop, & Field (2006), showed that rates of aggressive displays and contests are higher for females that are closer to inheriting the nest. However, there are periods of time where the hierarchy is less stable, such as during succession of the rank 1 position following the disappearance of the previous dominant. Given the potentially long wait to inherit, and the considerable benefits associated with being the dominant foundress, natural selection should favour subordinates that capitalise on opportunities to move ahead in the hierarchy, or 'queue-jump' (Bridge & Field, 2007). The previous chapters have already explored how aggressive contests with other foundresses are seemingly used to enforce one's rank or attempt to supersede others', although the focus of these points was largely regarding individuals soon to inherit dominance. It would be reasonable to expect there must be changes to the behaviour of lower ranking individuals also, lest the bottom end of the linear queue descend into chaos and foundresses lose their position in the queue each time a dominant dies.

This study investigates changes in helping effort of social *P. dominula* nests as a potential indicator of an individual's changing priorities when the dominance hierarchy is relatively unstable. The prediction is that during periods of instability of the hierarchy, such as during a period of succession, helping effort of all foundresses will decrease, because each individual spends a greater proportion

of time engaged in other behaviours such as defending their place in the queue or contesting a higher rank. We predict that foraging will be reduced until the linear hierarchy is stabilised again. In this study, foraging effort is taken as a proxy to helping effort, based on the logic of previous studies that highlight that foraging is the costliest task subordinates regularly undertake (Cant & Field, 2001). If this prediction is correct foraging effort should decrease immediately after removal of the current dominant, and then gradually return to its previous level when a new rank 1 is established. The expectation is that this decrease in effort will occur simultaneously with an increase in aggression (see Chapter 3) as contests over rank take precedent, and that aggression and foraging effort will both gradually stabilise over the same amount of time. Therefore, the results of this study can be paired with those from Chapter 3 to get a broader idea of what dynamics occur during succession. The prediction of reduced foraging effort during succession made here is contrary to what was discovered by Strassmann *et al.* (2004) and should go some way towards supporting or opposing these findings, thus giving us a more reliable understanding of the reality of the situation.

Methods

The objective of this chapter is to identify the changes in foraging behaviour of the entire nest before, during, and after succession of a new dominant. After the sample nests had been censused sufficiently to determine the order of the hierarchy, as described in Chapter 2, the experimental and control treatments were applied to the nests and videos taken for analysis. Half of the nests were randomly assigned to the experimental treatment, where the top-ranking foundress was to be removed after a day of videoing, and the

remainder assigned to the control treatment, where a low-ranking foundress was removed instead. A pre-removal video was recorded for each nest, where no removals of any foundresses occurred. The purpose of these videos was to set a baseline of the typical behaviour for each nest, to which the behaviour displayed in post-removal videos could be compared. These videos were recorded with unmanned camcorders mounted on tripods between 12:00 and 16:30 in order to capture the hours when the wasps were most active with minimal disturbance. The camcorders were checked every 30 minutes to an hour to ensure they were still positioned correctly and unobstructed. For the purposes of analysis, pre-removal videos were categorised as Day -1 videos. The same procedure was followed for post-removal videos. In the case of a nest subjected to the experimental treatment, the dominant foundress was removed in the same manner as those being collected for marking. For control nests the foundress removed was the lowest rank present at the time of removal. These removals ideally took place the day after the pre-removal videos were taken. The first post-removal videos, categorised as Day 0 videos, were taken on the same day the target wasp was removed. Where possible, another two post-removal videos were taken: one the day after removal, and the other 2 days after removal. These were categorised as Day 1 and Day 2 videos respectively. Where weather conditions made videos of successive days impossible the unsuitable days were not recorded, nor were they replaced with later days. In this way all post-removal videos were taken at standard intervals following the removal of the target wasp; no nests were given longer to adjust to the removal than others. Once all the videos for a nest were taken, the removed wasp was released next to its nest.

For the purposes of scoring, each nest was randomly assigned four 10-minute sample intervals using a random number generator to decide how many minutes after 13:00 to begin the interval. These intervals had to fall between 13:00 and 16:00 if possible, in order to capture a representative trend of the most active time of the day. Before behaviour scoring commenced, videos of each nest were quickly assessed to confirm the accuracy of the predicted dominance hierarchy, and to determine whether any foundresses had disappeared between the censuses and the recording. Notes were made of which foundress on each nest was on the nest the most, receiving the least aggression, and displaying submissive behaviour. Assuming these blind observations did not reveal that the predicted dominant consistently submitted to another foundress, the recorded hierarchy was assumed correct and scoring commenced. In instances where a dominant did consistently act submissively towards another foundress the nest was not included in the analyses, as this could have implications on the treatment the nest was subjected to. Where the rank 2 and 3 foundresses, whom were observed on the nest for similar durations during the censuses, displayed consistent dominance interactions that implied that the rank 3 was in fact the more dominant of the two, adjustments were made to the hierarchy. A note was also made of any foundresses that appeared to be consistently absent, despite being recorded in the censuses. On all days the number of exits and returns for all marked wasps were recorded, along with the identity of the foundress departing or returning, the number of wasps on the nest, the treatment group, the nest it was from, and whether it returned with a resource or not. Recording this information allowed an accurate record of foraging behaviour to be made for all foundresses on the nest. Behaviours had to meet specific conditions to be counted, as shown in

Table 3. In particular, exits were only counted if the foundress was out of the camera shot for at least 30 seconds. This was to differentiate foraging trips from airborne nest defence and avoid inflating the number of foraging trips, using criteria for what was considered a departure in a study by Gamboa, Greig, and Thom (2002). Overall 36 nests were scored in this fashion; 28 were from the main Conil de la Frontera site used in 2016 (WK), 2 were from the auxiliary 2016 site in the same region (BF), and 6 were from the Medina-Sidonia site used in 2017 (AP). 17 were used as control nests; 16 from WK and 1 from AP. The other 19 were subjected to the experimental treatment: 12 from WK, 2 from BF, and 5 from AP. To avoid overparameterising, the individual differences in conditions between these nests is accounted for by including nest ID as a random factor in the analyses.

Productive returns per foundress was taken as a measure of foraging effort for the nest. This was calculated as such:

$$\text{Productive returns per foundress} = \frac{\text{Number of productive returns}}{\text{Group size}}$$

As productive returns per foundress was calculated for each day, after the removal of a foundress the calculation became:

$$\text{Productive returns per foundress} = \frac{\text{Number of productive returns}}{\text{Group size} - 1}$$

Productive returns, rather than total returns or number of exits, were used to account for instances where foundresses left the nest for purposes other than foraging, such as in response to a disturbance or to visit another nest that the individual was a member of, as the censuses revealed occurred occasionally. Moreover, in *P. dominula*, there is evidence that foundresses continue to search until they find food, and then bring it back; if foundresses are provisioned with food away from the nest they do no further foraging and quickly return to the

nest (Donaldson *et al.*, 2014). Hence the number of productive returns is a good measure of the overall effort invested in foraging. The use of the number of productive returns as a measure of foraging effort allowed us to account for foundresses that departed the nest before our arrival.

Table 3. Categories of foraging activities. The descriptions of these behaviours were adhered to strictly in order to ensure video scoring was as standardised as possible.

Name of activity	Abbreviation	Description
Exit nest	EX	Leave the nest, often on wing, and not returning for at least 30 seconds.
Return to the nest with forage	RNF	Return to nest with food, nectar, or wood pulp, evident from balling motion or nectar-sharing* immediately after returning.
Return to nest without forage	RWF	Return to nest without food or pulp in its mandibles.

* Note that nectar-sharing was characterised as the returning foundress opening her mouthparts to another foundress, and remained entirely passive and unrestrained while the other foundress locked mouthparts together and appeared to start drinking.

Data Analysis

The statistical analyses were performed in R 3.1.1. (R Development Core Team, 2014). Productive returns from any of the foundresses during the sample period were tallied as a count. A return was considered 'productive' if the foundress was clearly carrying a resource in its mandibles, demonstrated a 'balling' motion on unseen forage in its mandibles, or engaged in nectar-sharing activity immediately after returning to the nest. The frequency of productive returns was then divided by the total number of foundresses recorded on the nest during the censuses (reduced by 1 after the removal). This value of productive returns per foundress was used as a measure of foraging effort which was comparable between groups of different sizes. This productive return

per foundress data was subjected to a square-root transformation to improve the fit of the data to the assumptions of normal errors and homoscedasticity. The fixed effect in this LMM was an interaction between treatment and day, testing the significance of any differences in foraging effort between manipulated and control nests, and how foraging effort differed before, during, and after the removal of a nest-mate. Nest ID was included as a random effect to account for inherent structure in the data linked to the location, developmental stage, or conditions surrounding a nest. All 36 of the nests scored were analysed in this way.

The R package 'lme4' (Bates *et al.*, 2015) was used during these analyses. The data were fit to a LMM using the 'lmer' function. A series of likelihood ratio tests were used to compare the maximal model to the model without each fixed effect. Non-significant interactions were removed to allow for the testing of the main effects.

Results

The interaction between treatment and day had no significant effect on foraging effort (LMM$_2$, X^2_3 = 5.783, P = 0.1227, Table 4) so the interaction was removed and the fixed effects were included independently for subsequent analyses. The results indicated there was no significant difference in foraging effort between manipulated and control nests (LMM$_2$, X^2_1 = 0.7203, P = 0.3960, β = 0.07982 $\pm$ 0.09627). There were, however, significant differences in foraging effort between the days sampled (LMM$_2$, X^2_3 = 10.9689, P = 0.0119, Table 4). The results for this analysis are presented in Table 4.

Table 4. Linear mixed model of productive returns per foundress (LMM$_2$). An interaction between two fixed effects is shown using an asterisk (*) between the relevant variables. Significant p-values are indicated by bold font.

Fixed effect		B	SE	df	X^2	P
Intercept	with interaction	0.57352	0.09037			
	without interaction	0.65768	0.08065			
Treatment*Day	Manipulated on Day 0	-0.20855	0.13136			
	Manipulated on Day 1	-0.14524	0.13136	3	5.783	0.1227
	Manipulated on Day 2	-0.32071	0.14072			
Treatment	Manipulated	0.07982	0.09627	1	0.7203	0.3960
Day	Day 0	0.04859	0.06627			
	Day 1	-0.14828	0.06627	3	10.9689	**0.0119**
	Day 2	-0.11092	0.07104			

As there was a significant effect of day on productive returns per foundress, post-hoc tests were applied to determine which days had significantly different foraging efforts. Three post-hoc pairwise T-tests were performed to determine which days were responsible for the significant difference identified in the second analysis. For each of these tests the α level was subjected to a Holm-Bonferroni correction to reduce the risk of Type I errors while maintaining more statistical power than a standard Bonferroni correction (Holm, 1979). This involved ranking each test in order of significance, from lowest P value to highest, and then calculating each test's adjusted α level ($α_1$) using the calculation:

$$\alpha_1 = \frac{\alpha}{n - rank + 1}$$

where α is the original alpha level, in this case 0.05; n is the number of post-hoc tests done in total, in this case 3; and $α_1$ is the Holm-Bonferroni adjusted α level. The outcome of these corrections is presented in Table 5.

The first test undertaken was between the day before removal and day of removal (Paired T-test$_1$, T_{35} = -0.66625, P = 0.5096, $α_1$ = 0.05), in order to

determine whether foraging effort changed immediately after a foundress was removed. The next test was between the day before removal and the last day (Paired T-test$_2$, T_{28} = 1.2, P = 0.2402, α_1 = 0.025), with the purpose of seeing if foraging effort returned back to its pre-removal level or not. The last test was between the day of removal and the last day (Paired T-test$_3$, T_{28} = 3.5827, P = 0.00127, α_1 = 0.017) to determine whether behaviour changed over the period of absence of the foundress. These results are presented in Table 5, where it can be seen that there is a statistically significant difference between the foraging effort on the day of removal when compared to the final day after removal.

Table 5. Post-hoc pairwise T-tests of foraging effort versus day of experiment, including Holm-Bonferroni corrected α levels (α_1) for each. Tests with a p-value lower than the Holm-Bonferroni corrected α level are considered statistically significant, and are indicated by bold font.

Days Compared	Mean of Differences	df	T	α_1	P
Day -1 & Day 0	-0.048595	35	-0.66625	0.05	0.5096
Day -1 & Day 2	0.09895545	28	1.2	0.025	0.2402
Day 0 & Day 2	0.1870404	28	3.5827	0.016667	**0.00127**

Discussion

This experiment intended to investigate changes in foraging behaviour as a result of succession and the associated need to defend one's own rank, with the hypothesis that foraging effort would decline during these periods. The results of the analyses provide evidence that foraging effort does decrease following the disappearance of a foundress. However, this does not seem to be restricted to instances when the rank 1 is removed, and thus does not reflect a response to periods of succession specifically. No significant difference in foraging effort was apparent between treatment groups, as displayed in Figure 2, yet foraging

effort decreased significantly across all nests from the removal of a foundress, regardless of her rank, to the end of the three day sampling period. The decreased foraging effort appears to persist for the duration of experiment, suggesting that there is a delay between the absence of a foundress and the subsequent contests to establish ranks. By the end of the three days there was no significant difference in foraging efforts from those prior to removals, suggesting that foraging rate was gradually increasing again by this point.

The most likely explanation is that the disappearance of any foundress, not just the dominant, results in some hierarchical instability that drives foundresses to spend a greater portion of their time on the nest asserting their position in the inheritance queue. During periods of instability, it may pay each foundress can make their presence known to the rest of the group to signal that they are still a present, active member of the hierarchy, and possibly capitalise on the opportunity to queue-jump during this time. Alternatively, subordinate foundresses may be investing more time in nest defence and brood care as opposed to foraging in response to the potential threat that may have been responsible for the disappearance, ensuring they are not absent should the nest need a strong force to deter any predators. This is a similar response to that observed in cichlids by Brouwer, Heg, and Taborsky (2005), whereby helpers feed less and attend the breeder more when group size is experimentally reduced. However, unlike the study on cichlids, this cannot be as heavily attributed to kin selection due to the fairly low levels of relatedness in some *P. dominula* colonies.

There were some limitations to this study. As with aggression, one factor that would have provided some insight if it could have been included would have been developmental stage of the nest. Naturally the foraging efforts should

increase with greater numbers of larvae and demand for larger nests. Unfortunately, due to practical limitations in recording this information, developmental stage of the nest could not be included. However, any studies intending to build on the findings of this one should consider a good measure of nest development and ensure that it can be assimilated into the model. It was also difficult to distinguish sharing of nectar from dominance displays when they were not on the very front of the nest. While even the most inconspicuous wood pulp and food forage could be identified by the behaviour of the foundress as she balled up the produce, nectar sharing behaviour was considerably easier to miss if one of both of the foundresses interacting were obscured by part of the nest or surrounding scenery. At times it was clear that one foundress was opening her mandibles to offer a droplet, but at others the orientation of the foundresses on the nest often meant only the recipient antennating could be seen clearly. Furthermore, as hypothesised by Pardi (1948), foundresses may regurgitate a liquid offering as submissive behaviour. This makes any sharing of liquid between two foundresses hard to classify, as it could be newly foraged liquid being shared freely or regurgitated liquid offered as an act of submission. With both dominance and foraging being investigated simultaneously, fluid sharing was classified according to how soon after returning to the nest it occurred, which is an imprecise means of doing so. However, if liquid sharing could be accurately classified on a set of standard criteria, such as with the rest of the behaviour, it may have given a more representative model of foraging effort for each nest. Finally, as with the calculation for per capita aggression rates in Chapter 3, there were limitations in the assumption that foraging effort and group size have a linear relationship. The calculation used to determine productive returns per foundress involved dividing counts of productive returns

to the nest by the total number of foundresses on the nest when it was censused. This, however, suggests that foraging efforts increase linearly with group size, which is unlikely to be the reality of the situation. Increasing group size is correlated with a number of other factors, which means that the relationship between group size and foraging effort is probably not truly linear, as it was taken to be for the purposes of analysis.

The prediction that foraging effort would decrease specifically during periods of succession as a result of heightened contests over rank was not supported. On the other hand, foraging effort did decrease significantly following the removal of a foundress regardless of rank. It seems the most plausible explanation for this is that removal of any foundress introduces opportunities for queue-jumping, consequently changing individual priorities from foraging to defence of one's position in the inheritance queue. It would be interesting to see if foundresses dart at passing organisms more frequently after removal of a foundress, which would help to test the alternative explanation that the entire nest becomes more vigilant and defensive following a disappearance of any nest-mate.

Future research may benefit from investigating how foraging rates change across the season with progressive development of the nest, which would provide further studies with something of a calibration curve between foraging and nest developmental stage. There may also be interesting patterns to be discovered by analysing whether there are any specific, subtle differences between fluid sharing as an act of provisioning and fluid sharing via regurgitation as a submissive display. It should also be noted that this study took foraging effort, measured as productive returns per foundress, as a proxy to help effort. However, future studies may consider creating an index of help effort that incorporates other tasks such as cell checking, nest building, and

feeding the brood. While these tasks are less costly than foraging, which requires prolonged flight, carrying produce, and a greater risk of predation, they are arguably more altruistic since a portion of any food foraged will likely go to the foundress that acquired it. Nest maintenance is arguably in the interest of a foundress that intends to inherit the nest at some point, but brood care only serves to sustain the current dominant's offspring and limit survival of eggs by other foundresses. It would be interesting to see whether these behaviours, which have less intrinsic benefits to the subordinate foundresses, decrease during a period of succession.

Beyond *P. dominula*, these findings provide evidence of how individual reactions to changes in colony dynamics can have substantial holistic effects on the productivity of an entire colony. Strategies such as pay-to-stay and kin selection mean that events such as succession or reduction in group size can have knock on effects on the behaviour of the rest of the hierarchy (Brouwer, Heg, & Taborsky, 2005) and, as this study has shown, a consequence of these behavioural changes being a product of individual reactions means that an important role such as foraging may be neglected in favour of a hypersensitive response to defending one's own position and potential fitness investments.
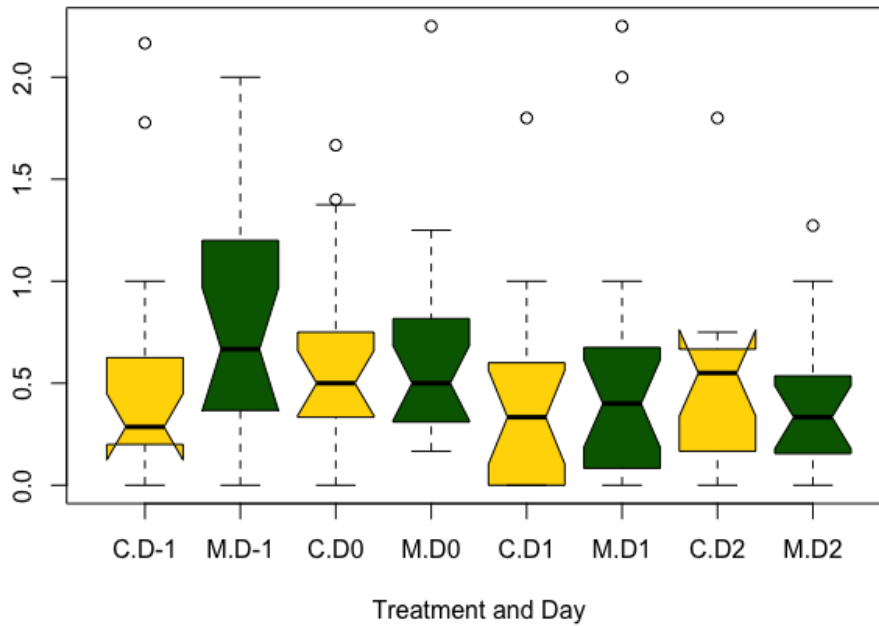
Figure 2. Productive returns from foraging per foundress on each day of the sample period for both treatment (M) and control (C) nests. Days referred to as "D-1" are days prior to removals. Days referred to as "D0" are days on which removals took place; in these instances the removals were undertaken the morning before the video was recorded.
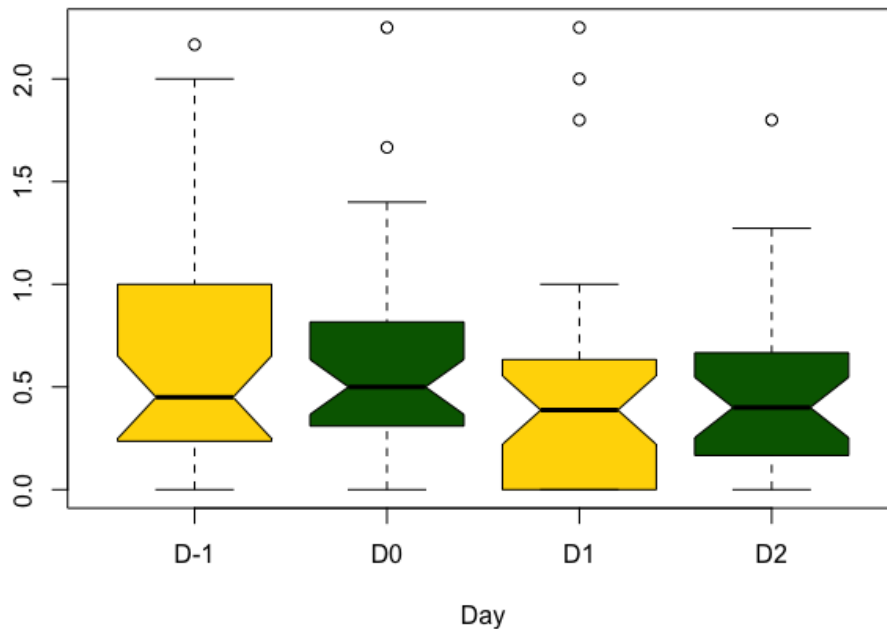

Figure 3. Productive returns from foraging per foundress on each day of the sample period without segregation by treatment type. Days referred to as "D-1" are days prior to removals. Days referred to as "D0" are days on which removals took place; in these instances the removals were undertaken the morning before the video was recorded.

CHAPTER 5: GENERAL DISCUSSION

Synthesis

The research that went into this thesis aimed to accurately determine the dynamics behind succession of a dominant foundress on social nests of *Polistes dominula*. By investigating the trends of rank 2 aggression and foraging activity of the entire nest, these experiments could contribute to the existing knowledge on how dominance is initially established and how such a stable linear hierarchy between reproductively viable foundresses is maintained. The predictions made were that removal of the dominant foundress would provoke a peak in aggression from the rank 2 immediately after removal, followed by a gradual return to the standard of aggression set by the last dominant, and that this would coincide with a trough in foraging effort that mirrors the spike in aggression. If these predictions were met it would have shown evidence that aggression is used by a new dominant wasp to re-establish the hierarchy before her chemical signature diverges to distinguish her from the rest. It would also have suggested that initial establishment of the hierarchy could be undertaken in this way. Meanwhile, foraging effort would decline as the nest adjusts to new leadership, meaning that there is a shift in priorities from foraging to provide for nest-mates and larvae to defending one's own position in the inheritance queue during these brief periods of succession.

The findings of this research did not support these predictions. Aggression rate of the rank 2 did not significantly change across any of the days, nor between treatments, standing as strong evidence against the predictions made. There was also no evidence that the aggression of the previous dominant had any influence of the aggression of the rank 2 before, during, or after her ascension

to dominance. Foraging effort did seem to decline, but this was delayed by a day or two after removals and did so as a result of a foundress disappearing, regardless of her rank. Statistical analyses seemed to indicate a gradual tend back toward the previous foraging level before removals, but with the limited sample period used it is difficult to say for certain. It therefore appears that neither aggression nor foraging effort fluctuate during periods of succession as predicted.

The big picture

The gaps that this study sought to fill were how dominance is established initially, and how behaviour of *P. dominula* foundresses fluctuates between selfish and altruistic acts depending on the nest dynamics at the time. More specifically, it investigated whether a rank 2 has to forcefully take up its rightful position as new dominant when the previous one disappears, and whether the resulting chaos of replacing a dominant drove the rest of the foundresses to act in their own self-interest by sacrificing foraging effort to commit more time to defending their position in the hierarchy.

This research indicates that, while aggression may constantly be playing a part in maintaining the linear hierarchy, the absence of a dominant is not met with an instant spike in aggression by the successor. This research also provides no evidence of succession specifically causing a reduction in helping effort, but the evidence does suggest that any disappearances, regardless of rank, instigate contests and some hierarchy instability that causes other foundresses to commit more time to establishing their presence and position in the group. This in itself suggests that the foraging and helping efforts of subordinates are not regulated by the dominant alone, and should in theory be prone to fluctuating

any time there the inheritance queue shifts. Interestingly, unlike the findings Strassmann *et al.* (2004), foraging effort did in fact decrease during the succession periods, even if this appeared to be more of a response to the disappearance of a foundress than the actual succession of the dominant.

Further research

As many of the predictions made by this research were not met, there is a call for future researchers to investigate the answers to the overarching questions that cannot be answered by the negative results of this study alone. It is still not known precisely how dominance is determined initially, and while it is suggested that the constant aggressive tests and displays which stabilise ranks may be responsible, this study suggests a limited effect of aggressive subduing of potential rivals in the establishment of dominance hierarchies. There is still also the question of how susceptible to queue-jumping are these linear dominance hierarchies, and are there other circumstances where individuals must commit themselves to contests over foraging lest they risk losing their position. Further to these potential research projects, there would be great value for researchers interested in the dynamics behind dominance and succession in undertaking analogous studies with adjustments to overcome the limitations of these experiments, such as using an experiment and model that allow factors such as nest development stage and date to be analysed.

The unexpected results that arose from this study have pathed the way for further potentially insightful research questions. One question worth future consideration is how a foundress determines that a dominant has disappeared, rather than simply left to forage. Given the contrast between the delayed decrease in foraging effort, which had not entirely returned to its original level

for at least three days, and the studies mentioned in Chapter 1 which suggest an almost immediate reaction to an absent dominant, it would be interesting to investigate whether the lag between absence and reduced foraging effort more closely. Of particular interest would be whether the rest of the foundresses wait a certain length of time, after which an absence is considered a disappearance, or whether an individual's rank is in danger of being 'queue-jumped' the instant the individual leaves the nest to forage. Furthermore, investigation into the effects of disturbance on the nest as a whole may also provide valuable insight into planning methodologies for future research, since it is difficult to say that the findings in Chapter 4 were not as a result of disturbing the nest to remove the target wasp.

For a species that has been so well researched there are still a great many questions still unanswered regarding their life history and social dynamics, even beyond the hotly contested role of clypeal marks. If future research can disentangle the factors influencing the creation and maintenance of the linear hierarchies optionally formed by aggregations of *Polistes dominula* it may contribute a wealth of knowledge and understanding regarding dominance hierarchies across taxa, and perhaps even reveal details about the evolutionary origins of altruism.

Dominance hierarchies are ubiquitous across taxa, and yet the mechanisms of their formation and maintenance remain poorly understood. Studies using well-researched and tractable species, such as *P. dominula*, are powerful investigatory tools for disentangling the mystery of sociality and hierarchy. One can conduct experiments using manipulations that are impossible in vertebrate systems in order to reveal the forces that stabilise and destabilise social hierarchy, with general implications for the evolution of sociality, altruism, and

cooperative transitions throughout the animal kingdom. The value of using such

accessible model species should not be underestimated; it is the opinion of this

academic and many others that continuously building on the methods and

findings of previous studies using these tractable species is the key to

discovering the secrets behind the evolution of sociality.

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APPENDIX 1: PROJECT PROPOSAL

OUTLINE

The basis of my project plan is investigating the role of aggression between the alpha female and the beta female on foraging frequency, labour division, and succession in paper wasps. Throughout the project I will be performing removal and reintroduction experiments, in which I will remove the alpha female and reintroduce her only once a beta has succeeded her. This will result in four stages for each nest: before removal, during removal, during reintroduction, and after reintroduction. I will be using high-speed cameras to looks closely at aggression and foraging behaviour of the wasps in order to accurately quantify aggression behaviour and foraging frequency. I shall be investigating whether or not relationships exist between pre-existing aggression between the alpha and beta, foraging frequency, number of foraging workers, length of time until succession, and post-introduction aggression.

HYPOTHESES

I hypothesise that high pre-existing aggression on the beta by the alpha will cause a longer time until succession upon removing the alpha, greater post-introduction aggression between the old alpha and the new alpha (formerly the beta), and a larger difference in foraging frequency and number of foragers between times when an alpha is present and times when there is no clear alpha. I also hypothesise that aggression interactions will be followed by a dip in foraging frequency and number of foragers, and that this dip will be greater when pre-existing aggression was high.

PROJECT SCHEDULE

October 2016 – December 2016: Watch footage from high-speed cameras of paper wasp interactions and record data on aggression interactions, foraging, and succession following removal of an alpha.
December 2016 – March 2017: Analyse video footage data and use hypothesis testing to reveal any significant relationships.
March 2017 – May 2017: Fieldwork in Spain performing removal experiments and monitoring nest sites regularly.
May 2017 – August 2017: Further analysis and hypothesis testing to reveal relationships and reinforce results.
August 2017 onwards: Aim to complete thesis and publish papers.

APPENDIX 2: SUPERVISOR LEARNING AGREEMENT

College of Life and Environmental Sciences

Postgraduate Research Student Learning Agreement Form

Postgraduate Research Student: Alexander Brown

Supervisor 1: Mike Cant

Supervisor 2: Tom Tregenza

Supervisor 3 *(if applicable)*:

Supervisor 4 *(if applicable)*:

Programme of Study: MRes Biological Science

Project Running Title: The effect of increased aggression and decreased foraging efficiency on stabilising paper wasp society.

Location if the student is based off-campus: Medina, Spain (Spring Term)

The Learning Agreement form should be completed within the **first 8 weeks** of study, following discussion between student and supervisor, and reviewed on at least an annual basis. It should be considered as a useful tool to facilitate completion of a successful research project and degree programme.

This Learning Agreement should be considered as complementary to the University "*Code of Good Practice for Supervision of Postgraduate Research Students*" and does not replace or supersede it.

The original of this signed form should be kept by the student, and a completed copy must be uploaded to MyPGR.

Period to be covered for following agreement (e.g. preparation for research; initial research; writing-up etc): 24 months

Who is responsible for arranging meetings or other formal contact?:

Alexander Brown

Agreed frequency of meeting with supervisor(s), including the role of each of the supervisors, and the mode in which meetings will take place e.g. face to face, telephone, skype:

      a. It is expected that the frequency of meetings will vary throughout the degree period, with a minimum of one meeting a month where possible, but that any changes to the frequency must be agreed by both parties.

      b. It is the responsibility of the student to make a record of the formal contact with their supervisor(s) or mentor on MyPGR (10 events per year for full-time students, 6 events per year for part-time students, 3 events per year for writing up students).

Approach to Research (include aims, objectives and key milestones):

The research project aims to investigate how aggression of the dominant wasps, foraging efficiency of the entire nest, varies with removal of the Rank 1.

The objectives of this project are test the hypothesis that dominant aggression increases and nest-wide foraging efficiency decreases with the removal of the Rank 1.

General Roles of postgraduate researcher and supervisor:

My role is to plan the project and develop questions to explore. I will identify areas for improvement within my knowledge and skillset and undertake appropriate steps to improve these. I will undertake the data collection, data analysis, and writing of the thesis.

Mike's role is to provide support, advice and guidance on the project and subsequent analysis.

Topic Specific Roles:

The project will examine how aggression of the dominant wasps, and foraging efficiency of the entire nest, varies with removal of the Rank 1. I will be reading relevant theory and literature concerning...

Research Support:

This project relies on the availability and accessibility of videos of wasp nests. It also relies on accurate ranking of the wasps, success of the nests in question, and comparable timings for the videos.

Research Training (Project Specific):

Video scoring training specific to analysing wasp behaviour.

Research Training (Generic):

Statistics training for the subsequent analysis of video analysis data. This will likely include creating a GLM or GLMM on RStudio.

Consultation & Reviews:
Meet with Mike regularly to discuss progress with the research project. Be in contact with Mike, either through face-to-face meetings or email, to discuss research progress and address any issues. Consult with Feargus Cooney and other team members for answers to minor questions/intricacies.

Role of/interaction with collaborating organisation (if applicable):

NA

Ethical aspects of the research:
There are no ethical aspects of this research project as wasps are marked and videoed in a method involving minimal stress and no damage to the individuals.

Intellectual Property Rights issues:
There are currently no intellectual property rights issues. Any that arise will be dealt with appropriately.

This Learning Agreement is made on   19/10/2016

Between Alexander Brown
and Signed : Michael Cant Date: 3/11/2016

 (Supervisor(s) Names)
Prof Michael Cant

APPENDIX 3: SUBMISSION FORM

FORM OF APPLICATION FOR EXAMINATION OF A THESIS/DISSERTATION FOR DEGREES IN THE FACULTY OF GRADUATE RESEARCH

PART A - To be completed in FULL by the Candidate (please use BLACK INK or TYPE)

1   I, Alexander Theocharides Brown, Student Number 630027656 submit myself for examination for the

Degree of Masters by Research in Biosciences in the College of University of Exeter Title of thesis/Dissertatio

2   Date of initial registration for the degree: 01/09/2016

3   I enclose 2 paper copies of the thesis/dissertation for examination (one for each External and Internal Examiner).

4   I confirm they are in a form prescribed in the University's Statement of Procedures: Presentation of Theses/Dissertations for Degrees in the Faculty of Graduate Research and embody the results of research on which my candidature for the degree is based. (If sent by post, the copies should be sent by Registered Post or Recorded Delivery).

5   I confirm I have read the Handbook for Examination of Postgraduate Research programmes

6   I confirm I have read the Statement of Procedures: Periods of Registration and Changes to Registration Status for Graduate Research Students

7   I certify that the thesis/dissertation submitted does not include any material for which a degree has previously been conferred upon me and that I have identified any work which is not my own.

8   I understand that the decision on my thesis/dissertation rests with the examiners alone and that a favourable view from my supervisor(s) cannot guarantee the award of the degree for which I am being examined.

9   I understand that the award of my degree will not be conferred until I have satisfied the requirements for final submission after examination, as set out in the above *'Statement of Procedures – Presentation of Theses/Dissertations for Degrees in the Faculty of Graduate Research'*.

10   I agree that the University will regard the electronic version of my thesis, as submitted to the University's online repository ORE, as the definitive copy. Please note the University is a participating institution in the British Library's EThOS service and a copy of your ORE submission will be shared with them (subject to any existing embargoes). For further information see http://as.exeter.ac.uk/library/resources/openaccess/e-theses/

* Tick relevant boxes

11a [ ] * It is a requirement of the project sponsor that the contents of this thesis/dissertation are not made publicly available due to commercial sensitivities. The External Examiner will be sent a **confidentiality agreement** form to sign from the Postgraduate Administration Office.

11b [ ] * I wish to place an embargo on my thesis to be made universally accessible via ORE, the online institutional repository, for a standard period of 18 months because I wish to publish papers using material that is substantially drawn from my thesis. (NB: This option is only available if submitting electronically and will take effect from the date the thesis is uploaded to ORE)

11c   [ ] * I wish to place an extended embargo on my thesis and withhold consent for my

thesis to be publicly available on ORE or to the British Library until _____

(maximum 5 years initially) for the following reason(s) **#:** _____

 # A letter from your supervisor supporting the embargo must be attached.

N.B: see Q13 in the FAQs at http://as.exeter.ac.uk/library/resources/openaccess/e-theses/faqs/.
Unless an embargo is requested your thesis on ORE will have universal online accessibility.

An extended embargo may be required if your thesis contains any of the following:

- *unprotected intellectual property which you, your sponsor or any other 3ʳᵈ party has the intention to use*
- *sensitive information that may need to be withheld from public view*
- *commercially sensitive material that may belong to your project sponsor*

Please contact pgadmin@exeter.ac.uk if you require any further advice.

Attendance of the Supervisor at the Viva

12   [✓] I would like to invite the following Supervisor to be in attendance at my viva voce exam:

Name of nominated Supervisor: Professor Michael Cant

Note: Only one member of the supervisory team may attend the viva and this should normally be the 1ˢᵗ (lead) supervisor. They will be present as an observer only. Please refer to the Handbook for Examination of Postgraduate Research Programmes for the full regulations governing the attendance of a Supervisor at a viva.
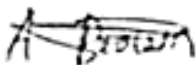
Please also note that it may not always be possible for your supervisor to attend your viva.

To be completed in full by the Candidate:

**   Please go to 'My Exeter' and check that both your home and contact address details are up-to-date. If not, please amend.

Name in full (print): ALEXANDER THEOCHARIDES BROWN

Signature   Date: 27/12/2018

Contact Address: 22 Smythe Close,

Clacton-on-Sea, Essex,

CO16 8FS

University email address: ab692@exeter.ac.uk

Personal email address: alex.theocharides@hotmail.com

PART B - To be completed by the Supervisor(s)

Please check with your College Office that you are correctly recorded in SITS as the 1st Supervisor (S1) or 2nd Supervisor (S2) and that the percentage weighting is also correct. This information will be used for the REF.

Please tick A1 or A2 as applicable.

A1 [ ]   I certify that I have seen and commented on a copy of the draft thesis/dissertation *(see 1.13 of the Code of Good Practice - Supervision of Postgraduate Research Students)*

A2 [ ]   For submissions by Publication: I certify that the thesis/dissertation meets the requirements of the University Regulations governing degrees by Publication and that I have seen and commented on a copy of the draft thesis.

B   I certify that the student is aware of the following Teaching Quality Assurance Manual Codes of Good Practice and Statements of Procedure:

- *Code of Good Practice: Supervision of Postgraduate Research Students*
- *Statement of Procedure: Presentation of a Thesis/Dissertation for degrees in the Faculty of Graduate Research*
- *Handbook for Examination of Postgraduate Research programmes*

C   Attendance of the Supervisor at the Viva – please refer to Part A, point 12.

Supervisors should make every effort to attend a student's viva if invited to do so and must contact the Internal Examiner to be included in the viva arrangements. Only one member of the supervisory team may attend the viva and this should normally be the 1st (lead) supervisor. They will be present as an observer only. Please refer to the Handbook for Examination of Postgraduate Research programmes for the full regulations governing the attendance of a Supervisor at a viva.

NB: If the student has requested an extended embargo by ticking box 11d above please attach your letter of support.

1st Supervisor Signature   Date   28 December 2018

Print Name: Prof Michael Cant

2nd Supervisor Signature_____ Date_____
(If applicable)

Print Name:_____

The completed form, together with copies of the thesis/dissertation as specified in 3 above, should be returned to:

- **Streatham and St Luke's Campus:** The Student Information Desk, University of Exeter Forum, Stocker Road
- **Cornwall Campus:** Academic Support Unit, Peter Lanyon Building