Rank and Inheritance in a Facultatively Eusocial Hover Wasp

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ABSTRACT

In each *L. flavolineata* colony only one 'dominant' female reproduces at a given time, but all of the females, apparently, have the potential to achieve dominant status. I provide detailed census data, which shows that the majority of wasps inherit dominance in an age-based manner, i.e. the oldest individual becomes the dominant when the previous dominant dies. However, I also provide evidence of 'cheats' that achieve dominance before older individuals.

Focusing upon 'cheating' individuals, I look at their relative size and genetic relatedness in relation to their nestmates to provide clues as to how they are able to 'queue-jump'. This study reveals that queue jumpers tend to be the sisters of wasps they jump in the queue yet queue jumpers are generally no larger than the rest of their nestmates. I then proceed to look at the prior foraging effort of queue jumpers before the queue jump took place. I conclude that queue jumping is an opportunist act performed when the dominant shows cues as to the imminent arrival of her death.

I provide data regarding the general genetic structure of *L. flavolineata* colonies, focusing particularly upon the relatedness of the dominant to subordinate ranks. This study reveals no correlation between rank and relatedness to the dominant.

Finally, I look at foraging effort and how it corresponds to rank and group size. Cant and Field (2001) have developed a Kin Selection model in which they predict the optimum levels of foraging effort for a subordinate individual according to its rank and the group size of its nest. *L. flavolineata* is a suitable species upon which to test this model as rank is revealed to be independent of relatedness to the Dominant. The results shown here are in good agreement with the predictions of the Kin Selection model.

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1 INTRODUCTION

1.1 Social Behaviour

The subject of sociality is a wide-ranging one, which has received a great deal of attention, in many varied disciplines, from anthropology through to computer science. Social behaviour is common to many organisms and seems all the more relevant to us, as humans, because of our social tendencies. Indeed, this behaviour at one point seemed inexplicable, as altruism was at odds with the theories of individual-based selection that were popular before W.D. Hamilton's work (see Section 1.2.6.1.2; Hamilton 1964a, 1964b). Therefore, the question of how such behaviour could have evolved has been the subject of much research.

1.1.1 Group Living and Sociality – A Distinction

A clear distinction must be made between the terms group living and sociality. Group living is often a very flexible phrase used to describe anything from mere gregariousness to close knit cooperative units. Coster-Longman *et al.* (2002) recently used group living to describe the gregarious nature of nesting in some species of Stenogastrine wasps. Therefore, to avoid confusion, I use the term sociality to indicate a cooperatively breeding unit i.e. a group in which reproduction is confined to one or more dominant individuals or mate pairings, which are assisted in their brood rearing by subordinate individuals. The term sociality can also be broken up into two distinctive groups i.e cooperatively breeding organisms, which are confined to some birds and mammals, and eusocial organisms, which are found within the insects. These terms will be discussed in greater detail shortly.

1.1.1.1 Factors Influencing Group living and Sociality

There are a number of factors that might have influenced the evolution of group living and sociality. It is easy to see how ecological constraints could often be responsible for gregarious group living due to isolated areas of suitable habitat limiting the opportunities for dispersal. Such constraints could also be implicated in the evolution of sociality, as I shall elaborate upon later.

Group living confers many benefits such as increased vigilance towards predators (Bertram 1980; Fernandez-Juricic *et al.* 2004), dilution effects (Simon 1979; Duncan & Vigne 1979; Calvert *et al.* 1979) and defence (Kruuk 1911). The benefits to these group-living individuals are obvious; yet such advantages are more ambiguous when examining groups that exhibit sociality. The behaviour in these circumstances seems more altruistic. Subordinate members within social groups do not breed directly and often help dominant individuals with brood rearing (Gaston 1978; Jarvis 1981; Emlen 1991; Komdeur 1994; Cockburn 1998; Kokko *et al.* 2002). Clearly, there must be strong forces influencing subordinate members to surrender or postpone their own reproduction. These factors are discussed in the following section (see Table 1.1 for a summary of factors influencing group-living and sociality).

Table 1.1 A Comparison of Factors which may have led to the Evolution of Group-Living and Sociality and the Benefits that each mode of living may confer upon its members

		Influencing Factor	Benefit	Example
GROUP LIVING	**Interspecific Interactions**	Predator Deterrence	Dilution Effect	Monarch butterflies (Calvert *et al.* 1979)
			Defence	Black-headed Gulls (Kruuk 1911)
			Predator Detection	Starlings (Fernandez-Juricic *et al.* 2004)
		Predation	Strategic Hunting	Lion packs (Cooper 1991; Eloff 1998)
	Ecological Constraints	Nesting Sites/Material	Ease of locating suitable nesting areas and nesting material	Dusky Moorhens (Shirley *et al.* 2003)
		Food Abundance	Ease of locating food Starvation avoidance	Colobus monkeys (Clutton-Brock 1975; Chapman & Chapman 1999)
SOCIALITY	**Physiological Constraints**	Sterility	Indirect Fitness gains	Hover Wasps (Field & Foster 1999)
	Genetic Predisposition	Haplodiploidy	Indirect Fitness Helpers at the Nest	Hymenoptera (Hamilton 1964a; Reeve 1993)
	Insurance Benefits	Length of Brood Dependence vs. Adult Lifespan	Assured Fitness Returns (Gadagkar 1990)	Sweat bees (Smith *et al.* 2003)

1.2 The Evolution of Eusociality

1.2.1 An Introduction to Eusociality

Eusocial organisms exhibit the most advanced forms of cooperation that exist within the natural world. Such organisms can be found in the three orders, Hymenoptera, Isoptera and Homoptera. Gadagkar (1994) suggested that the term eusociality be used to emphasise the similarities between these social insects and cooperatively breeding vertebrates, as both are composed of individuals that help rear each others offspring. Gadagkar (1994) suggested that the term eusociality be expanded to encompass both eusocial species and cooperatively breeding birds and mammals, in which individuals surrender their personal reproduction to aid conspecifics.

Advanced eusocial insects show, perhaps, the most familiar type of cooperation within the social insects. They are separated into different castes such as breeding 'queens' and sterile female workers. Here determination of caste depends upon the environmental conditions, which larvae experience during their development (Yanega 1989; Wheeler 1991; Hunt *et al.* 1996; Cnaani *et al.* 1997). They have three key characteristics:

1. Cooperative care of young
2. Sterile castes
3. Overlap of generations

However, the evolution of eusociality can be more effectively examined by looking at particularly primitive examples of eusociality. This can be found in the 'facultatively' eusocial insects. An invaluable insight into the evolution of eusociality can be found by looking at these species, as they contain individuals that show altruism even though they are capable of 'selfish' direct reproduction (Gadagkar 1994).

1.2.1.1 Facultatively Eusocial Insects

The facultatively eusocial Hymenoptera differ from advanced eusocial insects in that they have no specialised castes. That is to say, the workers are not sterile and may develop breeding status given the opportunity. The queen is also less constrained in that she is usually able to found a nest and care for her own brood without relying upon worker help. Therefore, the role of each individual is a great deal more flexible than in more advanced caste societies. The ability to adopt different strategies provides valuable insights into their immediate costs and benefits. For example, if two different strategies are adopted within a population, the benefits and costs that each strategy bestows can be compared between individuals. The important point here is that the individuals being compared belong to the same species. Therefore, many of the complications that arise from cross species comparison can be avoided, such as differences in life history, physiology and ecological constraints. Facultatively eusocial insects thus allow strategies such as independent nesting to be compared with that of remaining upon the natal nest and helping.

1.2.2 The Vespidae

One family that has been studied widely with regard to the evolution of eusociality is the Vespidae, as its member groups exhibit a wide range of social behaviours from solitary living to advanced eusociality (see Figure 1-1). Eusocial behaviour can be found in three of Vespidae's subfamilies, the Stenogastrinae, Polistinae and Vespinae.

1.2.3 The Stenogastrinae

The subfamily Stenogastrinae is regarded as very important in providing clues as to the evolution of eusociality because of their 'primitive' nature. They are a subfamily of the family Vespidae and consist of around 50 known species across six genera (Carpenter 1988; see Figure 1-2). The species that is investigated here, *Liostenogaster flavolineata*, like all Stenogastrine wasps, is distributed throughout many of the rainforests of South-

East Asia (Turillazzi 1991; see Figure 1-3). The Stenogastrinae are seen as one of the most important groups for studying social evolution because all females are capable of producing offspring and may therefore be free to adopt any number of reproductive strategies (see Section 1.2.1.1; Field & Foster 1999). Because of this, these wasps have received a greater deal of attention (Hansell 1985; Turillazzi 1986; Samuel 1987; Carpenter 1988; Turillazzi 1991; Cervo *et al.* 1996; Field *et al.* 1998; Sumner *et al.* 2002).

1.2.4 *Liostenogaster flavolineata*

The inheritance of dominance within *L. flavolineata* provides the main focus of this thesis. This facultatively eusocial hover wasp was first studied, in any great detail by Hansell *et al.* (1982). The first investigations of this wasp mainly focused upon the abdominal substance, which is unique to the Stenogastrines (see Section 1.2.4.4; (Keegans *et al.* 1992, 1993). Later work by Strassmann *et al.* (1994) looked at intra and internidal genetic relatedness, concluding that there was a high degree of movement between nests by individuals i.e joining events (see Section 3.1.4.1.1). More recently, this work was countered by Sumner (1999, 2002). Such a good level of background knowledge of this species makes it an ideal candidate to examine with regards to its dominance hierarchy, fitness decisions and reproductive strategies. The following sections describe the life history of *L. flavolineata* in greater detail.

Figure 1-1 Cladogram of the subfamilies of Vespidae (Carpenter 1982 from Ross & Matthews 1991)

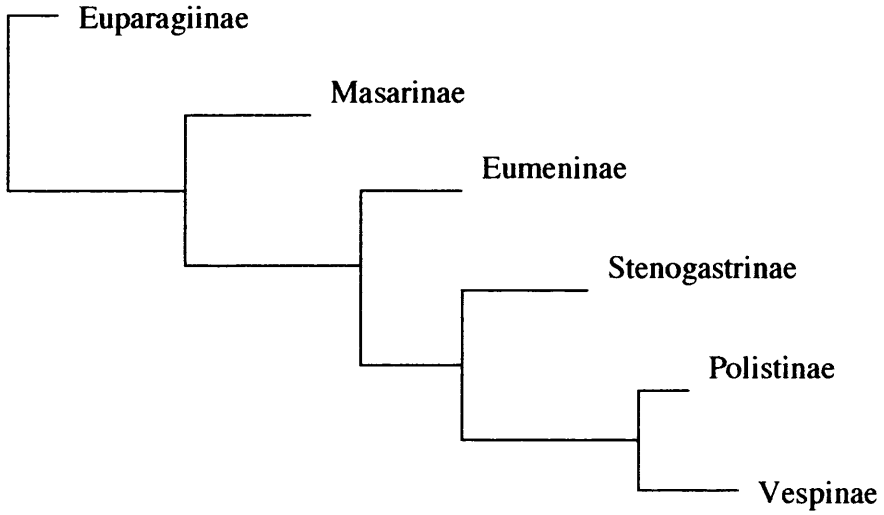
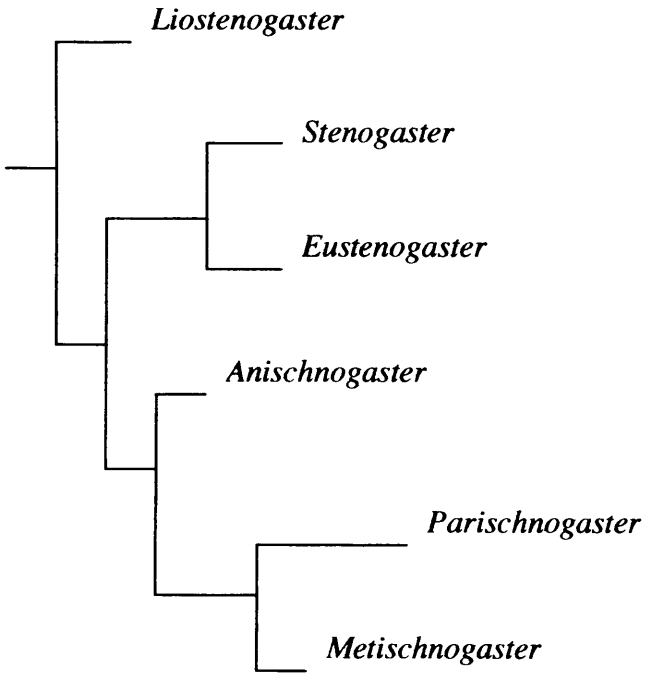


Figure 1-2 Cladogram of the genera Stenogastrinae (Carpenter 1988 from Ross & Matthews 1991)



1.2.4.1 Habitat

L. flavolineata is usually found in dark, moist and sheltered places such as under bridges and culverts (see Figure 1-4). Such an environment provides them with adequate supplies of mud for nest building as well as protection from extreme temperatures. Their nests can often be found alongside those of *Parischnogaster alternata*, another stenogastrine wasp, known as the Black Hover-Wasp.

1.2.4.2 Life History

L. flavolineata lives in groups averaging from 1 to 4 females (Field *et al.* 1998) and is similar to cooperatively breeding vertebrates in that it has no morphological castes i.e. it is facultatively eusocial. All of the females upon the nest are capable of egg-laying yet only one actually does at any one time: the dominant (Field & Foster 1999; Sumner 1999). Previous studies of *L. flavolineata* have indicated that dominance tends to be determined in an age-based manner (gerontocracy) and the implications of this will be discussed later (see Section 1.3.3.1 and Chapter 4). Sumner (1999) has shown through microsatellite studies that the dominant, reproductive, female is singly mated (see Chapter 3). These wasps have been the focus of much attention because, unlike many other facultatively eusocial wasps such as temperate *Polistes*, they have a continuous breeding season due to their aseasonal environment. Because of this a female may found a nest at any time (Samuel 1987).

Figure 1-3 Distribution of the Stenogastrinae throughout South East Asia

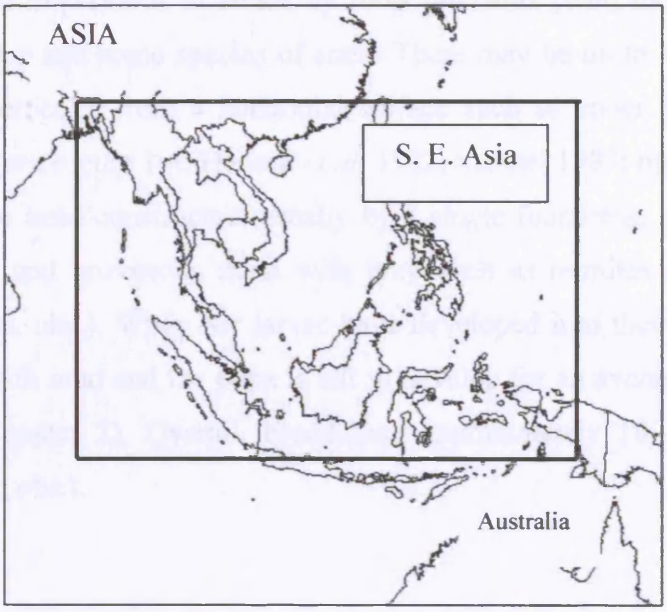
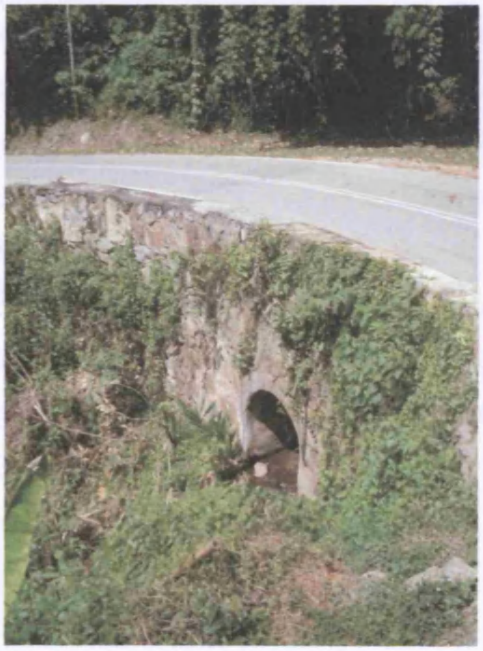
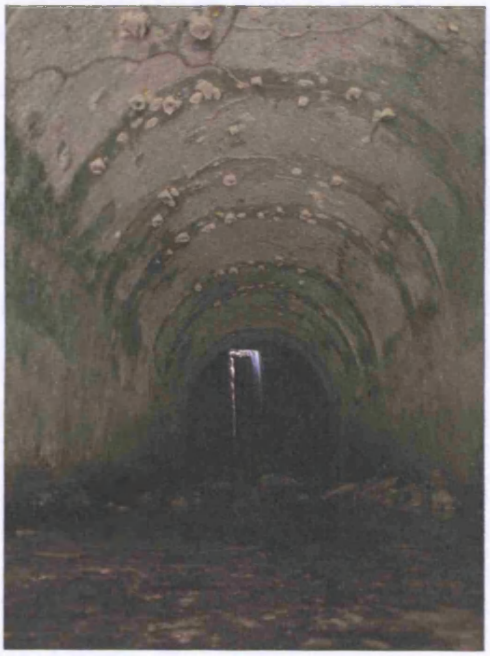


Figure 1-4 Typical Habitat of *L. flavolineata*

Outside (left) and inside (right) view of study site #8 (Photos courtesy of A.Cronin 2003).





1.2.4.3 Nest Construction

L. flavolineata nests are constructed from mud and are therefore very hardy structures, which seem almost impervious to attack by most predators (with the exception of the hornet *Vespa tropica* and some species of ants). There may be up to 100 cells in a nest that hang down vertically from a horizontal surface such as under a bridge or on an overhanging rock (see Figure 1-4; Hansell *et al.* 1982; Samuel 1987; pers. obs.). Once the first few cells have been constructed, usually by a single foundress, she lays an egg in some of the cells and provisions them with prey such as termites and arboreal ants (Samuel 1987; pers. obs.). When her larvae have developed into their pupal stage their cell is sealed off with mud and the pupa is left to develop for an average of 30 days (see Figure 1-5 and Chapter 2). Overall, brood take approximately 100 days to develop (Samuel 1987; pers obs.).

1.2.4.4 Brood Care

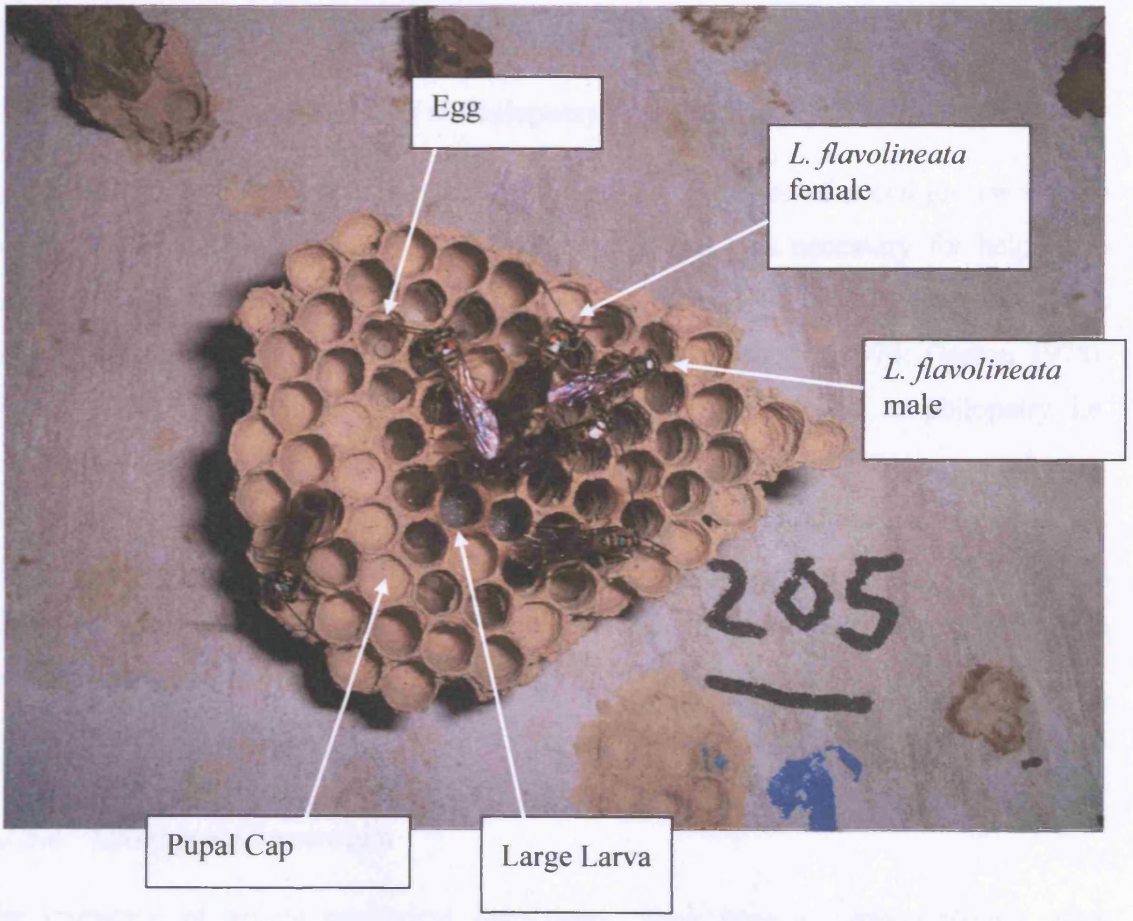
Each of the brood is held in position, within their cells, by a white 'abdominal substance' (Turillazzi 1985b). The dominant female, as well as any subordinate females that may be present, help to regularly provision these larvae. The abdominal substance is produced from the Dufour's gland of the female wasp and is mixed with fructose before it is deposited upon the brood. The use of this secretion is unique to the Stenogastrinae (Hansell 1977; Hansell *et al.* 1982; Turillazzi & Pardi 1982; Delfino *et al.* 1988). As soon as an egg has been produced, the female takes the Dufour's secretion in her mouth and places it upon the egg or larva. Keegans *et al.* (1992, 1993) have shown that the abdominal substance consists of fructose, water, linear alkanes and emulsifying agents. The wasp collects fructose and water, presumably in the form of nectar.

Turillazzi (1985a, 1989) proposes that the purpose of the secretion is fourfold:

1) For females to be able to handle their eggs after oviposition.
2) To serve as a substratum and protection for the larva.
3) To serve as a "dish" for larval food
4) To act as a reserve for the colony's liquid food.

Once the brood have developed, each newly emerged female may choose either to stay upon the nest and help rear sibs or leave and found their own nest. The implications of both strategies are diverse and will be discussed in later sections. A number of theories have been suggested to explain why facultatively eusocial organisms or cooperatively breeding vertebrates should choose to stay within their natal nest or group. Their lack of fixed caste systems means that each individual is not pre-disposed to remain within its group and could feasibly leave and reproduce directly.

Figure 1-5. The nest of *L. flavolineata* illustrating nest architecture and different stages of brood



(Photo Courtesy of A. Cronin 2003)

1.2.5 Factors influencing the Evolution of Eusociality

The following sections will outline some of the factors that may have influenced the evolution of eusociality. In each case, observations and experiments using *L. flavolineata* have been used to support or oppose a hypothesis.

1.2.5.1 The Causes and benefits of Philopatry

One of the first hypotheses put forward to explain the formation of social groups was by Emlen (1982a, 1991). He argued that the first step that was necessary for helping to commence was the separation of a population into small social units. These small units must retain adult offspring to be considered as eusocial (Brown 1974; Gaston 1978). Brown (1974) first promoted the idea that habitat saturation led to philopatry i.e. retention of adult offspring. He observed that, within cooperatively breeding vertebrates, offspring that stay at home generally do so when resources required for independent breeding are temporarily unavailable or of poor quality (Emlen 1997). The 'habitat saturation' hypothesis is now a widely accepted explanation for cooperative breeding in vertebrates (Gaston 1978; Koenig & Pitelka 1981; Koenig *et al.* 1992).

1.2.5.2 Ecological Constraints

The existence of severe ecological constraints, upon breeding independently, may underlie the evolution of familial philopatry in most cooperatively breeding species. An example of this was presented by Woolfenden & Fitzpatrick (1984) who found that helpers of Florida Scrub Jays (*Aphelocoma coerulescens*) would benefit more from forming their own breeding territory than by helping on their natal nest. In this bird, as soon as territory vacancies arise, helpers will usually leave their natal nest and fulfil such vacancies. In this case, staying and helping upon the nest may be seen as a "best-of-a-bad-job" strategy. The Azure-winged Magpie (*Cyanopica cyanus*) has also been observed to follow such a strategy as severe weather conditions strongly influence their decision to

stay upon the nest (Canario *et al.* 2004). Indeed, some species may switch their reproductive tactics from breeding to helping within the same breeding season due to seasonal variation (Maccoll & Hatchwell 2002).

Overall, there are two situations where grown offspring will have a low probability of successfully reproducing on their own:

1. When the species has specialised ecological requirements. (Brown 1974, 1978; Koenig & Pitelka 1981; Korb & Schmidinger 2004)

2. When the species inhabits a changing, unpredictable environment (Emlen 1982b).

As mentioned previously, the habitat saturation hypothesis is now widely accepted, yet some have suggested that it is the peculiarities of the breeding ecology of some cooperatively breeding species that limits their choice of habitats (Stacey 1979; Koenig & Pitelka 1981; Koenig *et al.* 1992; Davies *et al.* 1995). An extension of this idea was put forward in the 'life history' hypothesis. This hypothesis suggests that cooperative breeding tends to occur in species with low annual mortality because the turnover of territory owners is extremely slow and habitat saturation is therefore more likely (Brown 1974, 1987; Russell 1989). In support of this Arnold and Owens (1998) found that cooperative breeding does tend to be concentrated within certain families that are therefore likely to share some similar life history characteristics. More importantly, they found that increases in levels of cooperative breeding are strongly associated with decreases in annual adult mortality. Thus, slow turnover of territory ownership does seem to influence cooperative breeding (see also Arnold & Owens 1999; Hatchwell & Komdeur 2000)

1.2.5.2.1 Ecological Constraints in *L. flavolineata*

L. flavolineata is a eusocial organism whose offspring often remain upon the nest to help rear the dominant's brood. Ecological constraints are often suggested as a reason for adult offspring retention and previous experiments to test this within *L. flavolineata* are outlined here. This species lives in an aseasonal tropical environment so there is no pressure for a newly emerged female to leave and found a nest before winter encroaches. Field *et al.* (1998) suggested that such a situation makes it feasible for a newly emerged female to opt for the delayed dispersal strategies that are seen in many cooperatively breeding vertebrates. In such vertebrates, offspring may initially become floaters after dispersing from their group (disperse and search) or disperse when suitable vacancies arise (stay and foray) (Brown 1987). However, Krebs and Davies (1993) suggested that such a situation is not completely analogous to that of a cooperatively breeding vertebrate because a nest is a scarcer resource for an insect than a territory is for a vertebrate if nest building involves more effort and therefore incurs more costs. However the nest of *L. flavolineata* is a hardy mud structure that may remain intact for a number of years and is often re-used by founding females (Field *et al.* 1998; pers. obs.). Therefore the cost of founding a nest may not always be incurred by *L. flavolineata* if vacant nests are re-used.

Field *et al.* (1998) tested whether female *L. flavolineata* would leave their natal nests if given sufficient opportunity to do so, in terms of nest vacancies. They found that subordinates did not leave their natal groups when given opportunities to nest independently without nest-building costs (see also Herbers 1986; Bull & Schwarz 1996). Field *et al.*'s (1998) results even suggest that nest-less females (floaters) prefer to wait for better nesting opportunities rather than adopt nests consisting solely of younger brood. This is likely to be due to the fact that if nests with no older brood are adopted, an adult female would probably die before she was able to rear the brood all the way to adulthood (see Section 1.2.5.3; Gadagkar 1990; Queller 1989, 1996).

1.2.5.3 Insurance Benefits

Field *et al*'s (1998) experiments upon the adoption of nests within *L. flavolineata* (see above) provides valuable evidence for the role of insurance-based advantages in the evolution of eusociality (see also Queller 1994, 1996; Kukuk *et al.* 1998). If the period in which offspring depend upon parental care exceeds that of an adult's lifespan this can pose huge problems upon lone parent nests. This is often the case within species of polistine wasps where 38 – 100% of nests with lone foundresses fail before offspring can be reared through to adulthood (Queller 1996). In accord with this Gadagkar (1990) proposed that cooperatively breeding groups benefit from Assured Fitness Returns (AFRs). If a helper dies before she is able to rear offspring through to adulthood, there are surviving group members to continue her work. Indeed, Gadagkar (1990) has shown that such a mechanism can favour helping even if the relatedness between the helper and offspring is very low.

1.2.5.3.1 Insurance benefits within *L. flavolineata*

Field *et al.* (2000) carried out an experiment upon *L. flavolineata* to test whether AFRs were a clear benefit to helpers within this species. They found that after a helper dies, most of the brood that she has part-reared would be reared through to adulthood by the rest of the group. The removal of helpers in this way leaves a nest with more brood than it would normally rear; it was the large brood that tended to be raised through to adulthood with smaller brood more likely to be sacrificed to feed these larger, more valuable brood. This result shows a clear benefit to helpers within these nests. This work was later supported by similar results found in *Polistes dominulus* (Shreeves *et al.* 2003)

1.2.5.4 Physiological Constraints

Some species may contain helpers within their groups for the simple reason that these helping individuals are in some way constrained from breeding e.g. poor fertility. This has been tested in a number of species by providing vacant territories for breeding opportunities. When this was tested on the Superb Blue Fairy Wren (*Malurus cyaneus*) male helpers did fill the vacancies if a female was available in that vacancy to mate with (Ligon *et al.* 1991). Male helpers of the Seychelles Warbler (*Acrocephalus sechellensis*) will also take advantage of vacant breeding territories provided that they are of a high enough quality (Hatchwell & Komdeur 2000). Therefore it is clear that in both these species, helpers are not physiologically constrained from breeding and will seize the opportunity to breed independently should an adequate opportunity arise.

1.2.5.4.1 Physiological constraints of *L. flavolineata*

Field and Foster (1999) have shown that each female *L. flavolineata* is capable of reproducing and indeed females have been observed to leave their natal nest and reproduce directly (Sumner 1999; pers. obs.). Therefore breeding constraints cannot explain why females stay upon their natal nest in *L. flavolineata*.

1.2.5.5 Inheritance Benefits

Inheritance of dominance or a territory is potentially one of the major incentives for remaining within the natal group, yet has been largely overlooked in past studies. This incentive may play an important role within some cooperatively breeding groups, especially if dominance is inherited in a hierarchical manner (i.e. a social queue). A subordinate within such a queue can stay within its natal group and wait to inherit a breeding postion (for an in depth discussion of social queues see Section 1.3.4; Kokko & Johnstone 1999; Buston 2004). Territorial inheritance has often been cited as an incentive for philopatry, indeed almost half of Florida Scrub Jay male helpers, which outlive their

parents, acquire a part or the whole of a breeding territory by inheritance (Woolfenden & Fitzpatrick 1978). This is supported by the models of Pen and Weissing (2000), which indicate that territory inheritance should always promote cooperative breeding.

1.2.5.5.1 Inheritance Benefits within *L. flavolineata*

Past studies of *L. flavolineata* have revealed a social queue for dominance within *L. flavolineata* nests (Samuel 1987; Field *et al.* 1998, 1999). Field *et al.* (1998) suggested that females of this species may be more likely to stay upon their natal nests because the probability of a subordinate inheriting is unusually high compared with other social insects due to their small group size (see Section 1.2.4.1).

1.2.6 The Benefits of Helping

Helping within a group or nest is rare within the animal kingdom. It is predominantly seen when helping individuals are offspring that remain upon the natal nest to help their parents (this accounts for about 80% of species of birds and mammals which have helpers Macdonald & Moehlman 1982; Brown 1987). These offspring are known as 'helpers at the nest'. We have seen that some organisms remain within their natal group due to ecological constraints such as lack of breeding territories (see Section 1.2.5.2; Woolfenden and Fitzpatrick 1984) or, as has been suggested in *L. flavolineata*, due to their likelihood of achieving dominance. However these explanations for eusociality only go some way in explaining how helping itself might have evolved within groups since helping may be costly for the individual. A good example of the cost of helping can be found in the Stripe-backed wren (*Campylorhynchus nuchalis*): helpers that provision at high rates suffer lower survival (see Section 1.2.7; Rabenold 1990). Thus, helping might not always be a good strategy whilst waiting for a breeding position, because helping reduces the chance of surviving to gain a breeding position (Komdeur 1992).

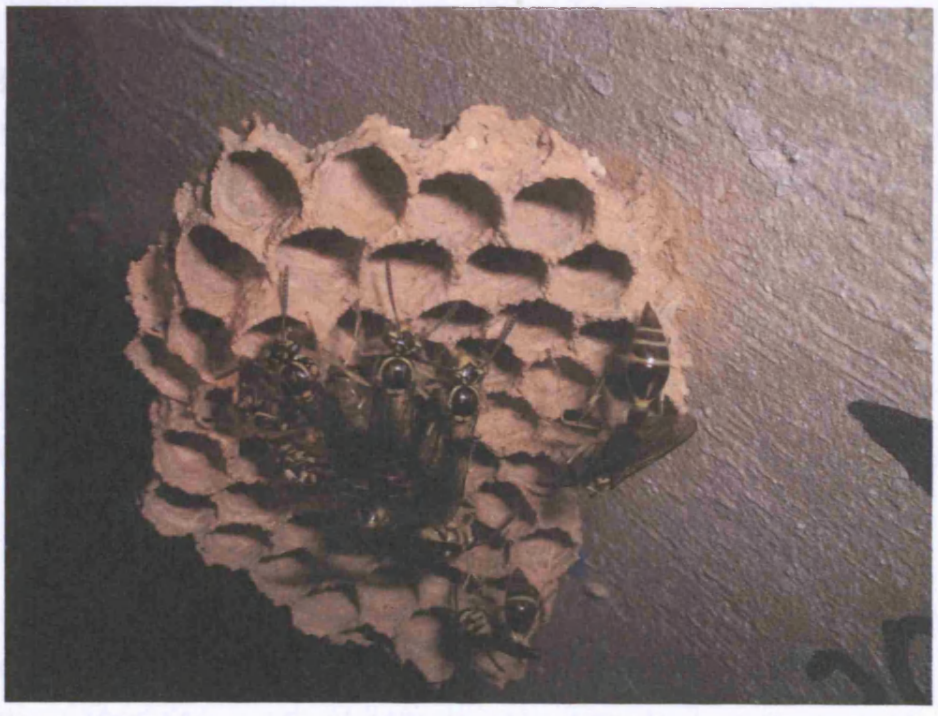
1.2.6.1 Genetic Benefits

Helping might seem to be an altruistic act, in which a donor individual suffers in order to lend help to a recipient individual, and therefore the evolution of such an act appears unlikely. Many investigations have been made into the benefits that helping might bring to a donor. In terms of genetic benefits, the advantages of helping within the natal nest or group tend to be great as long as such behaviour is directed towards kin. These ideas were encapsulated in 'Kin Selection Theory' (see below).

1.2.6.1.1 Kin Selection Theory

Haldane (1953) used the idea of 'kin selection', initially put forward by Fisher (1930), to explain the evolution of altruistic behaviour. Maynard Smith encapsulated these ideas in the Kin Selection Theory (Maynard-Smith 1964). In converse to the idea of helping for the good of the group, these theories proposed that there was a selfish element to cooperation. They proposed that the decision to help was based upon degrees of relatedness shared between donor and recipient individuals. Kin selection has now been found to play a profound role in predicting helping behaviour (Emlen 1982a; Brown 1987; Russell & Hatchwell 2001; Clutton-Brock 2002; Griffin & West 2003; Gilchrist 2004; Komdeur *et al.* 2004)

Figure 1-6 A nest of *L. flavolineata*. Individuals at the front of the nest are assuming a defensive posture whilst individuals at the sides provision brood.

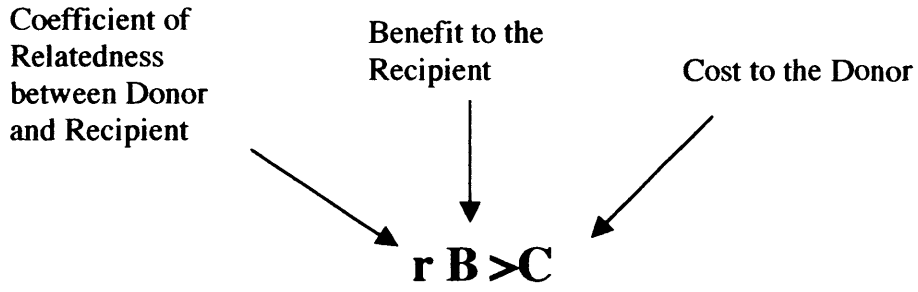


1.2.6.1.2 Hamilton's Rule

Kin Selection theory was expressed most clearly by Hamilton (1964a, 1964b) who created a rule by which one could predict the level of altruism that might occur between individuals. Hamilton's Rule is able to predict whether an allele that promotes an altruistic act will spread throughout the population.

Equation 1-1 Conditions for altruism to be favoured (Hamilton 1964a)

Helping is favoured if:



r corresponds to the coefficient of relatedness between the donor and recipient individual and therefore the 'helping' allele will spread only if the product of the coefficient of relatedness and benefit to the recipient is greater than the cost to the donor in providing the altruistic act. The use of Hamilton's Rule is limited in that it operates under a number of assumptions, as detailed below:

1) Random mating
2) Costs and benefits combine additively to determine the fitness of a given genotype.
3) Weak selection, all the genotypes have the same chance of selection.
4) Gene frequency is the same among potential donors and potential recipients.
5) Genetic relatedness is the only cause of genetic similarity.

(Williams 1966; Maynard Smith 1976; Dawkins 1976; Krebs & Davies 1993).

1.2.6.1.3 Direct and Indirect Fitness

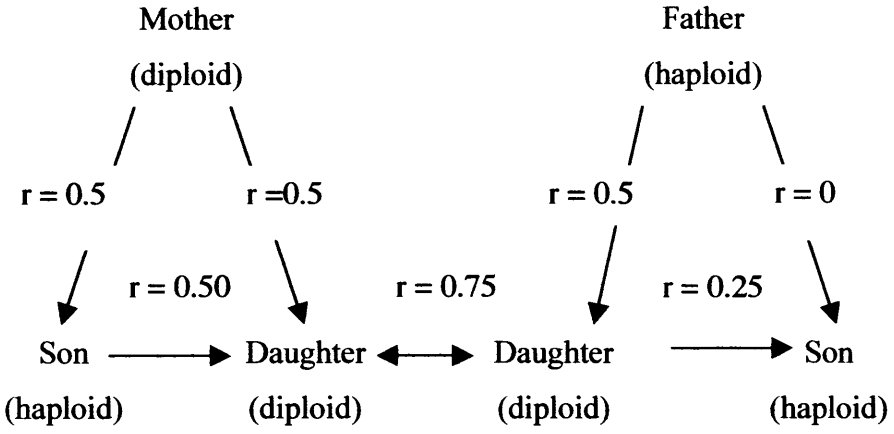
Brown (1980) proposed the terms "direct" and "indirect" fitness to explain the differences between the fitness gained through direct reproduction and through helping kin. Direct fitness could be increased through personal reproduction, whereas a donor individual was helping to raise her indirect fitness through helping a related individual.

1.2.6.1.4 Haplodiploidy and Indirect Fitness within the Hymenoptera

The Hymenoptera have a unique genetic incentive to stay upon the nest because of haplodiploidy. In haplodiploidy, males develop from unfertilised eggs and are haploid and females develop from fertilised eggs and thus are diploid. This situation means that daughters of the same male all inherit an identical set of genes from him. The other half of their genes come from their mother, who is diploid, and therefore daughters will share half of their maternally inherited genes with each other. Because half of the daughter's genomes will always be identical, this provides a situation where daughters are more related to each other ($r = 0.75$) than they are to their own offspring ($r = 0.5$; see Figure 1-7). For that reason, it was once thought that, females may gain greater fitness benefits through helping to rear their sisters than through producing their own offspring (Hamilton 1964a, 1964b).

It has become clear that haplodiploidy cannot provide a large enough incentive for helping on its own. If the sibs reared were solely female, haplodiploidy may provide such an incentive, however, males are also produced. Sisters are related to their brothers by 0.25, thus offsetting the genetic benefits of rearing sisters, to whom they are related by 0.75. Indeed, many social systems have been found to contain a much lower degree of intragroup relatedness than might have been expected through Hamilton's rule and haplodiploidy (Rissing *et al.* 1989; Hughes *et al.* 1993; Kukuk & Sage 1994; Strassmann *et al.* 1995; Danforth *et al.* 1996; Goodisman & Ross 1997; Queller *et al.* 2000). Consequently, there must be other factors that contribute to staying and helping upon the nest.

Figure 1-7 Haplodiploidy and resulting coefficients of relatedness



In summary, there are two main genetic benefits to living within a group:

1. Increased chance of survival or direct reproduction immediately or in the future (a direct benefit):
2. Enhanced production of non-descendent kin (an indirect benefit) (Moehlman 1979; Taborsky 1984; Woolfenden and Fitzpatrick 1984).

Interestingly, many studies do not show a positive relationship between the number of helpers and production of young (Magrath & Yezerinac 1997). Only some studies provide evidence that helper contribution leads to increased productivity (Brown 1982; Mumme 1992; Komdeur 1994) rather than territory or breeder quality.

1.2.6.2 Non-Genetic Benefits of Helping

Additional benefits to helping have been suggested that may promote cooperation within groups. For example, helping to feed offspring and interacting with fellow nest/group members must boost the experience of an individual, which could then be useful should this individual reproduce itself (Komdeur 1996; Heinsohn *et al.* 1988). Helping can also help to boost group size so that if a helper actually does inherit, she will have more helpers. This idea was termed "group augmentation" by Kokko *et al.* (2001). Others have suggested that some individuals have no choice but to help as this acts as a form of rent for which they are allowed to stay upon the nest (Mulder & Langmore 1993). This is particularly important if there is a chance of inheriting a breeding position (see Section 1.2.5.5). Additionally, some Florida Scrub Jay males help to expand their parents' breeding territory so that some young males are able to form their own small breeding groups at the edge of this territory (Woolfenden & Fitzpatrick 1978).

1.2.7 Costs of Helping

There are two main costs to an individual of helping within a group:

1. Loss of condition or weight
2. Heightened risk of injury or predation

(Reeve 1991; O'donnell & Jeanne 1992; Clutton-Brock *et al.* 1999; Nielsen 2001)

These costs are reflected in the fact that many cooperatively breeding organisms 'prefer' to help more closely related individuals to themselves e.g. Seychelles warblers (*Acrocephalus sechellensis*) (Komdeur 1992). There must therefore be a trade-off between the costs of helping and the benefits of helping kin (See Section 1.2.6.1). The cooperatively breeding cichlid fish *Lamprologus brichardi* illustrates the physiological costs of helping. In this cooperatively breeding fish helpers grow more slowly than nonterritorial fish (Taborsky 1984). Helpers of the White-winged chough (*Corcorax*

melanorhamphos) also lose weight in proportion to the time that they spend incubating upon the nest (Heinsohn & Cockburn 1994). In cooperatively breeding meerkats (*Suricata suricatta*) nonbreeding adults baby-sit young pups frequently which means foregoing foraging. The average babysitter loses 1.3% of its body weight compared with foragers that gain 1.9% of their body weight. It even seems that helpers in smaller groups perform a larger share of babysitting and are prepared to bear the greater costs involved (Clutton-Brock *et al.* 1999).

If helping does carry a cost for subordinate individuals this suggests that there may be a trade off between an individuals' investments in help, gaining indirect fitness benefits, and their own future direct reproductive success through inheritance (see Cant & Field 2001 and Chapter 4 for an in-depth discussion).

1.2.8 Rank and Helping Effort

Helping effort within a group is rarely the same for all individuals, yet there have been few studies investigating why this may be the case. One reason, which was initially suggested, was that genetic relatedness between subordinates and the dominant would affect the amount of effort put into helping. Individuals closely related to the dominant would be more closely related to her brood, and might therefore be expected to help more. However, studies of kinship and foraging effort have been mixed in their results, with some producing a positive correlation between kinship and effort and others failing to produce such a relationship (Clutton-Brock *et al.* 1999, 2000) .

Researchers then went on to suggest that helping effort might not be influenced by relatedness but by the actual costs of helping. If the costs of helping differ between individuals, and perhaps ranks, this may affect helping effort. Recent work by Cant and Field (2001) indicates that such variation in effort may be caused by differences between individuals in future fitness costs depending upon their rank. These findings are discussed in greater depth in Chapter 5: Rank and Foraging Effort.

1.2.9 Unrelated Helpers

It is clear that not all cooperative groups are composed of related individuals (for examples see (Bernasconi & Strassmann 1999; Queller *et al.* 2000). This appears to contradict kin selection theory (see Section 1.2.6.1.1); however studies have shown that helping behaviours in such groups are far from selfless. Two motives have been suggested for unrelated individuals to help upon a nest:

1. **Pay to stay**

If an unrelated helper has any chance of inheriting a dominant, breeding, position, helping can be seen as payment for permission to stay on that breeding territory. It has been shown that unrelated Dwarf Mongoose helpers can eventually inherit a breeding position, therefore helping in the group may arise through a 'pay-to-stay' tactic (Rood 1978, 1990). Such a tactic for remaining upon the nest has also been speculated to explain the helping behaviour of unrelated members within *P. dominulus* nests (Queller *et al.* 2000)

2. **Group Maintenance**

By helping upon the nest, an unrelated individual is helping to keep the group and its territory intact. Such behaviour will benefit the individual, if it inherits dominance, as this will also provide a ready-made group with helpers to rear its own brood. Any such helping behaviour which boosts the number of helpers available to the unrelated individual once it achieves dominance was named group augmentation by Kokko *et al.* (2001). The advantages and disadvantages of allowing an unrelated individual into a group were illustrated by a study on the Pied Kingfisher (Reyer 1980). Primary helpers in this species are related so their interests in helping are clear. However, secondary helpers are unrelated. Their help is accepted only when their help is effective at increasing the breeder's success. The disadvantages of unrelatedness are not confined to the unrelated helper. Whilst she gains no fitness returns from rearing the unrelated brood of the

breeder, in turn, that brood gain no fitness returns when they develop into adult helpers and help the unrelated breeder. Clearly there are numerous trade offs in allowing unrelated individuals to help.

1.2.10 Nest mate Recognition

Kin Selection Theory makes a large assumption that individuals can recognise their own kin (see Section 1.2.6.1.1). A number of mechanisms have been proposed, by which such recognition could take place. A simple idea put forward by Lorenz proposed that individuals recognise those they grow up with as kin. This behaviour was termed 'imprinting'. Evidence for such behaviour was put forward by Holmes and Sherman (1982) whose experiments determined that ground-squirrels rarely fight against individuals that they are brought up with regardless of whether they are related. However, they tended to act aggressively to sibs, which had been brought up separately from themselves. This idea, however, seems particularly susceptible to deceptive behaviour by unrelated individuals.

There has been considerable evidence of nest mate recognition by chemical means in insects (Cervo *et al.* 1996, 1996b). Such investigations have culminated in the discovery of non-volatile, long-chain hydrocarbons on a wasp's cuticle which are used in nestmate-recognition (Singer *et al.* 1998; Zanetti *et al.* 2001; Cervo *et al.* 2002; Destri *et al.* 2002).

1.2.11 Social Status Recognition

The evidence for nest mate-recognition through cuticular pheromones is convincing yet a similarly important question remains largely unanswered. Facultatively eusocial insects, and some cooperatively breeding vertebrates, rely upon recognising each other's rank. For example *L. flavolineata* and *Polistes dominulus* rely on rank recognition in order to form a structured dominance hierarchy, immune to cheating (Wyatt 2003). Therefore, there must also be a reliable cue by which many social insects can recognise social status. A correlation has been found between cuticular differences and reproductive status within *P. dominulus*, as the cuticular chemical profile of queens differs from those of workers in the percentage of certain alkanes and monomethylalkanes (Bonavita-Cougourdan *et al.* 1991). This is probably due to ovarian condition as the queen's offspring that had similarly developed ovaries matched the epicuticular profile of their mother (Dapporto *et al.* 2004). Similarly, differences in the chemical profile of *P. dominulus* foundresses have been found to correspond to hierarchical status at the end of the worker pre-emergence period (Sledge *et al.* 2001). However, cuticular compounds have recently been found not to be a general rank indicator in *P. dominulus* (Dapporto *et al.* 2004). Tibbetts and Dale (2004) have found that the facial markings of *P. dominulus* are used for rank recognition, perhaps in addition to epicuticular chemical profiles. Tibbetts (2002) has also shown that *Polistes fuscatus* females use facial and abdominal markings to recognise each other. As yet, however, the method of social status recognition in *L. flavolineata* is unknown in spite of recent investigations (Cervo *et al.* 1996, 2002).The facial markings of *L. flavolineata* are not as varied as those seen in *P. dominulus* and might therefore be an unlikely candidate for status recognition. Within *L. flavolineata*, however, rank correlates with age (see Chapter 4). Thus, the cuticular hydrocarbons of this wasp may change over time in order to provide a reasonably reliable indicator of their status. Such a system warrants further investigation.

1.3 Strategies for Achieving Dominance in Cooperatively Breeding Societies and Facultatively Eusocial Groups

The methods by which dominance is determined within cooperatively breeding societies and facultatively eusocial groups vary widely from direct interactions such as fighting to non-confrontational conventions. Within a cooperatively breeding vertebrate society, a separate hierarchy usually exists for both sexes in order to determine dominance and access to mating opportunities. A different situation usually exists for facultatively eusocial groups, in which the female members of the group predominantly vie for dominance: females carry out all of the foraging effort and brood caring responsibilities upon the nest. Each of the different mechanisms that have so far been discovered as the basis of dominance are detailed below.

1.3.1 Direct fighting

In some cooperatively breeding animals dominance and subsequent subordinate ranks are established purely through fighting ability. This is usually true only of male members of the group (Goessmann *et al.* 2000). These aggressive interactions are often costly so that dominance hierarchies are often established in order to minimise the frequency of such interactions (Clutton-Brock *et al.* 1982).

1.3.2 Ritualised fighting

Fighting is often costly to both competitors and the group as a whole if productivity is affected. Therefore, some animals have phenotypic characters to 'advertise' their fitness, such as colour or song. Such characters are likely to be strongly linked to an individual's physical condition rather than be genetically determined. In this way, fighting can be avoided, as the likely winner is determined beforehand through the comparison of characters. Such phenotypic characters are commonly named 'badges of status' as they

signal an individual's size or dominance. Badges of status are likely to be important between unfamiliar individuals in order for them to quickly assess their opponents fighting ability and may therefore be of greater use when unrelated, unfamiliar individuals fight for dominance, within a newly formed group, or for access to other resources (Strassmann 2004). One problem which arises with such a strategy is when individuals have a similar phenotypic quality, which may lead to escalated fighting as both individuals exhibit similar visual cues and expend energy in trying to achieve dominance (Bernstein 1981).

1.3.3 Conventions

A convention is a practice which is widely observed in a group in order to facilitate social interaction and ensure group stability. This method of acquiring dominance is purely based upon possessing an arbitrary attribute, unique to one particular individual, which ensures that the individual becomes dominant. Such an attribute is not based upon fighting ability or genetic quality but merely singles out one individual to become the sole reproducer. One example of this may be the attribute of being the oldest upon the nest. In this case a queue is effectively formed as each individual accedes to dominance according to its order of birth.

1.3.3.1 Gerontocracy

The convention of gerontocracy is otherwise known as age-based queuing (for dominance) or, to be more specific, dominance based upon seniority within the group. This term was first used by Strassmann and Meyer (1983) in their description of queen replacement within *Polistes exclamans*. There has been some evidence for such a convention within a few primitively eusocial insects such as *L. flavolineata* (Shreeves & Field 2002), yet it has not been investigated in any great detail until now (see Chapter 4). In this system an individual can inherit dominance through surviving to be the oldest individual upon the nest. Of course, such a method of achieving direct fitness is only

viable for subordinates in small groups due to the necessity of having to out-live older nest members (Kokko & Johnstone 1999; Ragsdale 1999; Shreeves & Field 2002).

In many social vertebrates one might expect that age correlates with strength and/or experience. However, the degree to which adult insects can grow is limited by their exoskeleton and there has been little evidence of greater breeding success due to increased experience. It would therefore appear that gerontocracy is purely a convention for establishing dominance and that seniority confers no particular advantage in itself. Reasons as to why such a convention should be used within some eusocial societies are largely unknown. Field & Cant (In Prep.) are currently investigating this problem by comparing the outcomes of a queuing convention with those of a random system in which individuals scramble to inherit the breeding position. They conclude that if a random system were to exist it would provide a disincentive for helping behaviour to take place. In an age-based system, in which older individuals inherit dominance, any brood that subordinates help to rear will join the bottom of the queue below the helping subordinate. This is not the case in a random system, where subordinates would effectively be rearing offspring that could compete directly with themselves for the dominant position. This is one of the most convincing arguments to date for the stability of gerontocracy within cooperatively breeding systems.

1.3.4 Social Queues for Dominance

If there is a high enough likelihood that an individual will eventually inherit a dominant position, this can provide a large incentive for staying upon the natal nest (see Section 1.2.6.2). Not only can an individual increase its indirect fitness through helping to rear kin, but it can also augment group size so that it may inherit a larger group to increase its direct fitness (see Section 1.2.6.2 and Kokko *et al.* 2001).

1.3.4.1 Group Size and Queuing

If an individual is born into a large group, the chance that it can attain dominance is smaller than if she had been born into a small group (Field *et al.* 1999). There must therefore be some benefits of a large group size that offset the decreased chance of achieving dominance. Reeve (1991) suggested that a larger group and therefore a larger number of helpers ensure that either more brood can be reared or the lifespan of the dominant is increased as she partakes in fewer risky foraging activities. With regard to *L. flavolineata*, Shreeves & Field (2002) suggested larger groups have two main advantages for dominants i.e. an increased reproductive output and insurance-based advantages (see Section 1.2.5.3.1)

1.3.5 Cheating

The advantages of becoming the dominant within a group are great, as the individual then acquires direct reproduction and, in the case of *L. flavolineata*, rarely has to leave the nest so avoids exposure to predation. In some cases, this may lead to cheating behaviour by subordinates, who may try to achieve dominance before higher-ranking individuals, or perhaps cheat in some other way such as exerting very little helping effort while they queue. Cheating commonly takes two forms i.e. signal or behavioural cheating (see Section 1.3.5.1 and 1.3.5.4).

Few social strategies are immune from cheating and therefore they cannot be considered as an Evolutionary Stable Strategy (ESS) i.e. a strategy that cannot be beaten by any other strategy. Therefore cheating may exist as part of a mixed ESS in some cooperatively breeding organisms. Cheating may be prevented from spreading throughout a population by the deleterious effects that this may have upon the group in terms of production i.e. through frequency dependent selection. Yet the population may be able to sustain a few cheating individuals i.e. those who have adopted a different strategy from the norm.

1.3.5.1 Signal Cheating

As discussed earlier, dominance is often established through the possession of phenotypic characters that advertise an individual's greater fitness through comparison with its competitors. This is seen in birds such as the Harris sparrow (*Zonotrichia querula*) in which dominance is established by possessing the darkest plumage (Rohwer & Rohwer 1988).

Animals usually perform ritualised aggression or fighting at an intensity that signals their resource-holding potential e.g. toads, red deer and antlered flies (see Halperin *et al.* 1998). Zahavi (1979, 1987) proposed that the evolutionary stability of such signalling lay in the fact that all such signals must be costly and would handicap their signaller. If the signaller was not sufficiently strong it could not carry out the signalling effectively because of the cost involved. Grafen (1991) elaborated upon the idea by stating that a stable communicative system could only remain stable if cheating on signalling increased the handicap associated with that display. The greater the degree of cheating, the greater the degree of cost incurred by the individual. Recent work by Halperin *et al.* (1998); Strassmann (2004) and Tibbetts and Dale (2004) have further suggested that there may also be social costs attached to cheating e.g. punishment from fellow group members or an increase in contests for dominance. Tibbetts and Dale (2004) have shown that this may be the case within *P. dominulus* as higher-ranking females (detected by a more broken facial pattern) and "experimental cheats" experienced more aggression from the dominant.

To summarise, the costs that are involved to a 'cheating' individual may be twofold:

1. Physiological.

2. Social i.e. the cheat has to take part in more contests for dominance or their cheating is detected and punished.

1.3.5.2 'Natural' Cheating within groups

Examples of natural occurrences of cheating are scarce within the scientific literature. Most cases used to back the handicap hypotheses of Zahavi (1979, 1987) and Grafen (1991) have relied upon artificially inducing cheating within a group (see Section 1.3.5.3) The few cases of natural cheating that have been found have primarily focused upon usurping male ranking orders for access to mating opportunities, within primates (Alberts *et al.* 2003). Forms of Cheating within social insects have rarely been studied and have mainly focused upon 'cheating' such as opportunistic egg-laying by workers rather than trying to achieve dominance (Bourke & Chan 1999; Oldroyd *et al.* 2001; Suzuki 2003).

1.3.5.3 Induced Cheating through Signal Augmentation

Natural occurrences of cheating are very difficult to detect. This is because cheating events can often be missed by an observer, for example if a young individual "jumps" ahead in a gerontocratic queue, over of one of its older nestmates, the action may not be immediately obvious. Any aggression involved with such an act may be very short-lived and the precise ranks of each individual within the group may be unknown leaving the behaviour undetected. Additionally, monitoring the inheritance of dominance by each group member, in order to detect a cheating event, can often be impossible due to the slow turnover of dominants which exists within many groups.

Because natural occurrences of cheating are so difficult to detect, a number of experiments have been carried out in order to induce such behaviour. The simplest way of inducing cheating is to augment the signalling power of a subordinate.

1. Painting

The Harris sparrow, *Z. querula*, establishes dominance through bearing darker plumage. Experiments have shown that cheating can be induced by painting subordinates black and injecting them with testosterone. However both procedures must be carried out, as merely being darkly coloured does not induce the dominant behaviour that is also necessary to gain dominance. In turn, the mere injection of testosterone induces dominant behaviour, yet is largely ignored by other subordinates because of the lack of dark colouring (Rohwer & Rohwer 1988). However, the question remains that if cheating is possible, why doesn't it occur naturally? It has been suggested that if a bird was to increase its androgen levels the success it would gain would be short-lived as it would be exhausted beyond it's natural capabilities (Silverin 1980; Roskaft *et al.* 1986). This provides good support for the Zahavi-Grafen model of signal handicaps.

A similar experiment has recently been carried out upon the facultatively eusocial wasp *P. dominulus*. As mentioned previously (see Sections 1.2.11; 1.3.2 and 1.3.5.1) this wasp has highly variable face markings that have often been thought to provide a means of individual recognition. Tibbetts and Dale (2004) have gone even further to suggest that these markings also indicate rank. Spot number and the percentage of clypeus that is pigmented black were found to predict head-width, which is highly correlated to overall body size (Eickwort 1969). Crucially, independent of body size, spot number also predicts dominance.

P. dominulus forms foundress associations at the start of their nesting season. The dominant individual that will produce the brood upon the nest is determined through aggression at the start of nest foundation. Tibbetts and Dale (2004) simulated this by collecting individuals from sites over 5km apart and pairing them within an arena for behavioural observation. Each pairing was matched in body size, however the facial markings of one of the wasps was manipulated according to one of three treatments, 1) a sham control which was painted without altering their facial markings, 2) a negative cheat, in which markings were joined up to make the wasp appear of lower rank and 3) a positive cheat, in which markings were broken up to make the wasp appear of a higher rank. The treatment had no affect on which wasp was able to achieve dominance, yet it strongly affected the way in which a beta positive cheat was treated after dominance establishment. Beta positive cheats received six times more aggression from alphas than the controls. Interestingly, beta negative cheaters received twice the aggression of controls. Tibbetts and Dale (2004) suggested that the alpha was able to detect a mis-match in the beta's facial marking and physiological strength, which led to this accelerated aggression. This experiment provides good support for the social cost theory, as the cost of a cheating wasp was so high compared with their non-cheating counterparts

2. Behavioural Priming

Halperin *et al.* (1998) tried to induce cheating within Siamese fighting fish, *Betta splendens*, by increasing the aggression of one fighting partner through behavioural priming. However, these primed individuals usually lost their fights because they exhausted themselves through their hyper-aggressiveness. Their un-primed opponents adopted a "smart boxer" strategy through waiting for their opponent to tire. Halperin *et al.* (1998) claim that the cheaters paid the cost of aggression most heavily and that they therefore support the Zahavi-Grafen model (Zahavi 1987; Grafen 1991).

1.3.5.4 Behavioural Cheating

Some forms of cheating in order to achieve higher status take a behavioural form rather than faking physiological quality through signalling. An example of this can be seen in the White Winged Chough. Helpers on the nest of these birds are sometimes seen cheating through 'fake' feeding behaviour. In 30% of cases young helpers carry food to the nest and place it in the mouth of nestlings before consuming it themselves. Even if the helpers do not feed the young in this manner, they proceed to preen them ostentatiously, behaviour which is often seen as an effort to raise their status (Boland *et al.* 1997).

1.4 Objectives of this thesis

This thesis focuses primarily upon the mechanisms involved in the inheritance of dominance within *L. flavolineata* nests and the consequences of these mechanisms. Previous studies have indicated the possibility of an age-based queue for dominance within this species (Samuel 1987; Field *et al.* 1999), yet no studies have been undertaken to confirm this. The main aim of this thesis is to present an investigation into the convention of gerontocracy within *L. flavolineata* revealing the stability of such a strategy and the possibilities for cheating (i.e. queue jumping) to occur. In addition to this the genetic relatedness of *L. flavolineata* colonies is examined to see whether it correlates with foraging effort or inheritance. These relatedness estimates can also be compared with the previous conflicting investigations of Strassmann *et al.* (1989) and Sumner (1999). The final investigation in this thesis looks at the effect of rank upon costly behaviour, such as foraging effort. As an individual moves up the queue, increasing in rank and nearing the inheritance of dominance, how does this affect the wasp's reproductive strategy? When the prospect of direct fitness nears, how much foraging effort is an individual willing to risk for the sake of increasing its indirect fitness? In investigating all of these ideas I hope to elucidate some of the benefits and costs of the strategies used within *L. flavolineata* and hope they may be used to support some of the main theories regarding the evolution of eusociality to date.

Main Aims:

1) To establish the genetic structure of *L. flavolineata* colonies.

2) To establish the mechanism for achieving dominance within *L. flavolineata*.

3) To establish the effect of rank upon foraging effort within *L. flavolineata*.

2 General Methods

2.1 Field Studies

The investigations were carried out upon a population of *Liostenogaster flavolineata* distributed near Fraser's Hill, Pahang, Peninsular Malaysia (see Figure 2-1). Some of the nesting sites within this population have been used in previous studies including Strassmann *et al.* (1994); Field *et al.* (1999) and Sumner *et al.* (2002). The study presented here took place over two field seasons, 23/03/01 – 17/09/01 and 06/05/02 – 11/11/02.

2.1.1 The Study Sites

The study sites consisted of culverts located along the road from Fraser's Hill Gap to Raub. Such structures provide an ideal habitat for the Hover-wasps *L. flavolineata* and *Parischnogaster alternata,* which often nest in dense aggregations in these areas (Hansell *et al.* 1982; Turillazzi 1986; Samuel 1987; Cervo *et al.* 1996). These culverts are constructed to carry water run-off from highland areas under the road and as such provide a more than adequate supply of moisture and mud for nest building. These sites also provide suitable observation points for censusing as they are sheltered and each of the nests is in close proximity to the observer. The nature of *L. flavolineata's* nesting aggregations and open nest structures also lend themselves to behavioural observation.

Figure 2-1 Situation of the Study Sites, located just outside Fraser's Hill (Bukit Fraser). (Map taken from Global Insight 2003)

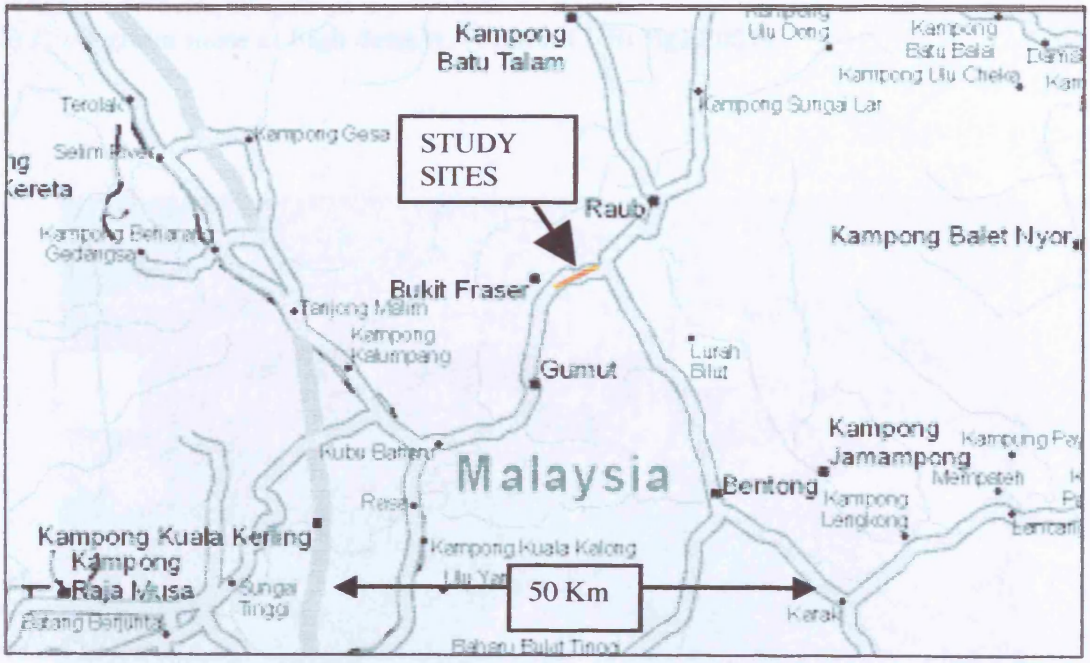
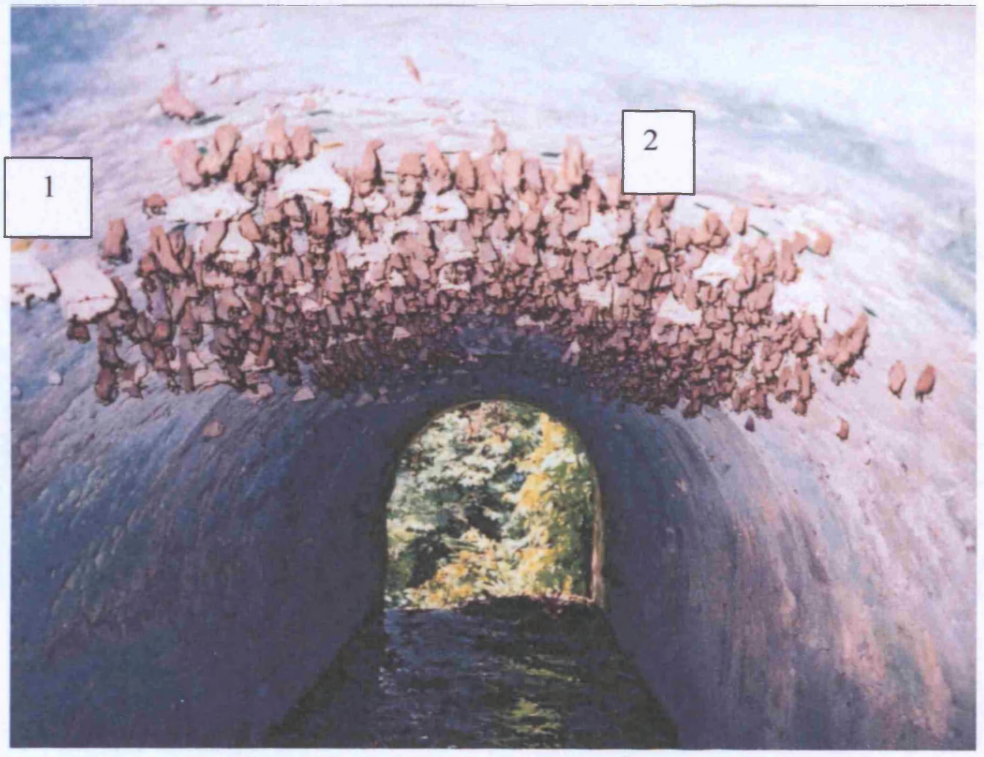


Figure 2-2 A culvert (Site 5) containing:

1) *L. flavolineata* nests

2) *P. alternata* nests at high density. (Photo: C. Bridge 2001)



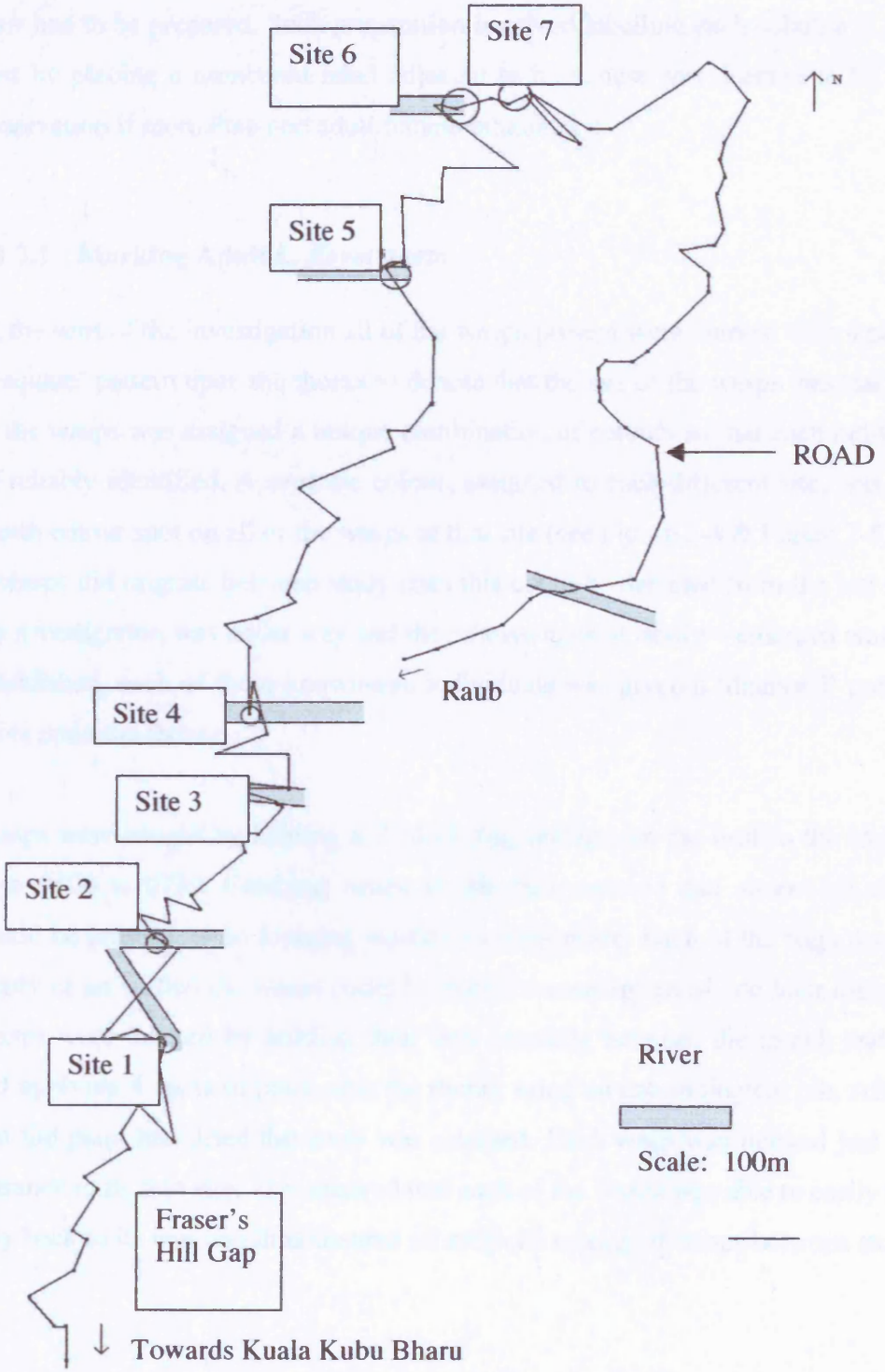
2.1.1.1 Site location

A map was constructed of the location of the study sites to calculate their proximity to one another. This map was constructed by driving along the route to the field sites and carefully noting each compass bearing with every turn in the road. The distance between each turn in the road was also noted. A map was then drawn to scale to reveal the proximity of each of the culverts in relation to each other (see Table 2.1 and Figure 2-1). Because the study sites are all quite close together and at a similar altitude, they were all exposed to similar environmental conditions.

Table 2.1 Direct distance between study sites (km)

		SITE							
		1A	2	3	4	5	6	7	8
SITE	1A								
	2	1.4							
	3	2.0	0.5						
	4	2.2	0.7	0.2					
	5	3.6	2.2	1.6	1.5				
	6	4.1	2.7	2.2	2.0	0.5			
	7	2.9	1.6	1.2	1.0	1.2	1.6		
	8	2.9	1.5	1.1	0.9	1.1	1.5	0.1	

Figure 2-3 Location of Study Sites in 2001; all sites are situated along the road from Fraser's Hill Gap to Raub.



2.1.2 Experimental Preparation

Before any experiments or behavioural observations could take place, each of the study sites had to be prepared. Such preparation involved labelling each suitable *L. flavolineata* nest by placing a numbered label adjacent to it. A nest was deemed to be suitable for observation if more than one adult female inhabited it.

2.1.2.1 Marking Adult *L. flavolineata*

At the start of the investigation all of the wasps present were marked with 4 paint spots in a 'square' pattern upon the thorax to denote that the age of the wasps was unknown. Each of the wasps was assigned a unique combination of colours so that each individual could be reliably identified. A separate colour, assigned to each different site, was used as the fourth colour spot on all of the wasps at that site (see Figure 2-4 & Figure 2-5). Therefore if wasps did migrate between study sites this could be detected from the last mark. Once the investigation was under way and the relative ages of newly – emerged wasps could be established, each of these known-age individuals was given a 'diamond' pattern of four spots upon the thorax.

Wasps were caught by holding a Ziplock bag underneath the nest in the early morning, from 0600 to 0730. Catching wasps at this time ensured that almost all of the wasps would be present, as no foraging would be taking place. Each of the bags was filled with plenty of air so that the wasps could be kept for a maximum of one hour during marking. Wasps were marked by holding their legs carefully between the thumb and forefinger, and applying 4 spots of paint onto the thorax using an entomological pin. After ensuring that the paint had dried the wasp was released. Each wasp was marked just outside the entrance to its nest site. This ensured that each of the wasps was able to easily navigate its way back to its nest and thus ensured no artificial mixing of wasps between study sites.

Wing length measurements were also taken in the field. This was performed by keeping the wasp in the bag before marking and gently holding the right fore wing against a white background. A pair of digital callipers was then used to measure wing length. Measurements were taken from the tegular at the wing base to the wing tip (to 2 d. p.).

Figure 2-4 Marks used to distinguish between known age and unknown age wasps.

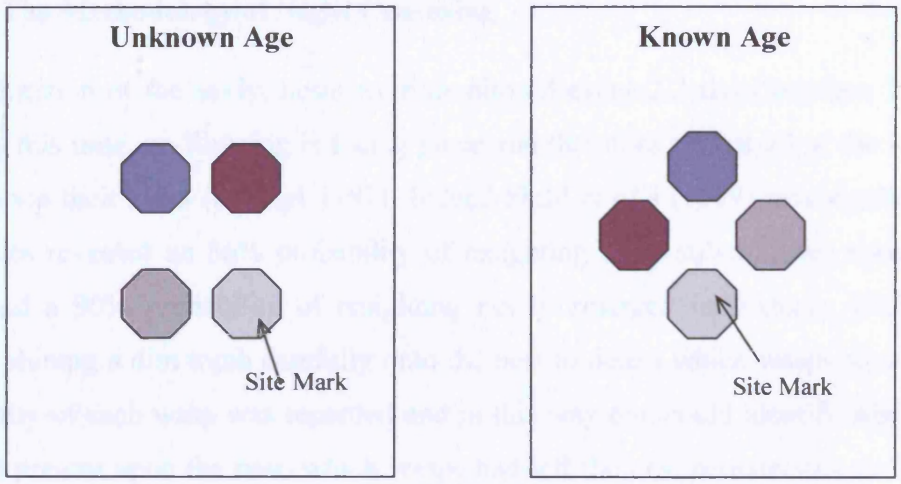


Figure 2-5 *Liostenogaster flavolineata* marked upon the thorax with 4 paint spots in a square mark to denote unknown age.

2.1.3 Nest Censusing

Two types of nest censusing took place, night censusing and day censusing.

2.1.3.1 The Methodology of Night Censusing

For the duration of the study, nests were monitored every 2-3 days between 21:00 and 23:00. At this time, no foraging is taking place and therefore almost all of the wasps are present upon their nests (Samuel 1987). Indeed Field *et al's* (1999) investigations upon this species revealed an 86% probability of resighting older subordinates upon a night census and a 90% probability of resighting newly emerged individuals. Each census involved shining a dim torch carefully onto the nest to detect which wasps were present. The identity of each wasp was recorded and in this way one could identify which wasps were still present upon the nest, which wasps had left the nest permanently and whether any wasps had recently hatched out.

A wasp was deemed to have left the nest permanently if it was not present on three consecutive night censuses. On rare occasions a wasp returned to the nest after being absent for a long period of time, indeed sometimes up to two months. However, most wasps were never seen upon their original nest again if absent for three night censuses. Therefore the method used here for assigning wasps as having left the nest is usually reliable.

Newly emerged wasps were marked as soon as possible i.e. one to two days after their emergence (see Section 2.1.2.1), so that another wasp hatching out did not confuse matters. If this occurred, the wasps could not be distinguished from each other and thus their ages were confounded. An emergence-to-marking interval of one to two days was needed because newly emerged wasps have to orientate themselves to the location of their natal nest. If the wasp was removed any earlier it may not be able to locate its own nest and nest-less "floaters" would be created (Field *et al.* 1999; pers. obs.).

2.1.3.2 The Purpose of Night Censusing:

1. At the start of the investigation, when all wasps were being marked, night censusing determined how many wasps were still to be marked upon each nest.

2. When the experiments were all underway, night censusing identified each of the individuals that was still present upon the nest. Day censusing cannot be used for this purpose, as many subordinate individuals are absent from the nest due to foraging.

3. As soon as all of the wasps were marked within a site and each of the nests were brood mapped (see Section 2.1.4), the investigations could begin. At this point night censusing helped to identify any newly emerged, and therefore unmarked, individuals upon the nest. Such individuals may be missed on day censuses if they begin to forage.

2.1.3.3 Day Censusing

Two types of day censusing took place, rapid nest censusing and intensive behavioural censusing

2.1.3.4 The Methodology of Rapid Nest Censusing

Rapid nest censusing took place between 07:00 and 12:30. This is the optimal foraging time for *L. flavolineata.* After this time period, subordinates tend to remain upon the nest and little or no solid food is brought back (Samuel 1987; Sumner 1999; pers obs.). Starting at 07:00, the identity of each of the individuals upon each nest was recorded. This census was repeated at half hour intervals. In this way, the identity of the dominant could usually be determined as she is almost always present upon the nest.

2.1.3.5 The Purpose of Rapid Nest Censusing

1. To establish the identity of the dominant upon each nest. The individual that is present upon its nest for the greatest proportion of time is invariably the dominant (Field and Foster 1999; Sumner 1999; pers obs.).

2. To determine the foraging effort of each individual.The foraging effort of each of the wasps can be measured according to the proportion of half hour time blocks that the wasp is absent from the nest. A census interval of 30 minutes is suitable as it allows time for each of the nests in a site to be censused. In addition, this time interval usually provides adequate time for the absence of an individual to be noted before its return. Each Rapid Nest Census was repeated over four consecutive days in order to build up a reliable estimate of foraging effort for each individual (the census was conducted on consecutive days to ensure that

environmental variance had minimal effect on foraging behaviour). If the identity of a dominant could not be established e.g. if two individuals were present upon a nest for an equal amount of time, an intensive behavioural census was needed.

2.1.3.6 The Methodology of Intensive Behavioural Censusing

Behavioural censusing took place between 07:00 until 12:30. During this time one to two nests were observed in order to record all instances of aggression and other forms of dominant behaviour. The census was repeated until the identity of the dominant was clear either through foraging effort or aggression.

2.1.3.7 The Purpose of Intensive Behavioural Censusing

1. To identify a dominant individual.

2.1.4 Brood mapping

Brood mapping was used to record the location of each different developmental stage of brood upon the nest. Brood maps were constructed by outlining each nest upon hexagonally lined paper. Once the outline was made, a strong torchlight was shone into each of the cells to identify each type of brood. This was then noted upon the brood map as:

- Egg or small larva (S),
- Medium Larva (L2),
- Large Larva i.e. a larva which fills the entire width of its cell (L3),
- Pupa (P)
- Hatched (H) - to denote a broken pupal cell from which a wasp has emerged.

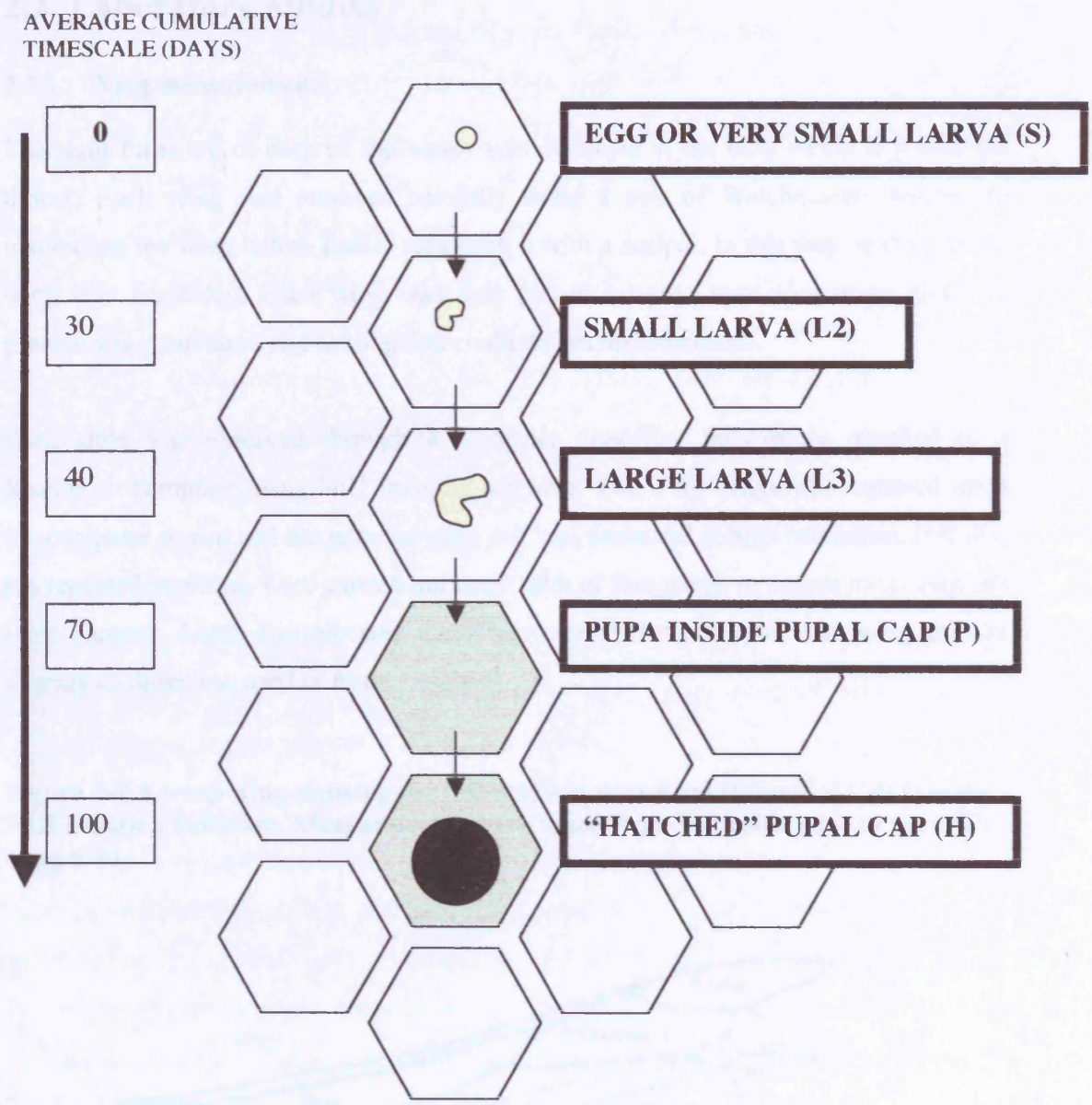
Such mapping was carried out every 13 days. This time period ensured that brood could be reliably tracked in their development and there was no chance that a wasp could develop and hatch upon the nest without being observed. It was particularly important to note the location of L3, as these would usually develop into pupae within 30 days (see Figure 2-6).

2.1.4.1 The Purpose of Brood Mapping

1. The pupae of *L. flavolineata* are enclosed by pupal caps therefore this can present a problem in distinguishing pupal cells from occasional cells that are filled only with mud. However, brood mapping can overcome this problem as it helps to identify mud-covered cells that previously contained larvae.

2. Brood mapping also helps to detect whether any adults have hatched out. Thus, whenever an unmarked wasp appears upon the nest, the brood map can be checked for any pupal caps that are missing. In this way one can clearly establish that an unmarked wasp is newly emerged and not an older wasp which has returned to the nest. When an unmarked female appeared upon the night census, the brood map was checked the following morning to minimise disturbance to the wasps at night.

Figure 2-6 Brood-Map illustrating stages of Brood Development



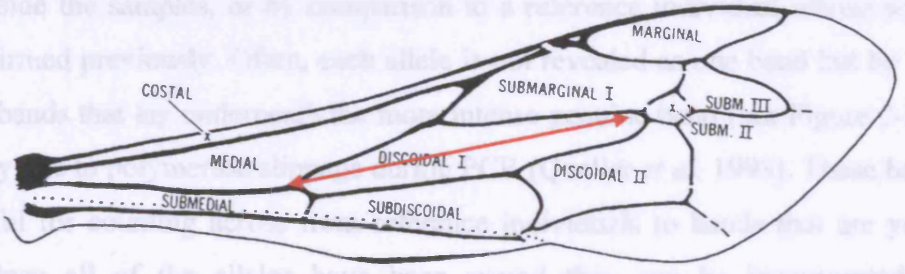
2.2 Laboratory Studies

2.2.1 Wing measurements

The right forewing of each of the wasps was dissected at the base where it joined the thorax. Each wing was removed carefully using a pair of Watchmakers forceps to manipulate the wing before finally removing it with a scalpel. In this way, tearing of the wing was prevented. Each wing was then placed between two microscope slides to prevent wing curvature and folding that could distort measurements.

Each slide was observed through a binocular dissecting microscope attached to a Macintosh computer using NIH Imaging software. The wing image was captured upon the computer screen and the relevant wing cell was measured at high resolution. Initially, ten repeated measures were carried out upon each of five wings to ensure measurements were accurate. Later, five repeated measures were carried out upon each wing and the average of these was used in further analysis.

Figure 2-7 A wasp wing showing the cell that was measured (Discoidal Cell I) using NIH Imaging Software. Measurements were taken from the inside edge of the cell's wing vein.



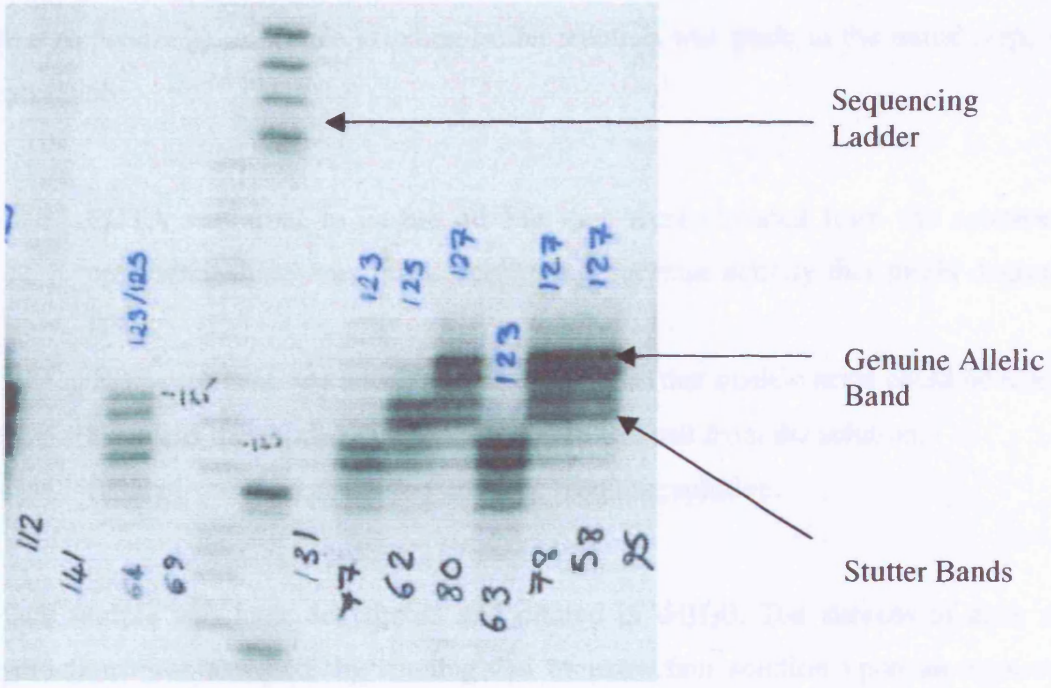
2.2.2 Microsatellite Analysis

Microsatellites are simple sequence loci that belong to a group known as variable number of tandem repeat (VNTR) loci (Nakamura *et al.* 1987). They consist of short tandemly repeated sequence motifs, which are up to 6 base pairs in length (Schlotterer 1998), and occur within non-coding sequences of DNA. Mutation rates for microsatellites are high with rates estimated at $10^{-2} - 10^{-5}$ per haploid genome per generation, a rate which is 2-3 orders faster than in protein allozymes (Baker 2000). This high rate of mutation is thought to be caused by polymerase slippage during DNA replication (Levinson & Gutman 1987). Such mutation rates are high enough to provide sufficient polymorphisms for resolution between populations and/or nests yet low enough to establish kinship within social groups (Schlotterer 1998).

Microsatellites often have multiple alleles that vary in the number of their tandem repeats (Choudhary *et al.* 1993). The allelic state of a microsatellite can be resolved by running it upon a polyacrylamide gel (e.g. Choudhary *et al.* 1993; Sumner 1999). Each allele travels a certain distance upon the gel according to its size i.e. number of repeats. Related individuals should share some of the same size alleles.

When using microsatellites, each sample should show either one or two bands of equal darkness. Each of these allelic bands can be scored by reference to a sequencing ladder, run alongside the samples, or by comparison to a reference individual whose score has been confirmed previously. Often, each allele is not revealed as one band but by a series of stutter bands that lay underneath the more intense genuine band (see Figure 2-8). This is probably due to polymerase slippage during PCR (Queller *et al.* 1993). These bands are often useful for counting across from reference individuals to bands that are yet to be scored. Once all of the alleles have been scored they can be incorporated into a relatedness estimator (see Section 3.1.2.1).

Figure 2-8 Genuine Allelic Bands and Stutter Bands upon a Polyacrylamide Gel



Six microsatellite markers were previously developed for genetic analysis in this species (Sumner 1999; Sumner & Field 2001). Of those developed, three were considered as most suitable for use as genetic markers in *L. flavolineata*. These were LF25, K18 and I3 (Sumner & Field 2001). The suitability and disadvantages of these markers will be discussed in turn.

2.2.3 DNA Extraction

A salt extraction protocol was followed in extracting DNA from the thorax of each wasp (see Appendix 2). A simple grinding buffer solution was made in the initial steps of the extraction:

- EDTA was used to ensure all Mg ions were chelated from the solution thus preventing these ions from accelerating nuclease activity that might degrade the DNA.
- SDS detergent was added to lyse the cells so that nucleic acids could be released.
- KAc was then added to remove the SDS and salt from the solution.
- Ethanol was then added to purify the resulting solution.

Each sample was later dehydrated and diluted in ddH_20. The success of each of the extractions was assessed, by running 2µl of extraction solution upon an agarose gel, before any further analysis took place.

2.2.4 PCR

The radioactive isotope ^{32}P was used as a label in the PCR mixture (For Protocol See Appendix 2). Each of the samples underwent PCR in which Thermoprime-Plus DNA Polymerase (ABGene - # 0301) was used in replicating the DNA template (see Appendix 2). The annealing temperatures of each of the primers had been previously optimised (Sumner 1999; Sumner & Field 2001), however a small increase in temperature was necessary to eliminate stutter bands in some instances. Tween-20 was used to increase the product yield.

2.2.5 Electrophoretic Analysis

Loading buffer was added to the resulting PCR mix to monitor its migration upon the polyacrylamide gel. This buffer contained EDTA to stop any enzymic reactions and therefore prevent any damage by nucleases. Each sample was heated for 3 minutes at 95° prior to loading upon the gel to ensure that DNA strands were separated. Formamide in the loading buffer also helped to ensure this.

The gel was connected to a BioRad rig and the bands were allowed to migrate to a desired distance according to the size of the alleles being sequenced and the degree of resolution required. The gel was then removed from the rig and dried for two hours to ensure that the strength of the radiolabelling was maximised (see Appendix 2). The gel was then juxtaposed to a sheet of Kodak Biomax MR-1 autoradiography film in a cassette. The cassette was placed at –80°C for exposure, the length of which depended upon the strength of the radioisotope.

The strength of the label could be increased with the addition of intensifying screens and thus the exposure time decreased. However, such screens tend to decrease the resolution of banding produced and were thus avoided if possible. The film was then developed using an automatic developer.

2.2.5.1 Gel Scoring

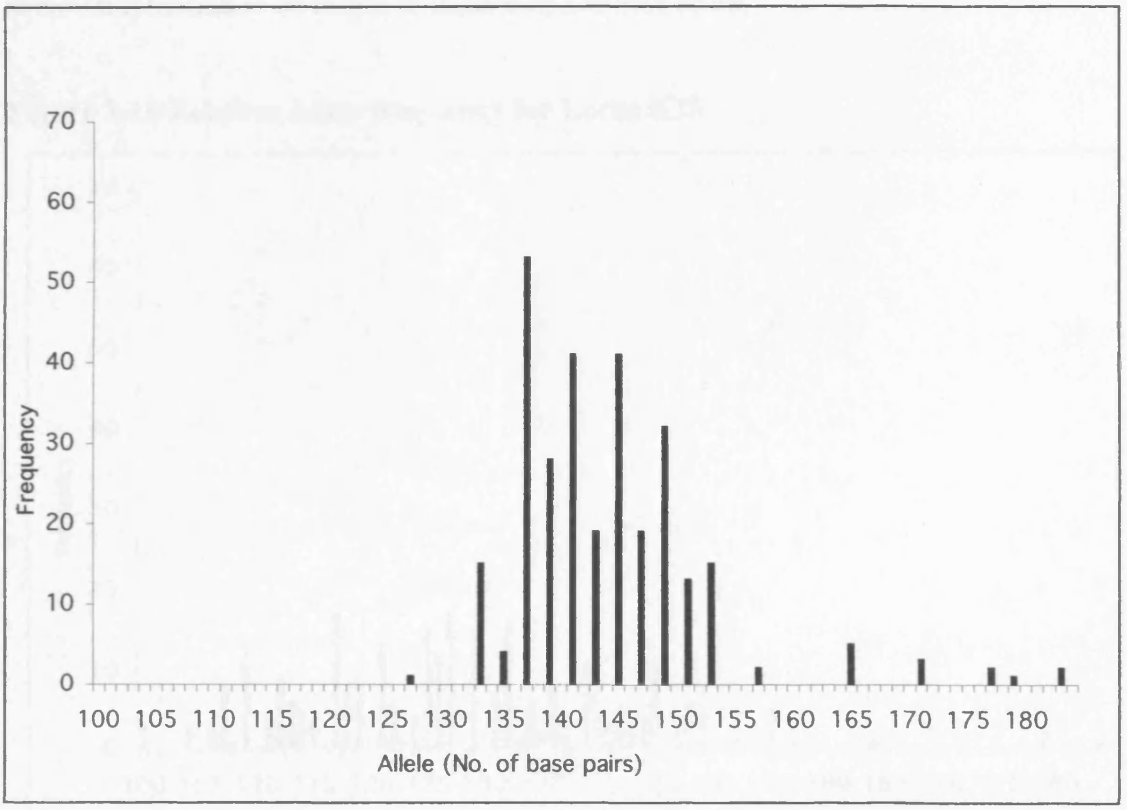
A sequencing ladder, consisting of M13mp18 ssDNA, was run alongside the samples in each of the gels to ensure accuracy in band scoring (see Appendix 2). This ladder was used as a standard to which each allelic band upon the gel could be compared (see Figure 2-8).

The three loci used in the investigations were all distinct in their particular banding patterns, therefore it was essential to familiarise oneself with each locus to ensure greater accuracy in band scoring.

2.2.5.1.1 Locus I3

I3 produced the easiest bands to score as alleles were two base pairs apart and the product range was generally small (alleles ranged in size from 133 to 183 base pairs, although sizes of 133 to 145 base pairs were typical; see Figure 2-9). There was very little variation in banding pattern, which normally consisted of a couplet with the upper band showing greater intensity. This upper band was the band that was scored.

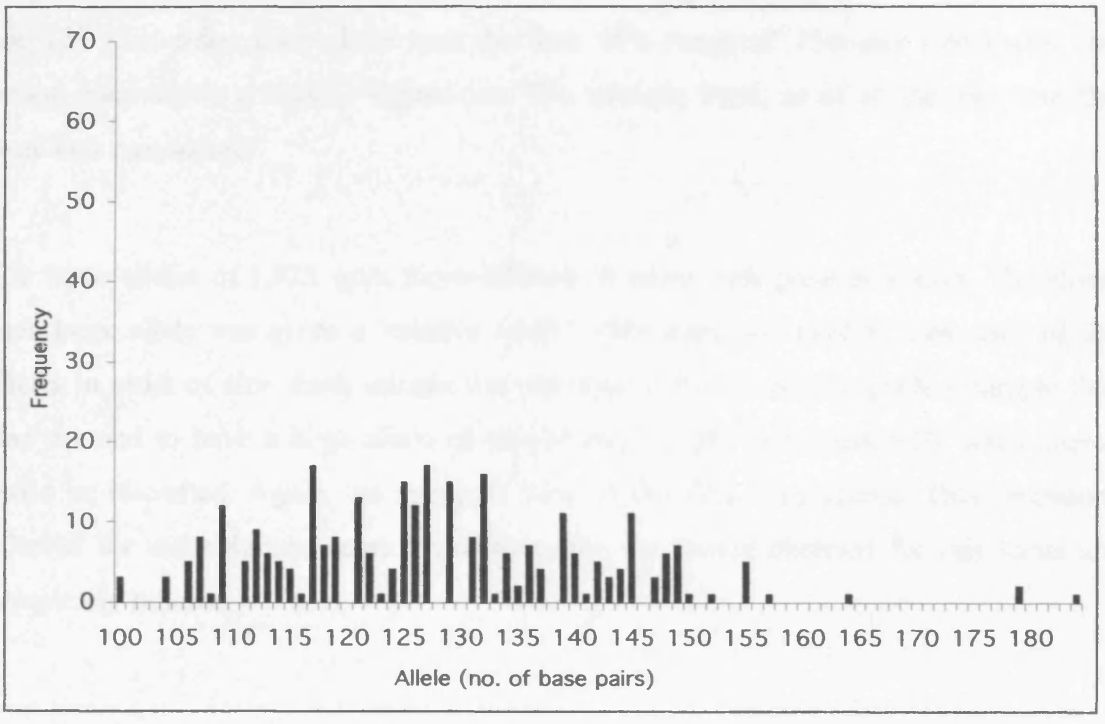
Figure 2-9 Relative Allele Frequency for Locus I3



2.2.5.1.2 Locus K18

K18 products ranged from 100 to 184 base pairs in size (see Figure 2-10). This is in contrast to Sumner (1999) who found the largest size K18 alleles to be only 142 base pairs in *L. flavolineata*. There were 47 alleles present among the 148 individuals analysed (29 nests/social groups), which makes this locus the most polymorphic of the three loci used. K18 was also found to be the most polymorphic in Sumner's (1999) data set. The bands produced by this locus were the most difficult to score due to the presence of some alleles differing by one base-pair in size only (Rubensztein *et al.*1995). The dinucleotide repeats seen at this locus also have the propensity to generate stutter bands, although these may sometimes aid the scoring process (see Chapter 3; Schlotterer 1998; Hauge & Litt 1993). An additional band that was sometimes found above the genuine allelic bands further confused the small difference in allele size. Schlotterer (1998) speculates that such banding was obtained through the terminal transferase activity of Taq DNA polymerase, which adds an A to the PCR product. Because of these problems in scoring, some samples had to be re-run to deduce an accurate score.

Figure 2-10 Relative Allele frequency for Locus K18



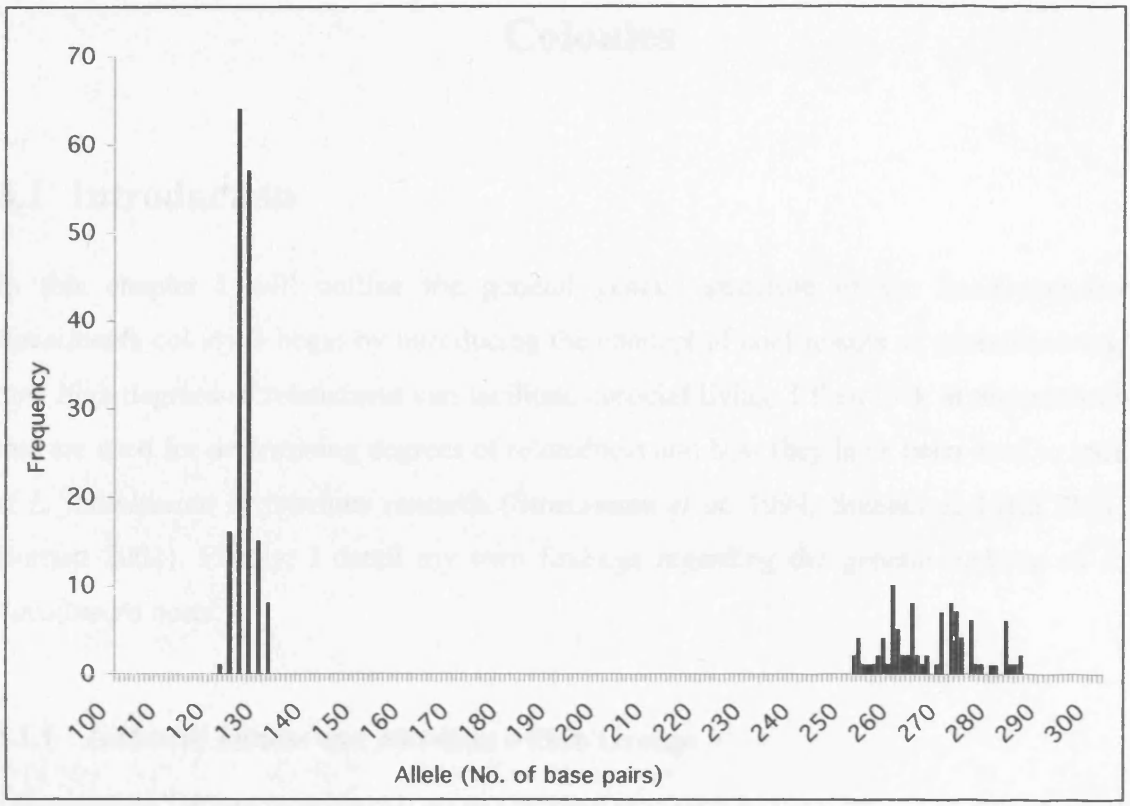
2.2.5.1.3 Locus LF25

LF25 products covered a very broad range of sizes, from 123 to 300 base pairs. Indeed, there is a strong bimodality in the allele frequencies (see Figure 2-11). A broad range of allele sizes was also found by Sumner (1999) at this locus. However, products in Sumner's study reached only 231 base pairs in size. Problems regarding biases in PCR amplification of this locus were experienced here and in Sumner's studies. There was a tendency for lower molecular weight alleles to amplify preferentially whilst larger alleles often failed to amplify at all. Therefore, each autoradiograph had to be checked carefully for very faint higher alleles and be left to expose for several days in order to score such higher alleles. Scoring difficulties arose from such extended exposure, especially if intensity plates were added to the autoradiograph, as this often obscured the banding pattern. Intensity screens were useful in decreasing exposure time for gels with simple banding patterns such as with locus I3, indeed they can increase banding intensity 10 to 14 fold but this does decrease resolution and is therefore of limited use for loci which produce alleles with one base pair differences or more complex banding patterns.

The small alleles of LF25 were relatively easy to score and ranged in size between 123 and 157 base pairs. Each allele took the form of a "couplet" (Sumner 1999) with one strong band above a slightly lighter one. The stronger band, as of all the loci, was the band that was scored.

The large alleles of LF25 were more difficult to score with great precision. Therefore, each large allele was given a 'relative score'. This score was used to rank each of the alleles in order of size. Each sample was run upon a PAGE gel alongside a sample that was deemed to have a large allele of similar size; in this way identically sized alleles could be identified. Again, the strongest band of the allele was scored. This procedure allowed for unambiguous scoring and therefore the results obtained for this locus are completely reliable.

Figure 2-11 Relative allele Frequency for Locus LF25



2.2.6 Statistics

Throughout this thesis statistics are presented in the following format: (Statistical test (significance level) (x – tailed test) (n1, n2))

3 The Genetic Structure of *Liostenogaster flavolineata* Colonies

3.1 Introduction

In this chapter I will outline the general genetic structure of the *Liostenogaster flavolineata* colony. I begin by introducing the concept of coefficients of relatedness and how high degrees of relatedness can facilitate eusocial living. I then look at the methods that are used for determining degrees of relatedness and how they have been used to look at *L. flavolineata* in previous research (Strassmann *et al.* 1994; Sumner & Field 2001; Hornett 2002). Finally, I detail my own findings regarding the genetic makeup of *L. flavolineata* nests.

3.1.1 Inclusive Fitness and Altruism within Groups

The presence of altruism within groups at first appeared to be converse to the ideas of natural selection. Classical mathematical models of natural selection do not allow for altruism within groups as this implies that some individuals help others to increase their fitness to the detriment of their own. W. D. Hamilton (1964a) was one of the first to realise the implications that coefficients of relatedness (r) could have upon offspring retention and helping behaviour within groups. He realised that helpers upon a nest gained fitness 'indirectly' through helping to rear their sibs (see Section 1.2.6.1).

3.1.2 Relatedness and its measurement

The methods I employed to determine genetic relatedness within colonies use microsatellites, as explained in Chapter 2: General Methods (see Section 2.2.2). Relatedness can be quantified as the expected proportion of alleles that are shared

between the genomes of the individuals in question. For diploid siblings, r = 0.5 and between haplodiploid siblings r = 0.75.

3.1.2.1 Relatedness Estimators

There are a number of relatedness estimators that have been produced in recent years. The program used most commonly in social insect studies has been that of Queller and Goodnight (1989). However, Casteele *et al.* (2001) compared four different estimators of pairwise relatedness to assess their reliability:

- Similarity index (Li *et al.* 1993)
- Regression-based estimator (see Section 3.1.2.3; Queller & Goodnight 1989)
- Correlation-based method-of-moments estimator (Ritland 1996)
- Regression-based method-of-moments estimator (Lynch & Ritland 1999)

These estimators showed a large sampling variance yet Queller and Goodnight's estimate proved to perform best for two bird species containing at least 50% of related pairs. Lynch and Ritland's estimate proved more successful when 60-70% of pairs were unrelated. They recommend the use of their estimator when loci are numerous or hypervariable, yet their estimate was outperformed by Queller and Goodnight even under such circumstances.

Casteele *et al* (2001) suggest that Lynch and Ritland's estimator may only be used if all of the loci have identical allele frequency distributions. The results presented here and previous genetic studies by Sumner (1999) have revealed this not to be the case within *L. flavolineata* populations. Due to the high relatedness levels found within haplodiploid nests, Queller and Goodnight's estimate would seem more useful as it proved more successful in looking at closely-related bird pairs.

3.1.2.2 Requirements for estimating relatedness

There are number of criteria which must be met in order to reliably estimate relatedness (Casteele *et al.* 2001):

1) Absence of null alleles.
2) Independent loci.
3) Random mating.
4) Limited levels of mutation.
5) Selectively neutral loci.
6) Accurate genotyping.
7) Known population allele frequencies.

If requirements 1 - 6 are not met this will lead to a deviation from Hardy Weinberg Equilibrium (HWE). This can be investigated using the software programme *Genepop* 3.1.d (Raymond & Rousset 1995).

1 **Null alleles** may result in an overall heterozygote deficiency within the data set as they give heterozygote genotypes the appearance of homozygous genotypes. This is because only one band will be shown upon the autoradiograph if a null allele is present (Chakraborty *et al.*1994; Pemberton *et al.*1995). These null alleles can arise due to a mutation, within the primer-binding site, which prevents the primers used from binding to them (Callen *et al.* 1993). Methods used for detecting the presence of null alleles use tests for heterozygote deficiency (Raymond & Rousset 1995; Rousset & Raymond 1995; Brookfield 1996) .

2 **Independent loci** can be tested for independence by examining the loci for 'linkage disequilibrium'. This was previously performed by Sumner (1999) who found that each of the loci used in this and previous analyses were independent of each other.

3 **Random mating** will be violated if inbreeding or assortative mating takes place. Other factors that might contravene random mating are geographic structuring, rare allele advantages and mating system effects such as one or a few males obtaining a disproportionate share of matings. The degree of inbreeding that occurs within a population can be measured by the *F* statistic (Wright 1951). The *F* coefficient is the difference between the HWE expectation and the observed number of heterozygotes (H), weighting this difference by the HWE expectation.

Equation 3-1 The F statistic for detection of inbreeding within a population (Wright 1951)

$$F = (H_{(HWE)} - H_{(observed)}) / H_{(HWE)}$$

4 **Limited levels of mutation.** Mutation is usually a weak force and rates of mutation are unlikely to vary between loci to any great extent. Mutation of genetic markers can be a problem when using minisatellites as they have very high mutation rates which will be picked up in any large study. Microsatellites provide a better alternative here as, although their variability is high, their mutation rates are usually below 10^{-4} (Schlotterer 1998). In addition, levels of homoplasy (i.e. allele size independent of common ancestry) are assumed to be minor within populations (Scribner & Pearce 2000). Convergence in allele size could elevate the probability of inferring false kinship within a population; nevertheless such homoplasy would not be expected for each locus and is therefore unlikely to bias any estimates of relatedness to a great degree.

5 **Accurate genotyping** may differ according to the loci that are used. For example, some allelic differences between individuals may only be one base-pair apart. In this case genotyping may be less accurate especially if residues from the PCR product obscure the banding pattern of an allele. The accuracy of genotyping may be improved by repeated analysis of each sample.

6 **Population allele frequencies** are determined through the analysis of all individuals from all study sites. If the sample size is large enough an accurate allele frequency may be determined for comparison with individual and intranidal allele frequencies.

3.1.2.3 Calculating Relatedness (using a regression-based estimator):

Equation 3-2 the Relatedness Calculation (Queller & Goodnight 1989)

$$r = \frac{\sum_x \sum_k \sum_l (P_y - P^*)}{\sum_x \sum_k \sum_l (P_x - P^*)}$$

Individuals to be analysed

x k l

Loci index

Allelic index
1 = haploid
2 = diploid

Px =the frequency of the allele at locus *k* and allelic position *l* within individual *x*. In a female stenogastrine i.e. a diploid individual this must be either 0.5 or 1.0.

P*y* = the frequency of the allele in *x*'s nestmates (or other such individuals for genetic comparison).

P* = Allele frequency in the entire population, with the exclusion of individual *x*'s likely relatives i.e. her nestmates. This factor is a bias correction to prevent genetically similar relatives from influencing the allele frequencies in *x*'s direction. This is particularly relevant if the sample size is small, as the contribution of a single set of relatives will heavily influence the resulting population relatedness values. For example, if a group of nestmates carry an allele that is particularly rare within the population, the inclusion of such members when calculating population allele frequencies will lead to an underestimate of individual *x*'s relatedness to her nestmates.

3.1.2.4 Standard Errors using *Relatedness* 5.0

Using the *Relatedness* 5.0 programme, the observed relatedness estimates compiled for each of the nests are compared to a null distribution generated by random permutations of data (Scribner & Pearce 2000). Relatedness uses the 'jackknife' resampling technique to assemble the null distribution and thereby calculate the standard error of each relatedness estimate. This technique is a parametric procedure, which assumes normality in the variable to be jackknifed. The statistic splits the data into groups and calculates relatedness by excluding one group of observations in each permutation. The average of the estimates is then taken to reduce any bias in the statistic. The variability among the estimates is used to calculate the standard error (Queller & Goodnight 1989). The group chosen for jackknifing depends upon the relatedness analysis to be performed:

- **Whole population relatedness.**

  The standard error of whole population relatedness can be calculated by jackknifing over loci or groups i.e. nests. In this case there are many more nests than loci and therefore jackknifing over nests is usually more informative.

- **Relatedness by nest.**
  The standard error of intranidal relatedness must be calculated by jackknifing over loci.

3.1.3 Kinship

The second programme used here in the analysis of relatedness within nests is *Kinship* (Goodnight & Queller 1999). *Kinship* uses likelihood methods to test hypothesised relationships among individuals. The programme works by coding each pedigree relationship in terms of the probability of the focal individuals sharing an allele identical by descent from the maternal or paternal line.

Kinship 1.2 (Queller & Goodnight 1989) was used to assign sibships within this study. In *Kinship,* a primary hypothesis must be put forward, for example that relatedness between individuals is 0.75. To do this, the probabilities under the primary hypothesis, that the individuals in question share an allele by direct descent from their father (rp) and mother (rm), must be entered. In the case of sisters, they inherit an identical set of genes from their father therefore rp =1. The mother's gametes undergo meiosis and therefore sisters have a 0.5 chance of sharing an identical allele from their mother so rm = 0.5.

A null hypothesis must also be entered with which to compare the primary hypothesis. Due to the limitations of my data set, such as the small number of loci used, *Kinship* is not able to discriminate between sisters and mother-daughter relationships with a sufficient statistical power and therefore a smaller degree of relatedness was used for the null hypothesis such as the aunt – niece relationship (r = 0.375). As the difference in relatedness between the primary and null hypotheses decreases, the chance of a type II error being committed increases. Therefore, the results here mainly use r = 0.75 as the primary hypothesis (seeTable 3.1).

Table 3.1 Primary and Null Hypothesis Pairings in *Kinship* and their effect upon the probability of committing a Type II error in my study.

Primary Hypothesis	Relatedness	Null Hypothesis	Relatedness	Type II error at p < 0.05 level
Mother – Daughter	0.50	Aunt - Niece	0.375	0.5235
Sister	0.75	Mother - Daughter	0.50	0.3188
Sisters	0.75	Aunt - Niece	0.375	0.0719
First Cousins	0.188	Sisters	0.75	0.028
Unrelated	0.00	Sisters	0.75	0.0029

Table 3.1 shows that *Kinship* cannot distinguish between sisters and mother-daughter relationships or mother-daughter and aunt-niece relationships with statistical power. However, *Kinship* can distinguish between sisters and aunt-niece relationships, first cousins and sister relationships and can distinguish between unrelated individuals and sister relationships, with high power.

Kinship then uses these relatedness values, the population allele frequencies from the data and the genotypes of the two individuals in question to identify whether the primary hypothesis is more plausible than the null hypothesis and with what statistical power.

If individuals within a nest are full sibs, they should agree with the following criteria:

a) Exhibit, at most, three alleles at each locus between them. One allele inherited from the father and two from the mother.

b) Share at least one common allele at each locus

c) Show relatedness values close to 0.75.

3.1.4 Previous Estimates of Relatedness in *L. flavolineata*

There have been two previous studies into the genetic composition of *L. flavolineata* colonies. The first of these was undertaken by Strassmann *et al.* (1994), using two closely situated study sites including the Gazebo Site, near the Genting Highlands, Peninsular Malaysia (see Figure 3-1). The Gazebo site was later used by Sumner (1999, *et al.* 2002) and lies approximately 63km from the sites studied in my investigation (see Figure 3-1). Relatedness in Strassmann's investigation was estimated from allozyme polymorphisms using six polymorphic loci.

3.1.4.1 Strassmann *et al.* (1994) Intranidal Relatedness Estimates

Strassmann *et al.* (1994) estimated relatedness between various classes of nestmates; (n = number of colonies in the analysis).

Results:

- Adult females from the same colony were related by 0.22 ($r = 0.22 \pm 0.1$, $n = 38$) a value which is significantly different from zero.

- Dominants were related to subordinates by 0.29 ($r = 0.29 \pm 0.07$, $n = 29$).

- Female pupae were related to each other by 0.79 ($r = 0.79 \pm 0.09$, $n = 5$) and were therefore probably sisters.

- Adult males were related to each other by 0.58 ($r = 0.58 \pm 0.15$, $n = 16$).

3.1.4.1.1 Movement between Nests (Joining)

The low relatedness values found between adult female nest mates was attributed to movement between nests. Strassmann *et al.* (1994) concluded that females must often join new nests containing nestmates to whom they are unrelated. Some of the observations of Samuel (1987) regarding joining behaviour in these wasps backed up this observation.

3.1.4.2 Sumner's (1999) Intranidal Relatedness Estimates

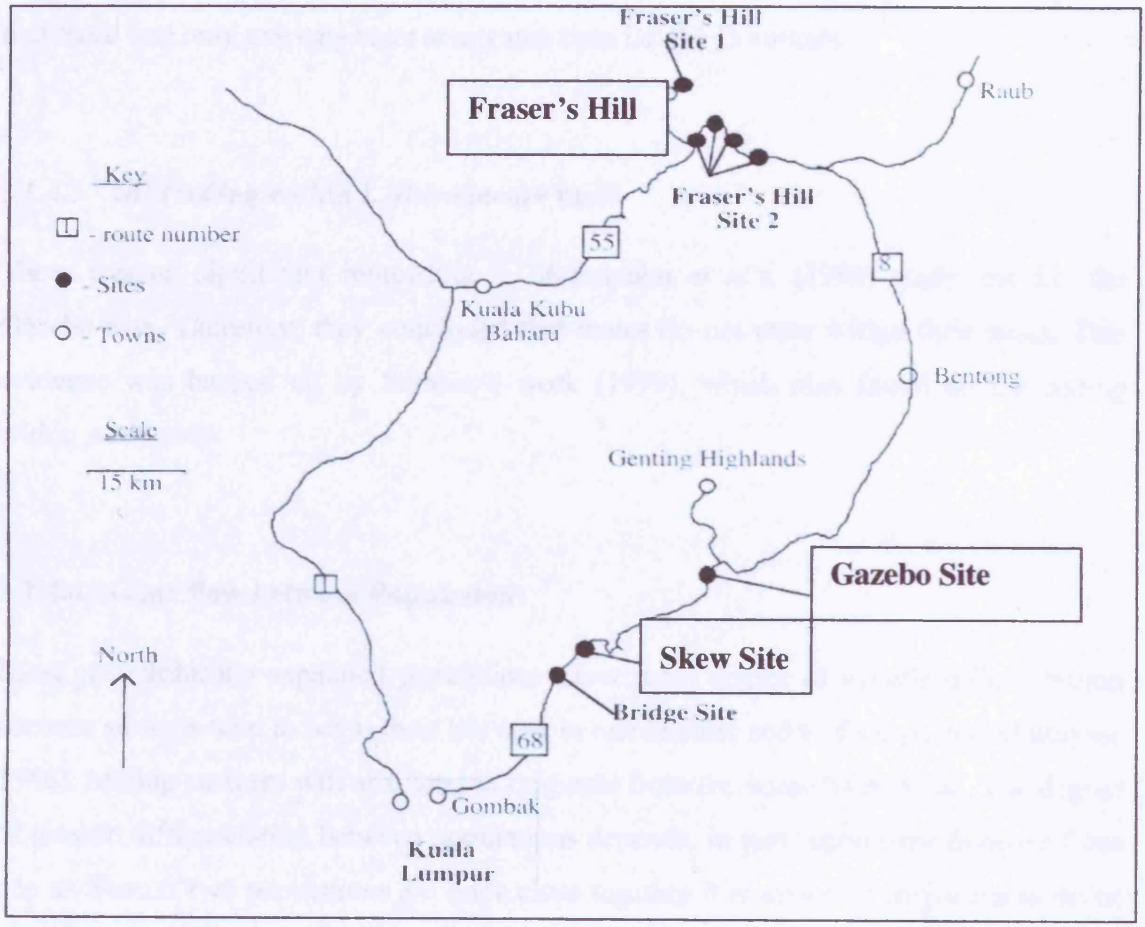
Strassmann's estimates of relatedness differ to those later found by Sumner (1999) who found intranidal relatedness to be much higher (see Table 3.2 for a summary of relatedness values). In Sumner's investigation polyacrylamide gel electrophoresis was used to determine allelic positions, using 3 polymorphic microsatellite loci. Sumner used samples taken from the Skew Site and Gazebo Site in 1995 and 1996 respectively (Figure 3-1).

Table 3.2 Comparison of Intranidal Relatedness Values Between Strassmann *et al.* (1994) and Sumner (1999).

Nest Member 1	Nest Member 2	Sumner (1999) Relatedness Values			Strassmann *et al.* (1994) Relatedness Values		
		r	Standard error	n (nests)	r	Standard error	n (nests)
Adult	Adult	0.49	0.073	40	0.22	0.1	38
Adult	Adult male	0.21	0.093	7	0.44	0.14	24
Adult male	Adult male	0.19	0.188	3	0.58	0.15	16
Dominant	Subordinat	0.40	0.060	10	0.29	0.07	29
Adult	Female	-	-	-	0.24	0.11	8
Female	Female	0.63	0.049	5	0.79	0.09	5

The reason for such a large difference in relatedness estimates between Sumner and Strassmann is difficult to identify. Sumner (1999) acknowledges that the difference in relatedness marker used between the two analyses is unlikely to have caused such a disparity. Allozyme markers are just as effective as microsatellites, the greatest disadvantage of allozymes being that they carry larger standard errors. This is because they display poorer resolution owing to their low level of variation (Seppa & Gertsch 1996; Sumner 1999). Microsatellites are more sensitive to changes in population breeding sizes and migration rate (Hughes & Queller 1993; Choudhary *et al.* 1993). The discrepancy is also unlikely to be caused by differences in site relatedness as Sumner and Strassmann both used the Gazebo site for their investigations and both found no population differentiation between their own respective study sites so it is unlikely that their populations were genetically isolated (see Section 3.1.4.4). Sumner concluded that the only event that may have caused the different relatedness estimate was a population change or the 5% error inherent when setting significance at $p < 0.05$ (Sumner 1999).

Figure 3-1 Map showing previously used study sites for investigating the genetic structure of *L. flavolineata* colonies. Sumner (1999), Sumner *et al.* (2002) and Strassmann *et al.* (1994) both studied the Gazebo Site population whereas the Skew Site population was studied by both Samuel (1987) and Sumner (1999, 2002). (Map taken from Sumner (1999).



3.1.4.2.1 Breeding Systems and Reproductive Skew within the *L. flavolineata* Nest

Sumner (1999) was also able to conclude that *L. flavolineata* females are singly mated. By amplifying DNA from dominant females and 2 to 5 eggs on each nest she found only a maximum of three alleles at each locus, two of which matched the dominant female. The relatedness amongst the female eggs collected ($r = 0.72 \pm 0.039$, $n = 13$) suggested that there was only one egg-layer at any one time i.e. the Dominant.

3.1.4.3 Inbreeding within *L. flavolineata* nests

There was no significant inbreeding in Strassmann *et al*'s. (1994) study site i.e. the Gazebo Site. Therefore, they concluded that males do not mate within their nests. This evidence was backed up by Sumner's work (1999), which also found no inbreeding within study sites

3.1.4.4 Gene flow between Populations

Most geographically separated populations show some degree of genetic differentiation because siblings tend to begin their life near to one another and to their parents (Futuyma 1986). Mating partners will also tend to originate from the immediate locale. The degree of genetic differentiation between populations depends, in part, upon their distance from one another. If two populations are quite close together it is easier for migration to occur between the two sites. If this takes place, gene flow between the populations will decrease their genetic differentiation. The investigations of Strassmann *et al.* (1994) and Sumner (1999, *et al.* 2002) have focused upon study sites that are situated near to one another, therefore it is likely that gene flow would have occurred between their study populations. The new investigation presented in this thesis focuses upon sites outside of Fraser Hill, which are approximately 63 km away from the Gazebo and Skew sites of

earlier investigations (Figure 3-1). Therefore there may be some genetic differentiation between the populations described here and those of Strassmann and Sumner.

3.1.5 Unexplored Factors that may affect Intranidal Relatedness within *L. flavolineata*.

Strassmann *et al.* (1994) and Sumner *et al.* (2002) both provided comprehensive studies of intranidal relatedness within their study populations. They also elucidated some of the factors that may influence intranidal relatedness such as joining events (Strassmann *et al.* 1994) and reproductive skew (Sumner 1999, *et al.* 2002). This thesis aims to present some additional factors that may influence intranidal relatedness (examples of these are detailed below).

3.1.5.1 Group Persistence and Intranidal Relatedness

The effect of group persistence upon relatedness in *L. flavolineata* nests has largely been neglected, yet it could be considerable. *L. flavolineata* lives in an aseasonal environment and therefore the persistence of a group (i.e. a group's lifespan) is not limited by temporal factors such as the end of a season. This may allow for a considerable overlap of generations as a number of individuals from each successive dominant may remain upon the nest. After several dominants have existed upon the nest, this may result in a complex mixture of sibships from different generations, which may have a profound effect upon the nest's overall relatedness. As a result of this, degrees of intranidal relatedness may vary with the lifespan of the group. For example, if a nest is newly established, degrees of relatedness may be very high (around $r = 0.75$). This is because the group may consist solely of a dominant and several of its offspring, which because *L. flavolineata* is singly mated, will share a high degree of relatedness. As time proceeds, this high intranidal relatedness may decline as aunts and cousins appear upon the nest. Providing some sisters remain upon the nest it is probable that degrees of intranidal relatedness would remain moderate (approximately $r = 0.5$).

3.1.5.2 Group Size and Intranidal Relatedness

If a single foundress newly establishes a nest, after about 100 days her daughters will emerge. At this point intranidal relatedness will be very high (approximately r = 0.75) regardless of how many daughters emerge. Therefore, in this case group size will have no effect upon intranidal relatedness. As time continues, some individuals from subsequent generations remain upon the nest and thus the addition of aunt and cousin relationships may decrease intranidal relatedness to some extent. The important point here is that nest persistence (as discussed above) is likely to have a greater impact upon relatedness than group size. As long as sibships are maintained upon the nest, this will counteract the effect of lower relatedness from more distant relatives. The effect of group size upon intragroup relatedness was recently investigated by Aviles *et al.* (2004). They found that the degrees of relatedness found within a group might sometimes be compromised in order to increase group size. However Aviles *et al*'s (2004) study mainly focused upon groups, which recruit unrelated individuals from the outside population in order to increase group size as quickly as possible. This kind of situation cannot be applied to *L. flavolineata* as their aseasonal environment allows for group size to be built up over time. Yet Shreeves and Field (2002) have shown that large group size can be an advantage to *L. flavolineata* dominants as they are more productive and live longer and large nests are also less likely to fail (see Section 1.3.4.1). The effect of admitting joining individuals to the nest might also be important with regard to *L. flavolineata* nests as Samuel (1987) and Strassmann *et al.* (1994) both noted such behaviour. The important question is whether such joining individuals are unrelated to the nest they join.

3.1.6 Why should an unrelated individual join a nest?

There are a number of factors that may influence an individual to leave its natal nest; indeed its nest may even be destroyed. However, establishing a new nest is often a difficult task, especially as a lone female must survive for at least 100 days in order to rear her brood into adulthood (Samuel 1987; pers. obs.). Indeed, only 10 – 30% of lone foundresses can expect to survive for such a long period. (Field *et al.* 1998). In joining

another nest the individual is becoming a member of an already established and productive group in which there may be a chance of inheriting the dominant position and achieving direct fitness. However, due to her lack of relatedness to the rest of the nest members she cannot gain any indirect fitness returns from helping to rear the nest's brood. Therefore such an individual might be expected to provide less helping effort. If the joining individual does help upon the nest this may be due to a pay-to-stay tactic to placate the nest members into letting her stay upon the nest, or it may be due to her trying to boost the number of helpers that will be present if she reaches dominance i.e. group augmentation (Kokko *et al.* 2001, 2002).

3.1.7 Why should a nest let an unrelated individual join?

The reasons why an individual may join an unrelated nest are perhaps easier to understand than why such an individual should be admitted to the nest. Whilst the joiner may stand the chance of inheriting dominance, the rest of the group stand the chance of gaining an unrelated individual as dominant and therefore rearing unrelated brood. An extra helper upon the nest could add a little to nest productivity yet it is difficult to see why an unrelated individual should be tolerated if there is a chance that nest members would have to sacrifice their indirect fitness whilst this individual was dominant. Buston's (2004) investigations into queuing within groups of the anemonefish (*A. percula*) revealed the presence of nine joining individuals. In each case the joiner entered the group at the bottom of the queue (Buston 2003) and never filled a breeding position. Even if breeding vacancies were created a joiner never filled them. Such a system within *L. flavolineata* would mean that nest mates would not have to surrender their indirect fitness by admitting a joiner. However, the benefit to the joining individual would be unclear. The anemonefish of Buston's (2004) investigations may have joined a group in order to avoid predation and the benefit from doing this may outweigh the cost of never achieving a breeding position.

3.2 Aims of this Chapter

1. **To determine whether there is a difference in average intranidal relatedness between a) years (1996 and 2001) and b) study sites.**

2. **To establish the effect of group size upon intranidal relatedness.**

3. **To investigate the typical kin composition of a *L. flavolineata* nest such as the number of sib groups that may exist at any one time.**

4. **To determine whether 'joiners' are related to the nestmates they 'join'.**

5. **To explore any factors influencing a) joining a nest and b) leaving the natal nest to join another nest.**

3.3 Methods

3.3.1 Sample Collection

Females were collected from a total of 44 nests, from 4 study sites (Sites 2, 3, 4 and 5; see Figure 2-3), using the procedures outlined in Chapter 2: General Methods. Most females removed were involved in the age-rank analysis (see Chapter 4 for more details). Five extra nests were sampled in order to gain more information on the background relatedness level. At the end of the period of dominant removal, the remaining nestmates were collected to investigate average intranidal relatedness.

3.3.2 Genetic Analysis

A more detailed description of microsatellite analysis can be found in Chapter 2: General Methods. The DNA was extracted from each of the adult females using a simple salt protocol. PCR analysis was used to amplify three microsatellites in the sample DNA strands; these were K18, I3 and LF25, previously identified by Sumner (1999) (see also Sumner & Field 2001) , which have been shown to be highly polymorphic in this species. The PCR products were then separated using Polyacrylamide Gel Electrophoresis (PAGE) (see Chapter 2).

3.4 Results

3.4.1 Requirements for Estimating Relatedness

In order to draw relatedness estimates from the study population it is necessary to ensure that the data set meets the criteria of Section 3.1.2.2.

3.4.1.1 Testing for Heterozygote deficiency within the Data Set

The data set was tested for heterozygote deficiency using *GenePop* 3.1 (Raymond & Rousset 1995). This program uses a Markov chain method to estimate the probability of heterozygote deficiency in the population. It is preferable to the Monte Carlo method in that it is sensitive to a small number of alleles (Guo & Thompson 1992). F_{is} (inbreeding coefficient) estimates are low for all of the loci used and therefore indicate minimal levels of inbreeding in this population (see Table 3.3; Weir & Cockerham 1984) The results show that there is no significant deviation from the Hardy–Weinberg equilibrium for any of the loci (All Loci $_{(0.05)}$, $p > 0.1$). These results also indicate that the presence of null alleles is not a significant factor, as there is no surplus of homozygote individuals (Schlotterer 1998).

Table 3.3 Tests for Heterozygote Deficiency within each Locus. Exact p-values were calculated using the Markov chain method (dememorization number 1000; batches 100; iterations per batch 1000) and estimate the probability of heterozygote deficiency.

Locus	P-Value (at 0.05 significance level)	W & C*
K18	0.1848	+0.026
I3	0.7004	-0.027
LF25	0.8638	-0.027

*W & C (Weir & Cockerham 1984) F_{is} estimate

3.4.1.2 Testing for Differences in Relatedness by Locus

It is essential to ensure that relatedness values calculated by using different loci give similar results. Sumner (1999) has previously shown that these loci are not linked and therefore give independent relatedness values. If any of the loci are under selection, and thus can no longer be considered as neutral markers, they could give distorted relatedness values. Each of the loci showed a normal distribution in relatedness values (see Shapiro-Wilk p-values Table 3.4); however the loci were significantly different in their variances (Levene $_{(0.05)} = 5.180$, $p < 0.01$) therefore a non-parametric Kruskal Wallis test was used. The test shows that the mean intranidal adult female relatedness estimates for each locus do not differ significantly ($\chi^2_{(0.05)(2)(29, 29, 27)} = 4.857$; $p > 0.1$)

Table 3.4 Mean intranidal adult female relatedness values for all nests using individual loci.

Locus	Mean Intranidal Adult Female Relatedness	Sample Size	95% Confidence Interval	Shapiro-Wilk test of normality (at 0.05 significance level)
K18	0.4825	29	0.0727	0.968; $p > 0.1$
I3	0.3569	29	0.0999	0.960; $p > 0.1$
LF25	0.5445	27	0.1433	0.947; $p > 0.1$

3.4.2 Mean Intranidal Adult Female Relatedness for 2001

The average intranidal adult female relatedness for the population as a whole is 0.457 ± 0.077, n = 29 nests; 146 individuals (see Table 3.5)

3.4.3 Difference in intranidal, adult female, relatedness between 1996 and 2001

The intranidal relatednesses of Sumner's 1996 data and my 2001 data presented here are normally distributed (See Figures 3.2 & 3.4; Shapiro-Wilk values of 0.987, $p > 0.1$ and 0.979, $p > 0.1$ respectively). Therefore a parametric test was used to compare the intranidal relatedness between years. There was no significant difference between intranidal relatedness in 1996 $_{(n = 27)}$ and 2001 $_{(n = 29)}$ ($F_{(0.05)(27, 29)} = 3.638$, $p > 0.05$) despite the two years' data coming from different sites.

Table 3.5 Intranidal adult female Relatedness Values for Nests from Sites 2, 4 and 5 in 2001. The first digit of each Nest number refers to its Site number.

Nest	Adult Female Relatedness	Sample Size	Jackknife/loci
26	0.5117	5	0.2606
27	0.3547	5	0.1032
215	0.6608	4	0.0496
217	0.2770	4	0.0988
220	0.4733	5	0.1305
227	0.4396	5	0.0305
2105	0.6397	6	0.0735
2109	0.4399	5	0.1557
2111	0.5096	5	0.1562
2112	0.3315	5	0.0756
45	-0.0031	5	0.0741
416	0.3203	4	0.1188
419	0.5643	3	0.2001
425	0.3253	6	0.0199
426	0.3944	6	0.0463
428	0.4393	4	0.0702
429	0.4473	3	0.1695
4102	0.7000	6	0.0757
4103	0.7064	5	0.0482
4104	0.4764	6	0.0687
519	0.2534	4	0.1069
5103	0.2348	5	0.2013
5105	0.8199	4	0.0755
5112	0.7612	4	0.2065
5113	0.4503	5	0.017
5114	0.1235	2	0.3814
5118	0.6805	6	0.0967
5126	0.3797	2	0.0646
5160	0.3066	4	0.261

Figure 3-2 Frequency Histogram of Intranidal, Adult Female, Relatedness in 2001

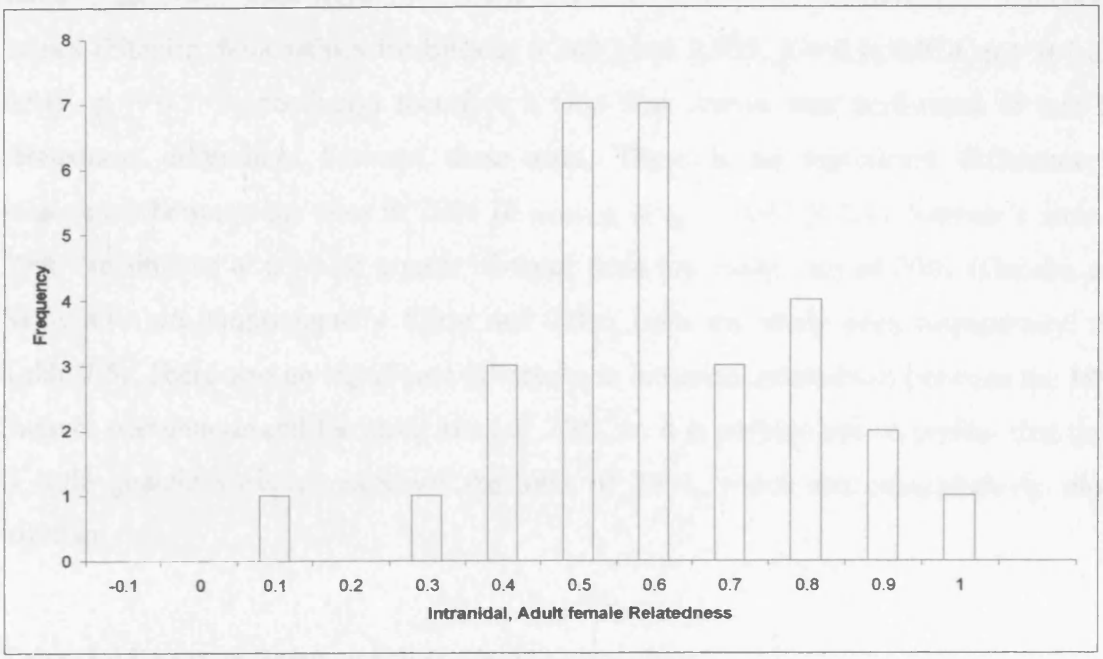
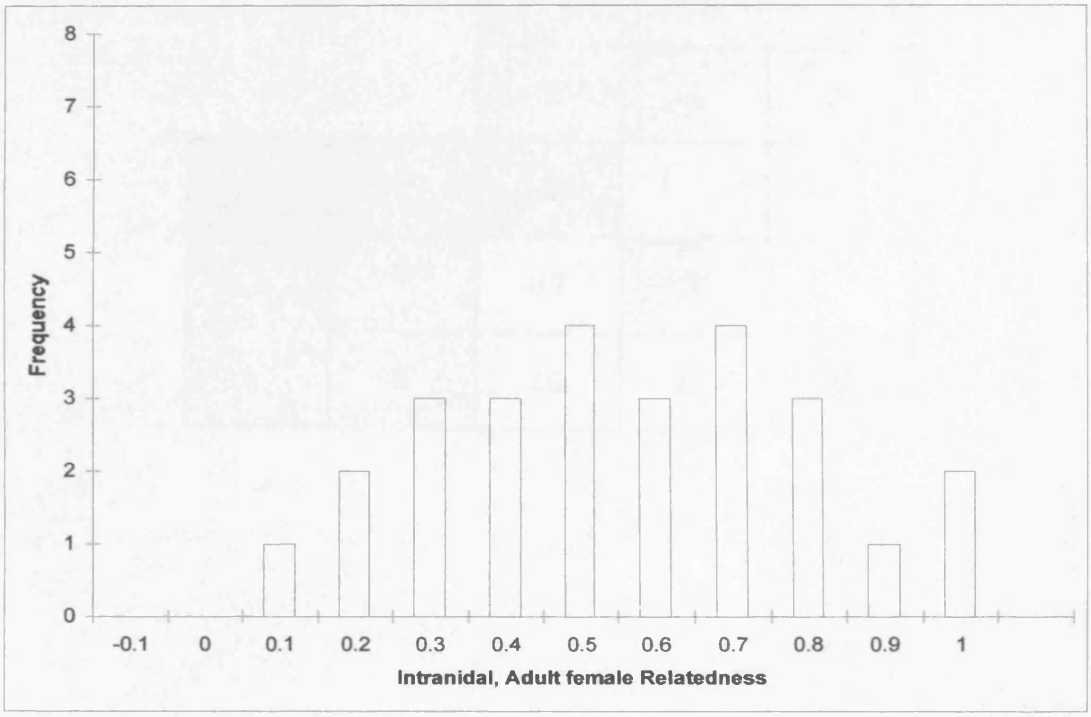


Figure 3-3 Frequency Histogram of Intranidal, adult female, relatedness in 1995-1996 taken from Sumner's (1999) data



3.4.4 Difference in intranidal, adult female, relatedness between study sites

Each of the study sites from 2001 show a normal distribution of intranidal relatedness values (Shapiro Wilk values for Sites 2, 4 and 5 are 0.955, $p > 0.1$; 0.924, $p > 0.1$ and 0.915, $p > 0.1$; respectively) therefore a One-Way Anova was performed to test for relatedness differences between these sites. There is no significant difference in relatedness between the sites in 2001 ($F_{(0.05)(10, 10, 9)} = 0.042$ $p>0.1$). Sumner's sites in 1996 are situated at an even greater distance from my study sites of 2001 (Gazebo and Skew sites are approximately 62km and 77km from my study sites respectively; see Table 3.6). There was no significant difference in intranidal relatedness between the 1996 Sumner populations and the study sites of 2001, so it is perhaps not surprising that there is little genetic variation between the sites of 2001, which are comparatively close together.

Table 3.6 Distance Between Study Sites in 2001 (km).

		Site		
		2	4	5
Site	2	X		
	4	0.7	X	
	5	2.2	1.5	X

3.4.5 The effect of Group Size upon Intranidal Relatedness

Both Sumner's (1996) data (see Sumner 1999) and the 2001 data presented here were combined to look at the effect of group size on intranidal relatedness. There was no correlation between group size and the degrees of relatedness within the nest (Pearson's $\chi^2_{(0.05)(2)(56)} = 0.174$, $p > 0.1$). The analysis was repeated with the exclusion of outliers yet this had little effect upon the significance of the correlation (Pearson's $\chi^2_{(0.05)(2)(56)} = 0.194$, $p > 0.1$). One of the assumptions of the Pearson's 'goodness of fit' correlation is that it assumes a linear and symmetric relationship between the test variables. Therefore the analysis (using data with the exclusion of outliers) was repeated using a non – parametric test, which does not make such assumptions. Nevertheless, there was still no correlation between group size and intranidal relatedness (Spearman's rho $_{(0.05)(2)(56)} = 0.164$, $p > 0.1$)

3.4.6 Kin Composition of *L. flavolineata* nests

The results shown here explore the kin composition of the *L. flavolineata* nests studied in 2001 (i.e the focal population of this thesis). The aim of such analyses is to investigate the typical kin composition of a nest in addition to detailing features such as the number of sibships that may exist within the nest at any one time.

3.4.6.1 Comparison of *Kinship* Data to Theoretical Relatedness Values, 0, 0.5 and 0.75

Each of the nest sites from 2001 was analysed separately to identify whether average intranidal relatedness values at each site differed significantly from zero (see Table 3.7). Relatedness at each of the sites did not differ significantly from a value of 0.5, but did differ from the expected sister – sister value of 0.75. This may be because groups contain cousin and aunt-niece relationships as well as sibships.

Table 3.7 Comparisons of Intranidal Relatedness Values from different Sites with Relatedness Values of r = 0, 0.5 and 0.75

Site	n	Mean Intranidal Relatedness	r = 0		r = 0.5		r = 0.75	
			$t_{(0.05)}$	p	$t_{(0.05)}$	p	$t_{(0.05)}$	p
2	10	0.464	11.772	0.000	-0.919	0.382	-7.265	0.000
4	9	0.437	6.716	0.000	-0.967	0.359	-4.808	0.001
5	9	0.446	5.327	0.001	-0.651	0.533	-3.640	0.007

3.4.6.2 *Kinship* data regarding the Number of Sib groups on each Nest

Kinship v 1.2 (Goodnight and Queller 1999) was used to assign each individual within a nest to a sib group. *Kinship* works by using the allele frequencies of the study population to calculate the probability that two individuals' genotypes could arise from being sisters (r = 0.75; the primary hypothesis) and calculates the probability again for them being cousins (r = 0.375; the null hypothesis) (for a more detailed explanation see Section 3.1.3). *Kinship* then calculates a log-likelihood ratio from the probability of being sisters to the probability of being cousins. In this analysis 10, 000 simulated pairs were used in order to estimate statistical power. The results that follow assign individuals to a sib group providing that the likelihood of being a sister rather than a cousin is significant at the $p < 0.05$ level (for which the chance of commiting a Type II error is 0.0719). If a female fits into more than one sib-group she is assigned to the group composed of the largest number of females with which she is likely to be a sister. A summary of the *Kinship* results can be found in Table 3.8; detailed results including log-likelihood ratios and the identity of each individual within a sib group can be found in Appendix 3. Overall, the number of sib groups ranged from two to four but the modal sibship size was two.

Table 3.8 A Summary of *Kinship* data detailing the number of Sib groups and the Group Size of Each Nest. The first digit of the Nest number refers to the Nest Site.

Nest	Group Size	Number of Sib Groups (using *Kinship* data)	Number of sibgroups using Census and *Kinship* data)
26	5	2	2
27	5	1	3
215	4	1	2
217	4	3	2
220	5	2	2
227	5	2	2
2105	6	1	2
2109	5	2	2
2111	5	2	2
2112	5	2	3
45	5	4	2
416	4	2	3
419	3	1	1
425	6	2	1
426	6	2	1
428	4	3	3
429	3	2	2
4102	6	1	1
4103	5	1	1
4104	6	2	2
519	4	3	2
5103	5	3	2
5105	4	1	1
5112	4	1	1
5113	5	2	2
5114	2	2	2
5118	6	1	1
5126	2	2	2
5160	4	3	3

3.4.6.3 Using Census Data to Support *Kinship* Data

Census data can be used in conjunction with *Kinship* data to provide greater accuracy regarding nest kin composition. For example, census data will reveal a female's date of emergence (and thus her developmental period) from which a likely mother – daughter relationship can be assessed, depending on which wasp was dominant when the focal individual was born. The census data also shows which individuals emerged at around the same time and are thus more likely to be sisters (see Appendix 3). By looking at the date of emergence of each female and tracing their development back to their time of birth it is possible to see which wasps were likely to have shared the same mother, thus allowing sibships to be inferred (see Appendix 3 for a detailed breakdown of each Sib Group).

3.4.6.4 Sib Groups within *L. flavolineata* nests

The number of sib groups found within each nest has no relation to group size within any of the nests (Spearman's rho $_{(0.05)\,(2)\,(29)} = -0.113$ and -0.201 for sibships established using *kinship* only and sibships using both *kinship* and census data, respectively. In both cases $p > 0.1$; see Figure 3-4). However, there is a strong correlation between the number of sibships and relatedness within a nest (Spearman's rho $_{(0.05)\,(2)\,(29)} = -0.796$, $p < 0.001$ for sibships deduced from *kinship* only and -0.549, $p = < 0.01$ for sibships deduced from *kinship* and census data; see Figure 3-5). Additionally, the number of sib groups found sometimes differs according to the method used in their allocation. In this analysis, *Kinship* was used to calculate the likelihood of being a sister rather than a cousin at the $p < 0.05$ level. *Kinship* was unable to deduce the likelihood of being a sister rather than a daughter due to the increasing likelihood that such calculations would produce a type II error (see Table 3.1). The census data allow for some inference regarding these relationships, based on date of emergence and the identity of the dominant at the time of an individual's birth. Therefore the census data were able to break down some sib groups produced by *Kinship* into more complex relationships.

Figure 3-4 Number of Sibships versus Group Size in *L. flavolineata* nests

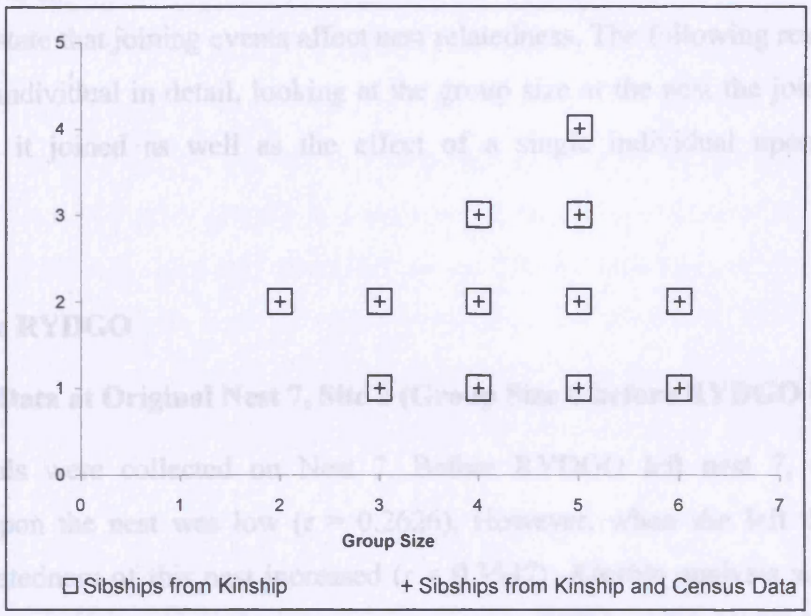
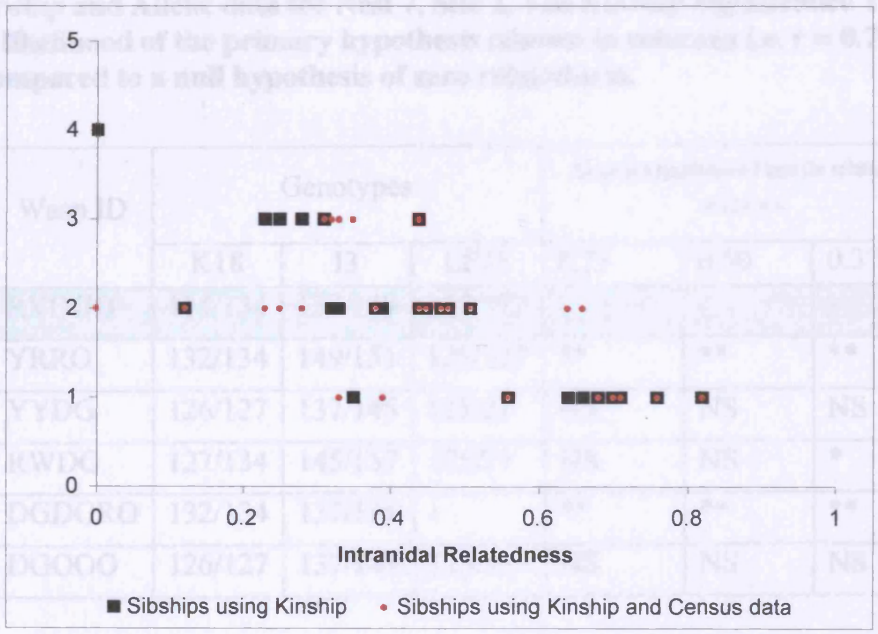


Figure 3-5 Number of Sibships versus Intranidal Relatedness in *L. flavolineata* nests



3.4.6.5 Joiners and their effect upon intranidal relatedness

In 2001 there were a total of 8 joining events. Only 3 of these nests (nests 6 (Site 2), 27 (Site 2) and 103 (Site 5)) were collected, therefore the sample size is not large enough to conclusively state that joining events affect nest relatedness. The following results look at each joining individual in detail, looking at the group size at the nest the joiner left and the nest that it joined as well as the effect of a single individual upon intranidal relatedness

1. Joiner RYDGO

Relatedness Data at Original Nest 7, Site 2 (Group Size 6 before RYDGO left)

All individuals were collected on Nest 7. Before RYDGO left nest 7, the overall relatedness upon the nest was low (r = 0.2626). However, when she left the nest the intranidal relatedness of this nest increased (r = 0.3547). *Kinship* analysis suggests that she was closely related to two of her original nestmates, YRRO and DGDGRO.

Table 3.9 *Kinship* and Allelic data for Nest 7, Site 2. The *Kinship* Significance Test refers to the likelihood of the primary hypothesis (shown in columns i.e. r = 0.75, 0.5, 0.375) compared to a null hypothesis of zero relatedness.

Nest	Wasp ID	Genotypes			*Kinship* Significance Flags (in relation to RYDGO)		
		K18	I3	LF25	0.75	0.50	0.375
7 (Site 2)	RYDGO	114/134	137/149	123/127	-	-	-
	YRRO	132/134	149/151	125/127	**	**	**
	YYDG	126/127	137/145	125/21	NS	NS	NS
	RWDG	127/134	145/157	125/21	NS	NS	*
	DGDGRO	132/134	137/151	-	**	**	**
	DGOOO	126/127	137/145	125/21	NS	NS	NS

Relatedness Data of Joined Nest 6, Site 2 (Group Size 7 before RYDGO joined)

Four individuals were collected from nest 6. Both the allele frequency and *Kinship* data for nest 6 indicate that the joining wasp was not closely related to the rest of her nestmates (see Table 3.10). The *Kinship* data show that the joiner was not significantly more likely to be related to her nestmates (on the 0.75, 0.5 and 0.375 levels) than unrelated (r = 0). However we are unable to conclude that the wasp was totally unrelated due to the absence of 3 other uncollected individuals, which were present at the point of joining. The allelic data show this wasp has unique alleles at all three of the loci. In the case of locus K18 its allelic state is unique which supports the theory that this wasp is unrelated. This wasp is of particular interest as it was able to join the nest at a rank one position and is therefore a queue jumper that will be investigated in the next chapter.

Table 3.10 *Kinship*, Rank and Allelic data for Nest 6, Site 2. The *Kinship* Significance Test refers to the likelihood of the primary hypothesis (shown in columns i.e. r = 0.75, 0.5, 0.375) compared to a null hypothesis of zero relatedness.

Nest	Wasp ID	Rank	Genotypes			*Kinship* Significance Flags (in relation to RYDGO)		
			K18	I3	LF25	0.75	0.50	0.375
6 (Site 2)	RYDGO	1	114/134	137/149	123/127	-	-	-
	OYWO	Low	121/122	147/153	127/127	NS	NS	NS
	OPRO	2	106/122	137/141	127/127	NS	NS	NS
	YYDGO	3	121/122	147/153	127/127	NS	NS	NS
	YPRO	Low	106/121	141/153	127/127	NS	NS	NS

2. Joiner PDGWO

Relatedness Data at Original Nest 29, Site 2 (Group Size 9 before PDGWO left)

Nest 29 at Site 2 originally consisted of nine females but they had deserted before the end of the investigation so that only two females could be collected. The intranidal relatedness of the nest 29 sample was very low (r = -0.0969) indicating that both wasps collected from this nest were unrelated. Indeed, the *Kinship* data show that the joiner is not significantly more likely to be related to her original nestmate (on the 0.75, 0.5 and 0.375 levels) than unrelated (r = 0).

Table 3.11 *Kinship*, Rank and Allelic data for Nest 29, Site 2. The *Kinship* Significance Test refers to the likelihood of the primary hypothesis (shown in columns i.e. r = 0.75, 0.5, 0.375) compared to a null hypothesis of zero relatedness.

Nest	Wasp ID	Genotypes			*Kinship* Significance Flags (in relation to PDGWO)		
		K18	I3	LF25	0.75	0.50	0.375
29 (Site 2)	PDGWO	123/184	133/149	125/129	-	-	-
	WPRO	108/133	139/145	-	NS	NS	NS

Relatedness Data of Joined Nest 27, Site 2 (Group Size 4 before PDGWO joined)

All individuals were collected from nest 27. The intranidal relatedness at this nest was high before the joining event occurred (r=0.7779). However after PDGWO joins intranidal relatedness decreases (r = 0.4396). The joiner has unique alleles at all three loci compared with the other nestmates which, combined with *kinship* data, indicates that it may be unrelated to the other nest members.

Table 3.12 *Kinship*, Rank and Allelic data for Nest 27, Site 2. The *Kinship* Significance Test refers to the likelihood of the primary hypothesis (shown in columns i.e. r = 0.75, 0.5, 0.375) compared to a null hypothesis of zero relatedness.

Nest	Wasp ID	Rank	Genotypes			*Kinship* Significance Flags (in relation to PDGWO)		
			K18	I3	LF25	0.75	0.50	0.375
27 (Site 2)	PDGWO	3	123/184	133/149	125/129	-	-	-
	DGWYO	1	126/127	141/141	7/32	NS	NS	NS
	RWRO	4	126/127	141/141	7/32	NS	NS	NS
	ODGOO	2	106/127	141/141	7/32	NS	NS	NS
	BrYBrO	Low	126/127	141/165	7/32	NS	NS	NS

3. Joiner WPOLB

Relatedness Data at Original Nest 138, Site 5 (Group Size 1 before WPOLB left)

WPOLB was the only individual present upon this nest.

Relatedness Data of Joined Nest 103, Site 5 (Group Size 4 before WPOLB joined)

All individuals were collected from this nest. The joining event here had little impact on the intranidal relatedness of this nest as relatedness increased from 0.2346 to 0.2348. This indicates that this wasp may share a low level of kinship with other group members and may therefore be a returning nest member i.e.an individual that left this nest before censuses began and had returned to the nest. The average relatedness value of nest 103 (see Table 3.14) was comparatively low, 0.2348 $\pm$ 0.8663 yet *kinship* data indicates that the joiner is significantly more likely to have an aunt – niece relationship with DGYOR and YRBrLB (at $p < 0.05$ and $p < 0.01$ levels respectively) than zero relatedness. The joining wasp also shares two common alleles at locus K18 with joined nestmates that

may indicate a degree of relatedness, but the absence of data for locus LF25 prevents any reliable conclusions from being drawn.

Table 3.13 *Kinship*, Rank and Allelic data for Nest 5103. The *Kinship* Significance Test shows the likelihood of the primary hypothesis (shown in columns i.e. r = 0.75, 0.5, 0.375) compared to a null hypothesis of zero relatedness.

Nest	Wasp ID	Rank	Genotypes			*Kinship* Significance Flags (in relation to WPOLB)		
			K18	I3	LF25	0.75	0.50	0.375
103 (Site5)	WPOLB	3	109/148	139/143	-	-	-	-
	PRLB	1	109/148	137/151	-	NS	NS	NS
	RPDGLB	2	145/147	149/153	23/27	NS	NS	NS
	DGYOR	4	109/109	133/151	127/131	NS	NS	*
	YRBrLB	Low	109/148	133/151	-	NS	NS	**

3.4.6.6 Factors influencing Leaving and Joining a nest

Table 3.14 shows all the incidences of joining at *L. flavolineata* nests in 2001. Most of the individuals were able to join a nest at a rank higher than that they left on their original nests. These ranks have been taken from behaviour and foraging effort rather than order of inheritance so they are not entirely reliable. However distinguishing a higher ranking female, i.e. rank 1 to 3, from a low ranking subordinate i.e rank 4 onwards is often obvious just by behavioural observation, and it seems that 6 out of 8 joiners were likely to have been able to join a nest at a higher rank. Group size did not seem to influence the decision to leave or join a nest although it is clear that by ORDGO joining a nest containing only one other individual an increase in rank is inevitable. In 3 of the 8 cases the joining individual was able to move straight into the dominant breeding position. One artificial factor that may have influenced leaving behaviour was the act of marking the wasps. As previously mentioned in Section 2.1.2.1, if a wasp has not had time to orientate itself to the position of its nest before it is taken away to be marked it may not be able to

find its way back to the nest (Field *et al.* 1999). In 5 out of the 8 joining events the individual did not return to its original nest after it was marked and therefore leaving behaviour in these wasps may just be an artefact of the experimental procedure. However, in 3 other cases the individuals returned to their nests, after marking, staying for a considerable length of time before they left to join another nest: RYDGO, PDGWO and WPOLB. These may therefore be considered as genuine joiners i.e. wasps that left their original nest of their own accord to join a new nest.

Table 3.14 Census Data for Joining Individuals. The first digit of the nest number refers to its site.

	ORIGINAL NEST				NEST JOINED			
Wasp ID	Nest ID	Inferred Rank	Group Size	Length of Stay after Marking (days)	Nest ID	Group Size	Inferred Rank at which joiner joins	Inheritance Rank
RYDGO	27	1	6	17	26	7	1 or 2	1
PDGWO	229	6 or 7	9	0	227	5	2	2
ORDGO	217	4 or 5	5	0	2113	2	2	Dies
RORW	428	4	5	0	423	3	3	Dies
WPOLB	5102	Alone	1	75	5103	5	1	Dies
OORLB	5138	Low	8	0	5106	6	1	1
RYOLB	5117	3	5	70	5106	5	2	1
OWOLB	5130	Low	2	0	516	5	3	3

3.5 Discussion

3.5.1 Differences in Intranidal Relatedness between Years

There was no difference in average intranidal relatedness between 1996 and 2001. This may be unsurprising as nest social structure is similar to that reported by Sumner (1999). Sumner (1999) found a similarly low level of joining events in her colonies (only 4% of nests contained a joining individual); therefore one might expect relatedness values between the two years to be of a similarly high value. Figures 3.2 and 3.3 show that there is a wider spread of relatedness values in the 1996 population, although levels of variance are not significantly different (Levene $_{(0.05)}$ = 0.003, p>0.5). The same techniques and microsatellite loci were used in both the investigations therefore the difference in the spread of relatedness values is unlikely to be due to experimental procedure. The low levels of intranidal relatedness, which are more numerous in Sumner's (1999) study, may be due to particularly high numbers of sibships within some nests. As previously mentioned, the low relatedness values are unlikely to be caused by joining events by unrelated individuals

3.5.2 Differences in Intranidal Relatedness between Study Sites

There was no significant difference in intranidal relatedness values between sites. This may be expected as all sites are quite closely situated to each other, the greatest distance being that between sites 2 and 5 i.e. 2.2 km (see Table 3.6). Map Figure 2-1 shows that streams may connect many of the study sites and provide corridors for migration. Therefore there is probably a high level of gene exchange between sites. Sumner's data supports this as she also found little difference in intranidal relatedness between her sites ($t_{(0.05)(2)(27,\ 12)}$ = 1.07, p > 0.05) (Sumner 1999). All sites contain nests with similar social structures and levels of joining; therefore similar relatedness values might be expected.

3.5.3 Group Size and Relatedness

There was no significant correlation between group size and relatedness within the population. If the nests were investigated from their founding state onwards, one might expect to see either an increase or decrease in relatedness depending upon the number of foundresses. Nests are usually founded by one wasp (Sumner 1999) and therefore as group size increases the group will become composed of her daughters, forming a highly related sibship. As group size increases further, through the reproduction of one of these daughters after she inherits the dominant position, nieces and cousins become incorporated into the group and can therefore decrease the average relatedness (see Appendix 3). Thus, the question of how relatedness should change with group size is a complex one. There was no significant correlation between group size and intranidal relatedness (see Section 3.4.5). This may be due to joining events by unrelated wasps decreasing nest relatedness but can equally be due to the fact that both large and small groups can exhibit high levels of relatedness according to the number of sibships present. In accordance with this there was a highly significant correlation between intranidal relatedness and the number of sibships within a nest. Differential survivorship within the colony leads to sibships composed of varying numbers of individuals and therefore group size does not correlate with the number of sibships and cannot be used to predict levels of relatedness.

3.5.4 The typical kin composition of *L. flavolineata's* nest

The number of sib groups that may exist within the nest at any one time ranges from one to four but most commonly nests possessed two sib groups. The number of sibships was highly negatively correlated to the intranidal relatedness of a nest thus confirming the point that the number of sibships rather than group size influences intranidal relatedness.

3.5.5 The effect of joiners upon intranidal relatedness

As mentioned previously, there were 8 joining events in the 2001 population (see Table 3.14). However there are only genetic data for 3 of these nests. In 2 of these nests it is likely that the joining individuals were unrelated to the nest they joined based on kinship and census data.

3.5.6 Factors influencing joining and leaving a Nest

RYDGO was originally a rank 1 or 2 individual upon nest 7, Site 2, one of the larger groups with 7 individuals. It left the nest after 17 days of being initially marked. This is significant as this shows that the act of marking itself is unlikely to explain why it left its original nest. Some wasps are removed from the nest for marking before they have had time to orientate to the position of their nest and can therefore become artificially created "floaters". This is unlikely to have happened in this case, especially as RYDGO was so high ranking and therefore likely to have been on the nest for a lengthy period. RYDGO was able to join nest 6 at a similarly high position to rank 1 or 2, and finally inherited the dominant position. Kinship data suggest that this individual is unrelated to the group (see Section 3.4.6.4). Nest 6 is likely to have consisted of a single sibship before it was joined by RYDGO. Therefore one cannot explain the ability of this wasp to join the nest by the presence of previously low levels of relatedness. The group size of the nest RYDGO joined was similarly high to the nest it left therefore it is unlikely that the decision to join nest 6 was due to an attempt to inherit a larger group.

PDGWO differs from the previous case of RYDGO in that it was originally a low ranking wasp upon a large nest of 9 individuals. This wasp was then able to join a rank 2 position on a 5 female nest. It is similar to RYDGO in that it appears to be unrelated to the nest it joins, as indicated by the *Kinship* data (see Section 3.4.6.4). The nest it joins appears to consist of just a single sibship. Therefore, low levels of relatedness on the 'joined' nest, again cannot explain this wasp's ability to join. PDGWO did face a long wait for inheritance of dominance upon it's original nest therefore it's decision to join

another nest at a higher rank may have been influenced by this. It is possible that the advantage of inheriting a group in a shorter time outweighed the fact that the group size of the nest it would inherit would be smaller.

WPOLB was a lone-nesting wasp, which remained upon its own for 75 days after marking. It then joined a nest with only two wasps and was able to take over the dominant position whilst these wasps were still present upon the nest. However, its time as dominant was short, so that the original, second ranking wasp was able to inherit. *Kinship* analysis in this case is not very informative as there is no allelic information for locus LF25. However, this wasp does share two alleles at locus K18 with PRLB, DGYOR and YRBrLB so may be related to the nestmates it joins at some level (see Table 3.13). This may have determined WPOLB's decision to join this nest. It is possible that WPOLB was an original member of this nest, which left before the investigation began in order to found its own nest. However upon failing to do so it may have decided to return to this original nest.

Overall, two of the joiners were likely to have been unrelated to the nests that they joined: RYDGO and PDGWO. These two wasps came from relatively large group sizes so that their decision to join another nest was not based on an attempt to inherit a larger nest. PDGWO was a low ranking wasp upon its original nest that was able to increase in rank by joining another nest; therefore the benefit of joining for this individual is perhaps the most apparent. RYDGO was already the dominant upon its original nest and therefore the reason for its departure is unclear. The pattern that seems to emerge from the joiner data is that joiners tend to join a nest at a higher rank (see Table 3.14). Therefore, for the most part, joining may be a strategy used to increase rank in the shortest possible time. Group size seems to play no part in an individual's decision to leave or join a nest.

3.6 Conclusion

In this chapter I have found that the average relatedness within *L. flavolineata* colonies is high and not significantly different between sites or between years. This indicates that allele frequencies have remained stable within these populations and may be maintained between these sites through frequent migration events. The relatedness values of the nests do not differ significantly from the value of 0.5. *Kinship* and census data provide evidence that these nests are composed of a maximum of two sib groups. Therefore high relatedness values from these sibships probably combine with low values of relationships such as cousins to produce the relatedness average of 0.5.

There was no correlation between group size and intranidal relatedness. The number of sib groups within a nest seemed to be the most important factor in determining intranidal relatedness. Group persistence (see Section 3.1.5.1) is also likely to have an important effect upon intranidal relatedness yet it is difficult to investigate this as it would involve a long term study of each nest all the way through from its foundation.

It was possible to look at the frequency of joining events in detail. There seems to be no link between the nests that were joined yet the data have shown that joining individuals can often join the queue at a higher rank than that they left behind. This may be a determining factor in an individual's decision to join a nest as it would allow a joiner to increase its rank in a short space of time. Other factors such as group size appear to have no influence upon a joining decision. The relationship between rank and age is investigated in the next chapter in order to establish whether there is a system of gerontocracy within *L. flavolineata*.

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4 Inheritance of Dominance in *Liostenogaster flavolineata*

4.1 Introduction

In this chapter I look at the inheritance of the dominant, egg-laying, position within *L. flavolineata* nests. Past studies of these wasps have suggested that rank position within each nest is determined by age i.e. through gerontocracy (Samuel 1987; Field & Foster 1999; Field *et al.* 1999; Shreeves & Field 2002). Other studies of social organisms have also pointed to such a system in determining rank. If rank is determined by seniority, this effectively leads to a queuing structure within the group (see Section 4.1.2). However, all such studies to date have failed to show how strict the age convention actually is. The observer may have a rough idea of the age of each individual in a group but unless the time of birth or emergence of each individual is monitored to a high degree of accuracy he cannot comment upon the rigidity of the gerontocratic convention. The study presented here attempts to resolve such questions since the emergence of each wasp was monitored carefully over a six-month period. In this way, it can reveal whether inheritance rank (order of inheritance) corresponds to age and whether any exceptions occur.

4.1.1 Strategies for Achieving Dominance

As discussed in Section 1.3 there are a number of methods that may be used within a group for the inheritance of dominance. Such methods are outlined below with each benefit and detriment, to both the individual and the group, considered briefly. As fighting is often a common strategy for achieving dominance (see below), it provides a convenient starting point for comparison with alternative strategies and conventions such as ritualised fighting and gerontocracy.

4.1.1.1 Fighting for Dominance

If rank is determined through fighting, as is common in many vertebrates (Zuk *et al.* 1998; Cote 2000), a dominance hierarchy may emerge which is largely based upon body size (Veiberg *et al.* 2004). Larger individuals are likely to be the strongest and most effective of contenders when fighting for dominance (Appleby 1983).

Personal Benefits: Stronger members of the group have a greater chance of achieving dominance.
Group Benefits: If fighting ability is correlated with components of fitness, the "fittest" of group members is likely to achieve dominance thus increasing group 'genetic quality' and productivity.

Personal Detriment: Weak members of the group have little chance of achieving dominance
Group Detriment: Injuries may be sustained whilst fighting for dominance thus damaging the productivity of the group.

4.1.1.2 Ritualised fighting for Dominance

Some organisms possess phenotypic characters which advertise their genetic fitness such as colour or song: indeed dominance established by colour has been observed in a number of bird species (Parsons & Baptista 1980; Rohwer 1985) and *Polistes* (Tibbetts & Dale 2004). If the health and genetic quality of an organism can be established through such a character, the group may be at its most productive if a brightly coloured specimen is able to achieve dominance.

Personal Benefits: Injuries to the inheriting individual and her helping nest mates are avoided.

Group Benefits: If colour intensity indicates "good genes", the fittest of group members is likely to achieve dominance thus increasing group genetic quality and productivity. Injuries are also avoided, as the quality of each competitor is assessed through colour intensity rather than fighting.

Personal Detriment: Poor quality members of the group have little chance of achieving dominance.

Group Detriment: None

4.1.1.3 Conventions for Achieving Dominance

As discussed previously, social groups sometimes depend upon conventions to facilitate group functions and efficiency (see Section 1.3.3). In order to acquire dominance using a convention, an individual must possess a unique attribute that does not indicate its quality but merely singles the individual out as the next Dominant.

4.1.1.3.1 Gerontocracy

Some organisms, including *L. flavolineata*, have been observed to exhibit an age-based system of rank inheritance (Wilson 1975; Clutton-Brock *et al.* 1976; Masure & Allee 1934). It has been proposed that *L. flavolineata* females inherit dominance in a seniority-based manner i.e. the oldest individual becomes the dominant (Samuel 1987; Field & Foster 1999). The reverse of this, i.e. the youngest inherits the dominant position, is exhibited by some other organisms, such as some ants (Ito & Higashi 1991). Studies into seniority-based inheritance or gerontocracy, especially in insects, often use rough age estimates and fail to find the exact ages of each individual (Samuel 1987). Such estimates could mean that some incidences of non-age based inheritance go un-noticed.

Personal Benefits: Dominance can be inherited regardless of fitness and without risk of injury.
Group Benefits: Dominants are established without fighting and therefore no injuries are sustained and the productivity of the nest is unaffected

Personal Detriment: Low-ranking individuals in large groups may have little chance of achieving dominance before dying.
Group Detriment: Individuals of comparatively low quality with regards to the rest of the group can inherit the dominant position thus decreasing the overall fitness of the group.

4.1.2 Queuing for Dominance

If the likelihood of achieving dominance is large enough for a subordinate, this may provide a sufficient incentive for an individual to stay within its natal group (Wiley & Rabenold 1984). Some studies have even shown that inheritance can provide an incentive to stay despite low intra-group relatedness (Stacey & Koenig 1990; Dunn *et al.* 1995; Shreeves & Field 2002; Queller *et al.* 2000; Buston 2004). Additionally, indirect fitness benefits can be increased through helping to rear relatives whilst waiting to inherit (Emlen & Wrege 1989). New evidence provided by Buston (2004) indicates that non-breeders of the clownfish *Amphiprion percula* remain within their group due to the sole incentive of territory inheritance. In this fish, non-breeders cannot breed directly and their presence fails to enhance the dominant's fitness (Fricke 1979; Buston 2004) therefore they can only gain fitness benefits through the inheritance of territory i.e. the anemone. Helping within a group can also help to increase an individual's own direct fitness through "group augmentation" as any increase in group size, as a consequence of helping, may be inherited (Kokko *et al.* 2001).

4.1.3 Rank and Relatedness

The existence of an age-based queuing system within a group can have important implications for intra-group relatedness. Within the context of *L. flavolineata*, when a nest is first founded (usually by a single female see Samuel 1987; Field *et al.* 1998), the first generation upon the nest will consist of her sons and daughters. Upon the founding female's death, the oldest of her daughters will inherit the dominant position and produce her own kin. Assuming that no joining events occur and the Dominant is the sole reproductive, the oldest individuals, i.e. the higher ranks, will consist of the dominant's sisters and the lower ranks of her daughters. At this time there may be a relatively simple relatedness structure to the nest in which higher ranks exhibit higher relatedness to the Dominant than lower ranks. However, as time progresses upon the nest, a mixture of generations builds up. This may lead to a complex relatedness structure. The aseasonal environment in which *L. flavolineata* lives ensures that such an ensemble of generations can cohabit upon a nest. This may mean that the relatedness structure seen in *L. flavolineata* nests differs greatly when compared to seasonal nesting species. If relatedness to the dominant does correlate systematically with rank, this may have some important implications. For example, if relatedness to the Dominant decreases with rank, this may lead to lower ranks delivering less help to the Dominant's brood.

4.1.4 Cheating the Convention of Gerontocracy

The benefits of achieving dominance are clear, for example the dominant acquires direct reproduction and has less exposure to predation due to the minimal foraging that she may perform compared to subordinates. As previously discussed in Section 1.3.5, cheating commonly takes two forms i.e. signal or behavioural cheating. When considering the first two methods of acquiring dominance discussed in Section 4.1.1 (i.e fighting and ritualised fighting), the strength of an individual determines its success. However this is not true with 'conventional' inheritance systems, so that they may be perhaps the most vulnerable to cheating behaviour i.e. queue jumping to reach the dominant position: as

the dominant is not necessarily the strongest of group members and may therefore be usurped with greater ease.

4.1.5 Possible reasons for Queue Jumping within *L. flavolineata*

Queuing for dominance is clearly a viable option within *L. flavolineata* nests. The aseasonal habitat and small group sizes of these nests ensure that low ranking females may live long enough to inherit dominance (Field *et al.* 1998). However, given the opportunity, there may be distinct benefits for an individual that can queue jump:

1) Acceleration to Dominance

Queue jumping would allow an individual to inherit dominance without waiting for older individuals to inherit and die. This would decrease the queue jumper's chance of mortality before inheritance, as less overall time would be spent foraging and performing other risky subordinate behaviours.

2) Unrelated Joining Nestmates.

As mentioned previously, a subordinate who is related to the dominant can gain indirect fitness through helping to rear the dominant's offspring (Shreeves & Field 2002). This, of course, is not the case if the subordinate is unrelated to the dominant. This situation may arise if a wasp joins a nest from the outside population. Such behaviour has been noted previously and is supported by the results of this study (see Chapter 3). Sumner (1999) recorded joining events on 4% of her nests and in my study 14% of nests, from 2001, experienced joining events. Such a joining wasp may gain no fitness benefits from helping to rear unrelated brood, and could therefore face the greatest selection pressure to queue jump to dominance. However, there are two qualifications to this explanation for queue jumping, both involving the effects of group augmentation (Kokko *et al.* 2001). The first might be the benefit, through helping, of effectively increasing the size of the group she herself could inherit. However, any such benefit would be lost if the joiner died

before inheritance. The second benefit of increasing group size might be to increase individual fitness, for example through defence against predators (Cant 2003). This may offset the costs of rearing unrelated brood.

4.1.5.1 Body Size as a Possible Mechanism for Queue Jumping

Perhaps one of the most obvious ways in which an individual may be able to queue jump is through direct competition with older individuals. A younger wasp, which is larger than its older nestmates, might be expected to find such competition easier. At first, one might see this as advantageous to the group as the largest and 'fittest' of nestmates gains dominance. However there are a number of reasons why such behaviour could be detrimental to a eusocial group such as that of *L. flavolineata*:

a) Damage to Nest Productivity through fighting.

Any competition on the nest that involves aggression may lead to injury. Such injury may affect the ability of subordinates to forage and may, in turn, reduce the productivity of the nest.

b) Disincentive to helping.

It has been suggested that an age-based queuing mechanism facilitates subordinates remaining as 'helpers on the nest' (Field & Cant, In Prep.). If dominance was determined through body size for example, any brood that are reared by subordinates are, in effect, potential competitors for the dominant position. Such a situation may provide a disincentive for helping upon the nest.

4.2 Aims of this Chapter

Within *L. flavolineata* females:

1. **How strongly does Age correlate with Rank?**

2. **How does Relatedness correlate with Rank and what is the kin composition of each of the subordinate ranks in relation to the Dominant?**

If Queue Jumping Exists:

3. **Are queue jumpers related to their nestmates?**

4. **Are queue jumpers larger than their nestmates?**

5. **How is queue jumping carried out e.g. is aggression used?**

4.3 Methods

There were two field investigations over the course of the study. The first took place in 2001 to determine whether there was a strict age-based queue within *L. flavolineata*. Queue-jumpers were found during this study and therefore the 2002 study was used to look at the behaviour of queue-jumping individuals in more detail, before and after queue jumping took place, to try to determine how such behaviour was possible.

4.3.1 Age-Based Rank Investigation (2001)

In the 2001 investigation 56 nests were chosen (18 nests at Site 2, 1 at Site 3, 13 at Site 4 and 24 at Site 5. See Figure 2-3 for site locations). All of the nests present with more than one wasp were included in the study, to maximise sample size. Group size varied over the course of the study due to natural mortality and joining events.

Table 4.1 Frequency of Group Sizes for Nests at the beginning of the 2001 Age-Based Rank Investigation

Group Size	Frequency
2	7
3	14
4	6
5	14
6	3
7	8
8	3
9	1

In April 2001, each female wasp was marked with its own individual 4 spot paint-mark in a 'square' pattern upon the thorax (see Figure 2-4) to denote that it was of unknown age. In order to start the investigation, each of the nests was then brood-mapped to record the location of different developmental stages of the brood (see Figure 2-6). Five different stages are recorded, small, medium and large larvae, pupae and hatched cells. When a large larva develops into a pupa, a "pupal cap" of mud is built across the cell. When the newly developed adult is ready to emerge she bites through the cap so that an easily identifiable cell with a mud rim or hole remains known as a "hatched cell". Brood mapping allows brood to be monitored from the egg-stage to adult emergence; it is therefore easy to separate mud-capped cells that previously contained larvae from abandoned cells that merely contain mud. Unmarked adults that appear upon a nest for the first time can then be checked to verify whether they are newly emerged by looking for a broken pupal cap and comparing this with the nest's brood map. If the disappearance of a pupal cap can be matched with the appearance of an unmarked female, this wasp is designated as "newly-emerged".

Over the period of April to September 2001, newly emerged female wasps were marked with 4 paint spots upon the thorax in a diamond pattern to distinguish them from unknown age wasps (see Figure 2-4 & Figure 2-5). Male wasps were all given one white spot upon the thorax, as their identity was unimportant in this investigation. By the end of September 2001, many of the initial wasps of unknown age had died, so that most wasps were of known chronological age.

4.3.1.1 Establishing Age through Census Data

The relative age of each of the wasps, in relation to the rest of their nestmates, was obtained through frequent censusing of broken (hatched) pupal caps and unmarked individuals, over a period of four months. The date of emergence was assigned to each wasp and censusing visits were repeated frequently (every two days) to ensure that each

hatching event was recorded and could be pinpointed down to a short time interval. Complications arose when more than one wasp emerged between censuses; in such a case the relative age of these individuals was 'tied'. Such 'tied' individuals are excluded from the analysis.

4.3.1.2 Previous methods used to determine Rank

Foraging effort has been used in previous studies to designate dominance within a nest (Samuel 1987; Field *et al.* 1998). However, this becomes increasingly difficult as the foraging effort of lower ranks is examined. Lower ranks (below that of rank 3) spend a similar amount of time foraging so that it is almost impossible to assign rank based on foraging effort alone. In these cases dyadic interactions between ranks are often examined for signs of aggression that might indicate dominant behaviour by one of the individuals.

4.3.1.3 Determining Rank

The most effective way of determining rank is, of course, to look at the order in which the wasp inherits the dominant position. However, the lifespan of a dominant can be considerably longer than that of a subordinate, up to one year in some cases, (pers. obs) and therefore waiting for each individual within the queue to inherit naturally is usually unfeasible in the amount of time available. Therefore, the accession to dominance for many of the females was accelerated by removal of the dominant.

Through sequential removal of each dominant, the inheritance rank of each individual could be determined (see Table 4.2). There was a minimum interval of one week between removals to ensure that there was adequate time for the new dominant to begin to develop her ovaries and exhibit convincing dominant behaviour. After each dominant's removal each nest was left to 'stabilise' for 24 hours for each nest member to discover the absence of the dominant and begin to establish their new ranks. Two morning censuses (07:00 –

13:00) were then carried out, on consecutive days, to determine the identity of the new dominant through their foraging effort and aggression to fellow members. Removals were continued until at least ranks 1 to 3 could be ascertained. The number of removals carried out upon each nest varied according to group size, as an effort was made to ensure that some of the original nestmates remained to prevent brood from parasitism or predation.

Table 4.2 Successive Removal of Dominant individuals to determine Inheritance Rank

Inheritance Rank	Time →				
1	Dominant 1	Removed			
2	Subordinate 2	Dominant 2	Removed		
3	Subordinate 3	Subordinate 3	Dominant 3	Removed	
4	Subordinate 4	Subordinate 4	Subordinate 4	Dominant 4	Dominant 4

4.3.2 Identification of Queue Jumpers

An individual was defined as a queue jumper if it inherited dominance before one or more, older nest mates. Each of the queue jumpers and their nest mates were collected for microsatellite analysis and wing measurement.

4.3.2.1 Size of Queue Jumpers

The overall body size of each of the wasps was determined through the measurement of a particular wing cell (see Chapter 2: General Methods). The right forewing of each of the wasps was removed from the thorax at the wing joint. Each wing was then placed between two microscope slides to prevent wing curvature and folding, which could distort measurements. Each slide was then observed through a binocular dissecting microscope attached to Macintosh computer with NIH Imaging software. The wing

image was captured upon the computer screen and the relevant wing cell was measured at high resolution. Initially, ten repeated measures were carried out upon the same wing to ensure measurements were accurate. Five repeated measures were carried out upon each wing and the average of these was determined for further analysis.

4.3.2.2 Microsatellite Analysis of Queue Jumpers

Microsatellite analysis was carried out upon all queue jumping individuals and their nest mates from 2001, in order to determine whether queue jumpers were related to the rest of their nestmates or whether they commonly shared a particular relationship with those that they queue jumped e.g. are queue jumpers usually the sibs of queue jumped individuals. Individuals were also collected from nests in which queue jumping had not taken place in order to provide a background relatedness level with which to compare queue jumper to nestmate relatedness. Overall, females were collected from a total of 44 nests. Most females, which were removed, were involved in the age-rank analysis and therefore successive dominant individuals were removed over a total of 17 days (see Section 3.3.1 &). At the end of the period of dominant removal, most of the remaining nestmates were collected.

Three microsatellite markers were used in the genetic analysis; K18, LF25 and I3. A detailed outline of these markers can be found in Chapter 2: General Methods. DNA was extracted from each adult female and the PCR was used to amplify each of the three microsatellites within the sample DNA. These PCR products were then separated using PAGE (See Sections 2.2.5). Each allelic score was entered into the software programs *Relatedness* (Queller & Goodnight 1989) and *Kinship* (Goodnight & Queller 1999) in order to compare the intranidal relatedness of nests and determine the likely relationship between queue jumpers and their nestmates.

4.3.3 Queue Jumping Behaviour Investigations (2002)

Queue jumping individuals were identified in the 2001 investigation, yet due to the intensive nature of the study it was not possible to take a close look at their behaviour, particularly before the queue jump took place. Therefore, the 2002 investigation was designed to try and observe such behaviour.

In 2002, a total of 70 nests was censused over a period of six months to try and identify any queue jumping individuals. Rapid censusing was carried out in the morning (07:00 –12:30) on each nest over a period of 3 months. Most censuses were carried out on at least three consecutive days to reduce the effect of varying group size and environmental conditions upon foraging effort. In this way, an effort was made to ensure that variation in foraging effort was due to rank position alone.

4.3.3.1 Behaviour of Potential Queue Jumpers (2002)

As soon as an individual was identified, from censusing, which did not appear to show the expected amount of foraging effort for its rank, its nest was observed alongside a control nest i.e. a nest of similar group size in which foraging effort was as expected. In this way, foraging effort, aggression and cell inspection behaviours could be compared between potential queue jumpers and control individuals of the same rank before the queue jump actually took place. An example of an unexpected amount of foraging effort might be a rank five individual, which is present upon the nest as frequently as a rank two individual since foraging effort is expected to be higher within lower ranks. However, foraging effort is often similar in individuals below rank three so that a rank five female that shows the same amount of foraging effort as a rank four individual would not be automatically suspected as a queue jumper.

4.3.3.2 Confirmation of Queue Jumping Individuals

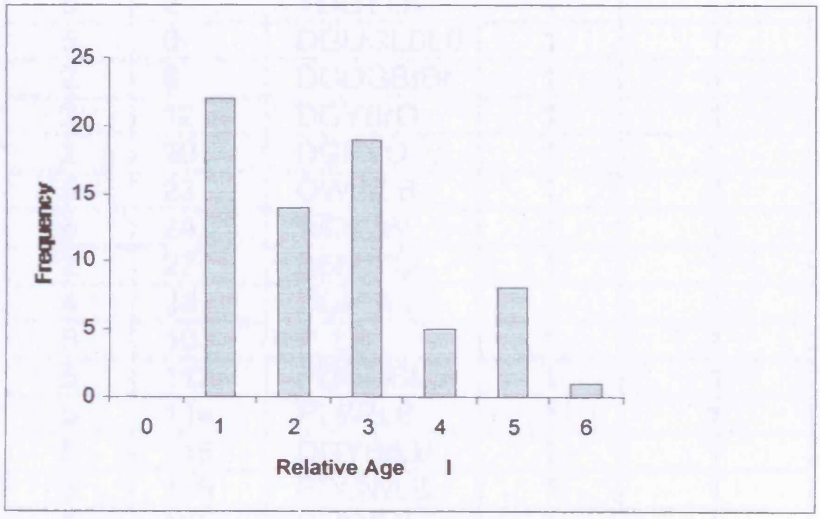
After behavioural observations were carried out, the queue jumping behaviour of the individuals in question was tested by carrying out dominant removals, as in 2001, to accelerate accession to dominance. Thus, it was determined whether females inherited dominance in an order in line with their relative age. Dominant removal was not necessary upon all nests as natural accession to dominance sometimes took place during the extended period of nest censusing in 2002 (see Table 4.3).

4.4 Results

4.4.1 How strongly does Age correlate with Rank in *L. flavolineata* groups?

The relative age versus inheritance rank data are not normally distributed due to an unavoidable bias in the collection of samples (see Figure 4-1, Kolmogorov-Smirnov $_{(0.05)}$ = 0.180, $p < 0.00$, Levene $_{(0.05)} < 0.00$). Such bias was due to varying group sizes, age 1 i.e. the dominant was obviously present upon all nests yet the same cannot be true of lower ages such as age 5 as there were a limited number of nests that contained 5 or more individuals (the mean number of individuals being 1 to 4 see Field *et al.* 1998). Therefore a non-parametric test was required to determine the relationship between relative age and inheritance rank.

Figure 4-1 Total Number of Samples from each Age Group for Age and Rank Analysis (1 = Oldest, 6 = Youngest).



Spearman's Rho was used to analyse the relative age: inheritance rank data. There is a highly significant, positive, relationship between Relative Age and Inheritance Rank (Spearman's rho $_{(0.05)\ (2)\ (69)}$ = 0.940, p = 0.000) (see Table 4.3 and Figure 4-2). Significantly, eight individuals possessed a relative age that did not correspond to their inheritance rank (see Section 4.4.5). It should be noted that only individuals that could be aged accurately in relation to the rest of their nestmates were included in the analysis (see Section 4.3.1.1).

Table 4.3 All known Relative Age and Inheritance Ranks for individuals from all sites (Individuals are grouped according to Relative Age) n = 69.

Relative age corresponds to inheritance rank

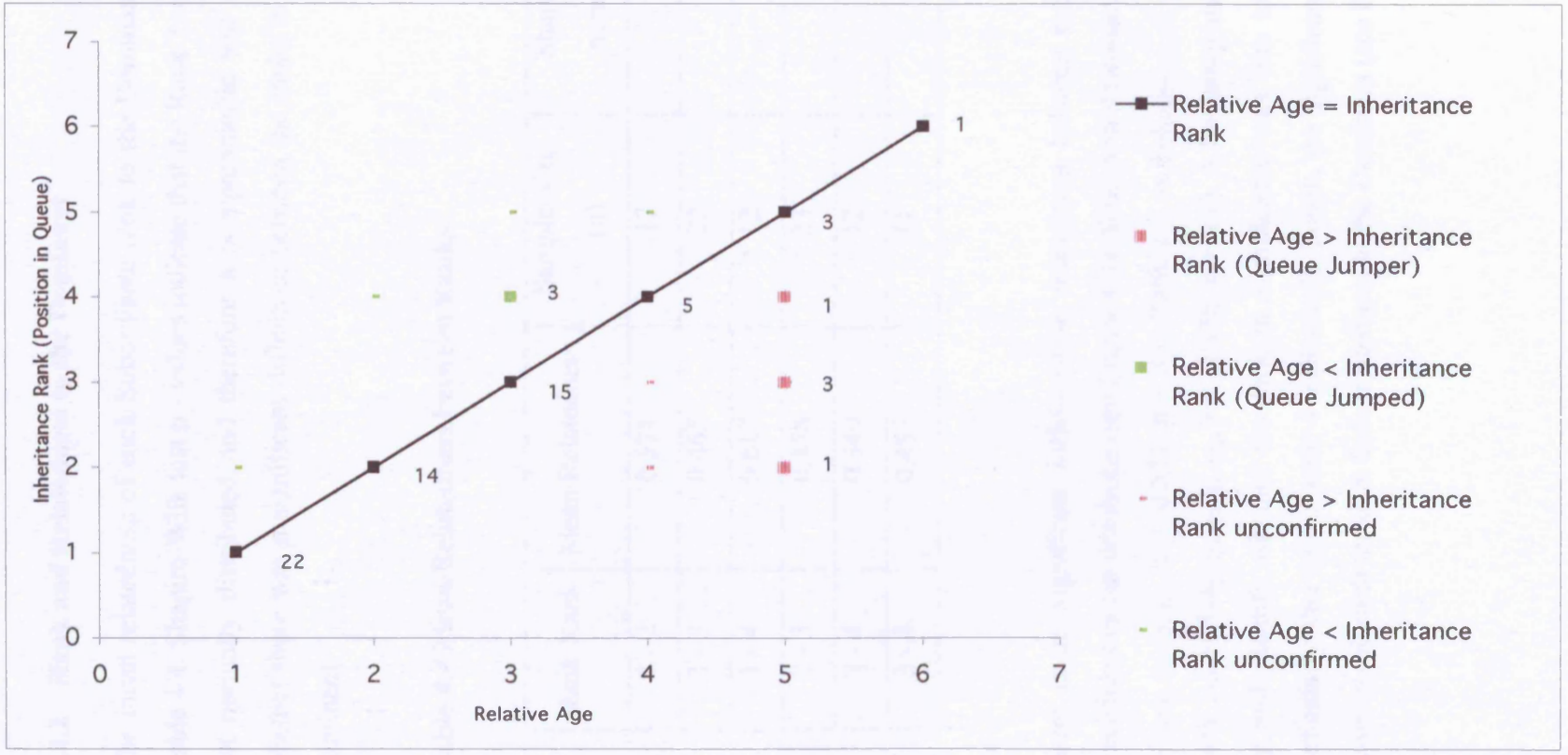
Relative age does not correspond to inheritance rank.

Site	Nest	Wasp ID	Relative Age	Inheritance Rank
5	2	YDGYLB	1	1
5	6	DGDGLBLB	1	1
2	8	DGDGBrBr	1	1
2	12	DGYBrO	1	1
2	20	DGPYO	1	1
5	23	OWWLB	1	1
4	24	WOWW	1	1
2	27	DGWYO	1	1
4	28	DGPRW	1	1
5	103	P_LB	1	1
5	112	PDGDGLB	1	1
5	114	PLBRLB	1	1
5	118	DGYBrLB	1	1
5	119	PDGWLB	1	1
5	123	RLBYLB	1	1
5	137	YRLBLB	1	1
5	138	PROLB	1	1
5	187	DGRYLB	1	1
2	113	WBrPO	1	1
5	161	BrWDGLB	1	1
3	1	DGYOP	1	1
4	103	LBRWW	1	1
5	2	OYRLB	2	2

Site	Nest	Wasp ID	Relative Age	Inheritance Rank
5	6	RYWR	2	2
2	8	OODGO	2	2
5	23	OWBrLB	2	2
4	24	WYDGW	2	2
5	115	WPWLB	2	2
5	119	RPPLB	2	2
5	123	RODGLB	2	2
5	137	BrRRLB	2	2
5	138	OYOR	2	2
5	187	RYRLB	2	2
2	113	ORDGO	2	2
5	161	DGDGOR	2	2
3	1	BrWPP	2	2
2	6	YYDGO	3	3
4	16	ODGPW	3	4
5	19	WODGLB	3	3
2	20	WPWO	3	3
2	23	ORPO	3	3
5	23	RDGOR	3	3
4	24	PPOW	3	3
2	25	YOBrO	3	3
4	25	PRDGW	3	3
4	28	DGROW	3	3
4	29	WYOW	3	4
4	102	PROW	3	4
5	103	DGYOR	3	4
5	105	DGWBrLB	3	3
5	106	DGYRLB	3	3
5	115	WPRLB	3	3
5	137	WPOLB	3	3
5	150	RRRR	3	3
5	160	RYOR	3	3
2	7	DGDGRO	4	4
5	19	YDGOLB	4	4
4	28	OYDGW	4	4
5	160	WBrBrR	4	4
2	111	YDGDGO	4	4
2	15	DGBrOO	5	5
4	26	PDGPW	5	2
4	28	WWBrW	5	5
4	29	UM	5	3

Site	Nest	Wasp ID	Relative Age	Inheritance Rank
2	105	RBrDGO	5	4
5	118	RYDGR	5	3
2	111	DGOPO	5	5
4	103	DGOWW	5	3
4	103	OROW	6	6

Figure 4-2 Inheritance Rank and Relative Age in *L. flavolineata;* 1 on the Inheritance Rank axis corresponds to the Dominant position, higher numbers correspond to Subordinates. Relative Age 1 indicates the oldest individuals upon the nest. The graph shows an overall increase in inheritance rank with decreasing individual age (increasing relative age). Numbers at each data point indicate the number of data entries. These numbers are only included for relative ages that are unambiguous.



4.4.2 Rank and Relatedness to the Dominant

The mean relatedness of each Subordinate rank to the Dominant (Rank 1) is shown in Table 4.4. Shapiro-Wilk test p - values indicate that the Rank 3 relatedness distribution is not normally distributed and therefore a non-parametric test was used to determine whether there was a significant difference between the ranks in their relatedness to the dominant.

Table 4.4 Mean Relatedness between Ranks.

Rank-Rank	Mean Relatedness	Sample Size (n)	Shapiro-Wilk test of normality (p-values)
1 - 2	0.531	27	0.077
1 - 3	0.465	22	0.002
1 - 4	0.617	12	0.710
2 - 3	0.458	23	0.063
2 - 4	0.349	12	0.411
3 - 4	0.453	11	0.971

There is no significant difference in relatedness between ranks 2, 3 or 4 in their relatedness to the dominant (see Figure 4-3). Wilcoxon's two-sample test =. Rank 1-2 vs $1\text{-}3_{(0.05)\ (2)\ (27,\ 22)}$, $z = -0.523$, $p > 0.1$; Rank 1-2 vs $1\text{-}4_{(0.05)\ (2)\ (27,\ 12)}$, $z = -1.177$, $p > 0.1$; Rank 1-3 vs $1\text{-}4_{(0.05)\ (2)\ (22,\ 12)}$, $z = -1.156$, $p > 0.1$). A bootstrap analysis was performed to try and identify whether the lack in significance was due to a small sample size, particularly that of the rank 4 individuals. Again, no significant difference was found between different ranks in their relatedness to the Dominant (see Figure 4-3).

Figure 4-3 Mean Relatedness between each Rank pairing within Nests

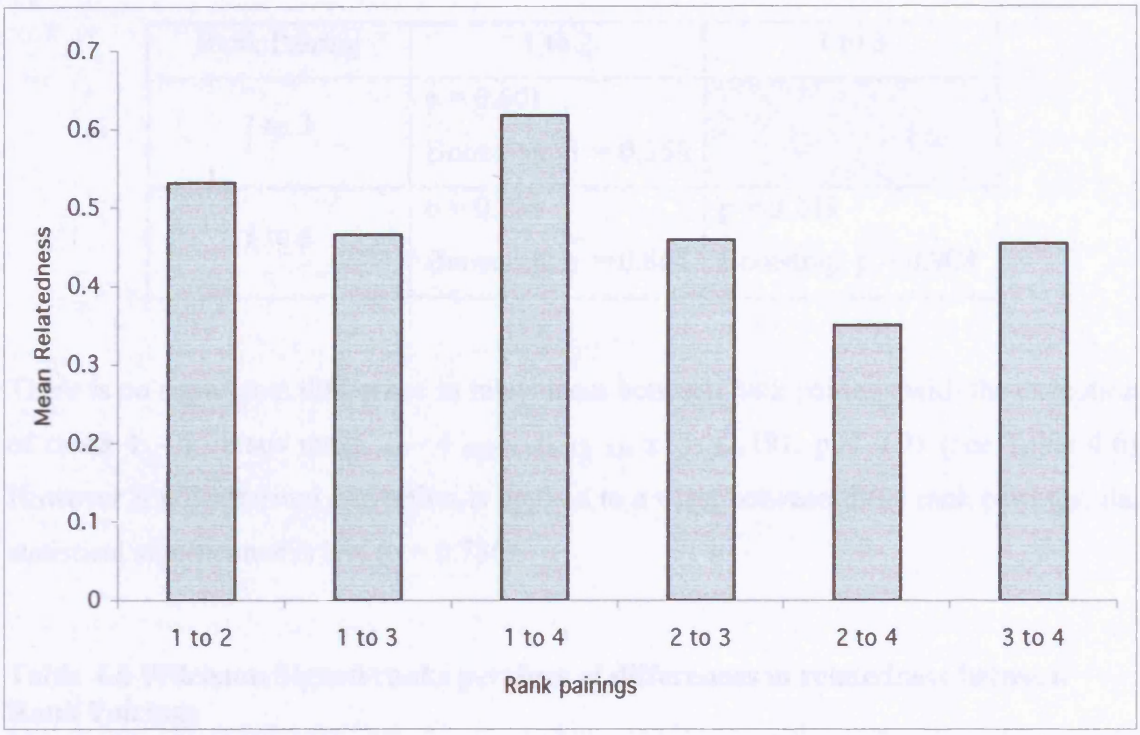


Table 4.5 Means and Wilcoxon Signed Ranks p-values of differences in relatedness distributions between different ranks to the dominant

Rank Pairing	1 to 2	1 to 3
1 to 3	p = 0.601 Bootstrap, p = 0.358	
1 to 4	p = 0.239 Bootstrap, p = 0.864	p = 0.248 Bootstrap, p = 0.908

There is no significant difference in relatedness between rank pairings with the exception of ranks 1 - 4 versus ranks 2 – 4 $_{(0.05)\ (2)\ (12,\ 12)}$ $z = -2.191$, $p < 0.05$ (see Table 4.6) However if a Bonferroni correction is applied to a t-test between these rank pairings, the statistical significance is lost ($p = 0.786$).

Table 4.6 Wilcoxon Signed ranks p-values of differences in relatedness between Rank Pairings

Rank Pairing	1 to 2	1 to 3	1 to 4	2 to 3	2 to 4	3 to 4
1 to 2						
1 to 3	0.601					
1 to 4	0.239	0.248				
2 to 3	0.639	0.906	0.155			
2 to 4	0.990	0.213	**0.028**	0.534		
3 to 4	0.959	0.722	0.128	0.721	0.214	

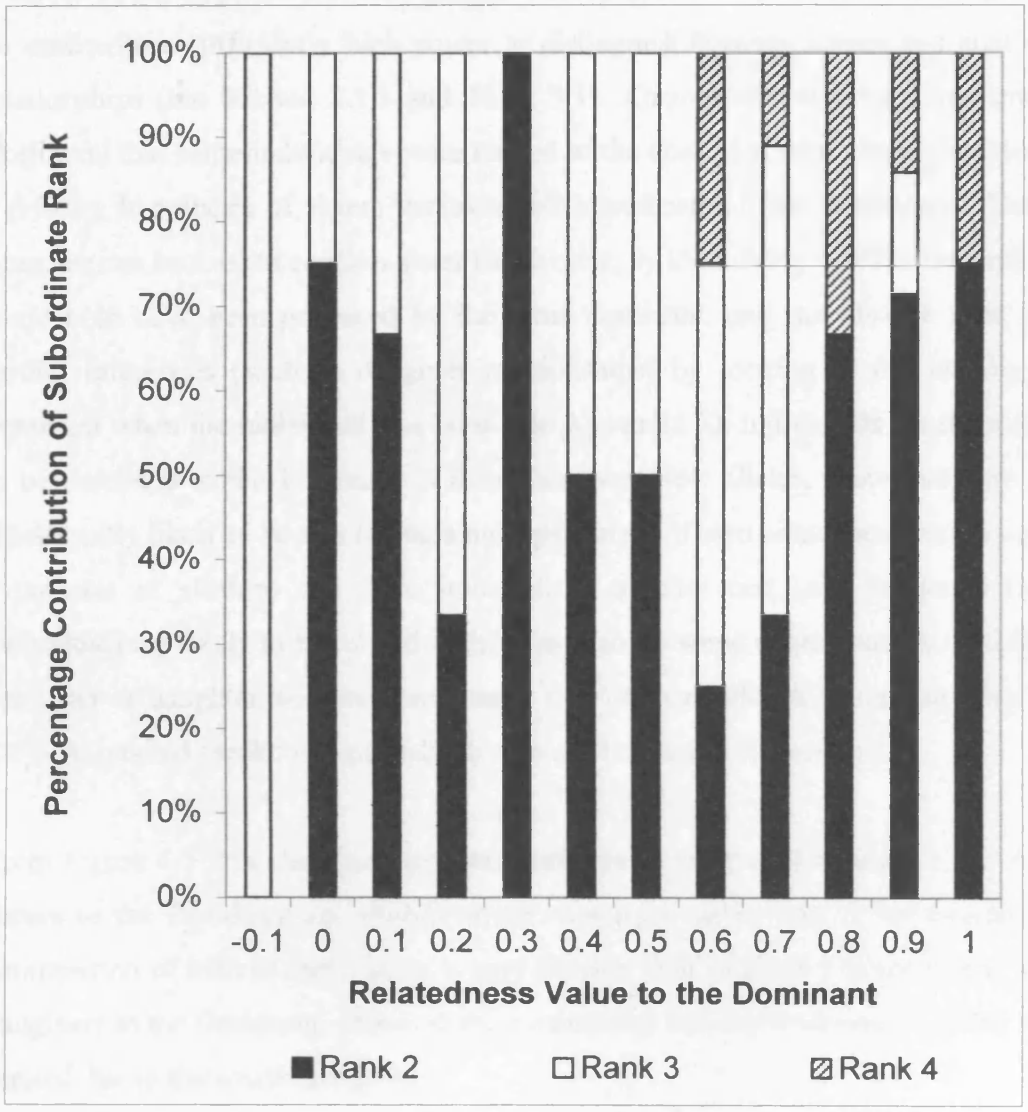
4.4.3 Trends in Relatedness between Ranks in their Relatedness to the Dominant

No significant difference was found between any of the ranks in their relatedness to the dominant. However, there are a number of trends in the distribution of relatedness to the dominant which can be seen across the ranks and which are worth describing in a little more detail. Such trends are clearly illustrated by Figure 4-4, which shows the proportion that each rank contributes to a particular relatedness level with the dominant. For example, Rank 2 shows a wide spread of relatedness values to the dominant, from 0 values, which indicate unrelatedness to the dominant, to highly related values of up to 1. Rank 3 shows a similar spread, although highly related values of 0.8 and 1.0 are absent. However, Rank 4 has no lower relatedness values and is therefore limited to high values of 0.6 onwards. Such high values for rank 4 would indicate that this group is mainly composed of daughters or sisters to the dominant (see Figure 4-4)

All of the dominant- subordinate pairing groups show a spread of relatedness values and thus indicate that each of the groups are composed of a number of relations to the Dominant, from more weakly related cousins to strongly related sisters. None of the groups are composed of one specific range of relatedness values.

Figure 4-4 Percentage proportion that each Subordinate Rank contributes to each Relatedness Value with the Dominant (within their nest) in 2001.

Rank 1 –2 (n = 27); Rank 1 – 3 (n = 22); Rank 1 – 4 (n = 12).

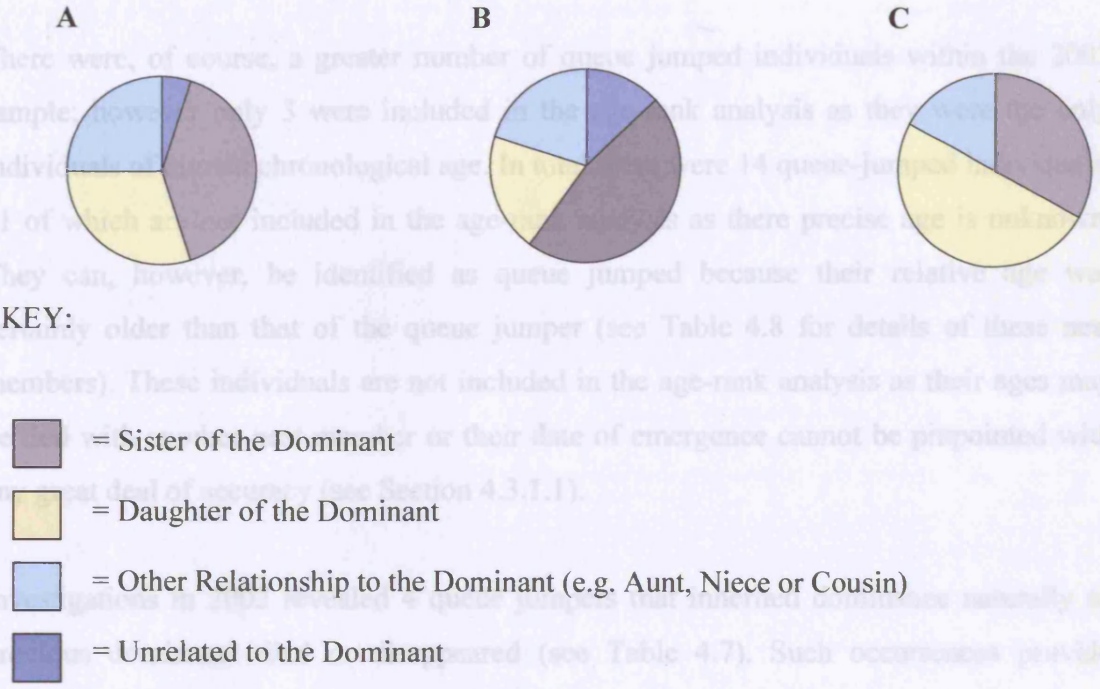


4.4.4 Rank and Kinship

A combination of Kinship and census data was used to establish the most likely relationship between each subordinate and their dominant. *Kinship* uses likelihood methods to test hypothesised relationships among individuals: however *Kinship* can only be used with a sufficiently high power to distinguish between sisters and aunt – niece relationships (see Section 3.1.3 and Table 3.1). *Kinship* was also used to identify the likelihood that some individuals were related to the dominant rather than unrelated using a primary hypothesis of sisters versus a null hypothesis of zero relatedness. Data from censuses can be used to confirm sister likelihoods, by identifying whether two individuals could both have been produced by the same dominant, and can also be used to draw further inferences (such as daughter relationships) by looking at the identity of the dominant when the individual was born (see Appendix 3). Individuals are deemed likely to be unrelated to the Dominant if they share very few alleles, show that they are not significantly likely to be sibs (using a null hypothesis of zero relatedness versus a primary hypothesis of sibship) and have joined from another nest (see Section 3.4.6.4). If individuals are likely to be related to the Dominant to some degree but are unlikely to be her sister or daughter they are listed under the "other relationship" category (see Figure 4-5). A detailed breakdown of each sib ship can be seen in Appendix 3.

From Figure 4-5 it is clear that unrelated individuals exist within Ranks 2 and 3. Likely sisters to the Dominant are slightly more numerous within rank 3 but overall the kin composition of both of these ranks is very similar. Half of Rank 4 is composed of likely daughters to the Dominant. However the conclusions that can be drawn from this rank are limited due to the small sample size.

Figure 4-5 Proportion of Relationships that Each Rank shares with the Dominant (compiled from all nests). A = Rank 2 (n = 27); B = Rank 3 (n = 22); C = Rank 4 (n = 12).



4.4.5 Do individuals Queue Jump?

From Table 4.3, which shows all individuals of known chronological age in 2001, it is possible to see that 9 individuals did not hold the expected inheritance rank for their relative age. Of these females, 6 were queue jumpers and these are detailed in Table 4.7. The remaining 3 individuals were queue jumped (see Table 4.3 and Table 4.7)

There were, of course, a greater number of queue jumped individuals within the 2001 sample: however only 3 were included in the age-rank analysis as they were the only individuals of known chronological age. In total there were 14 queue-jumped individuals, 11 of which are not included in the age-rank analysis as there precise age is unknown. They can, however, be identified as queue jumped because their relative age was certainly older than that of the queue jumper (see Table 4.8 for details of these nest members). These individuals are not included in the age-rank analysis as their ages may be tied with another nest member or their date of emergence cannot be pinpointed with any great deal of accuracy (see Section 4.3.1.1).

Investigations in 2002 revealed 4 queue jumpers that inherited dominance naturally as previous dominants died or disappeared (see Table 4.7). Such occurrences provide valuable evidence that queue jumping is not an artefact of artificial dominant removal.

Table 4.7 Queue Jumpers found in 2001 and 2002 investigations

Year	Wasp	Site	Nest	Relative Age	Inheritance Rank	Mode of Inheritance
2001	RBrDGO	2	105	5	4	Removal
2001	YYRW	4	16	4	3	Removal
2001	YWWW	4	102	4	3	Removal
2001	WPOLB	5	103	Joiner	3	Removal
2001	DGOWW	4	103	5	3	Removal
2001	PDGPW	4	26	5	2	Removal
2001	UM	4	29	5	3	Removal
2001	RYDGR	5	118	5	3	Removal
2002	PYWD	2	7	2	1	Natural
2002	OLBD	2	7	3	2	Natural
2002	LWOW	4	9	2	1	Natural
2002	ROYO	5	61	3	1	Natural
2002	BBRB	8	8A	2	1	Natural

Table 4.8 Queue Jumping and Queue Jumped individuals from 2001

		QUEUE JUMPERS (QJ)				QUEUE JUMPED (QJD)				
Site	**Nest**	Wasp	Date of Emergence	Relative Age	Inheritance Rank	Wasp	Date of Emergence	Relative Age	Inheritance Rank	**Age Difference (days) (QJD-QJ)**
2	105	RBrDGO	29/08	5	4	ODGRO	16/07-30/07	3	5	30-44
4	16	YYRW	19/08	4	3	ODGPW	28/06	3	4	52
4	102	YWWW	16/06-28/06	4	3	PROW	27/05-12/06	3	4	16-20
5	103	WPOLB	Marked 05/06	Joined 09/09	3	DGYOR	17/08	3	4	73
						YRBrLB	27/08	4	5	83
4	103	DGOWW	19/06	5	3	YYDGW	27/05-16/06	3	4	3-23
						RWBrW	27/05-16/06	3	5	3-23
4	29	UM	29/08-06/09	5	3	WYOY	22/05	2	4	96-107
						YWOW	25/06	3	5	65-73
5	118	RYDGR	20/08	5	3	PWOLB	05/07-08/07	2	5	46-49
						YDGRLB	05/07-08/07	2	4	46-49
4	26	PDGPW	20/06-22/06	5	2	DGYRW	Marked 05/04	1	3	77-79
						OYBrW	13/06-19/06	3	4	3-7
						RRPW	13/06-19/06	4	5	3-7

Table 4.9 Queue Jumping and Queue Jumped Individuals from 2002.

		QUEUE JUMPER (QJ)				QUEUE JUMPED (QJD)				
Site	**Nest**	Wasp	Date of Emergence	Relative Age	Inheritance Rank	Wasp	Date of Emergence	Relative Age	Inheritance Rank	**Age Difference (days) (QJD–QJ)**
2	7	PYWD	2002	2	1	PiORD	2001	1	3	> 360
4	9	LWOW	24/05	2	1	WDYW	Marked 29/04	1	2	>36
5	61	ROYO	04/06	2	1	OWDO	Marked 09/05	1	2	>26
8	8A	BBRB	23/07	2	1	OLRB	01/06	1	2	53

4.4.6 Age Differences between Queue Jumpers and Queue Jumped Individuals

From Table 4.8 and Table 4.9 it can be seen that the minimum difference between queue jumpers and their queue jumped nestmates is 3 days. This occurs in only 4 instances with most differences being of at least 20 days. Therefore it is unlikely that the relative ages between the queue jumpers and queue jumped could be confused. If two wasps hatched out at a close interval of 1 – 2 days, there is a chance that these wasps may be mis-assigned relative ages especially if the first to hatch out leaves the nest so quickly that it cannot be marked and then later returns to coincide with another hatching event.

4.4.7 Relatedness of Individuals upon Nests in which Queue Jumping has taken place

There are 2 hypotheses that will be addressed when investigating the relatedness between queue jumpers and their nestmates:

I. Queue Jumpers are unrelated to their nestmates, in particular those ahead of them in the queue

If the queue jumper is a joining wasp it may face the greatest pressure to achieve dominance and thus direct reproduction (see Section 3.1.6 and 4.1.5)

II. Queue Jumped individuals are less related to the rest of their nestmates than the Queue Jumper.

If the queue jumper is more closely related to its fellow nest members (that it does not queue jump) than those it queue jumps it may face little opposition in its bid to achieve dominance. The rest of the nest may support a more closely related individual achieving dominance in order to increase their indirect fitness.

A number of analyses are detailed below in order to answer these questions. Such tests are conducted using *Relatedness* (Queller & Goodnight 1989). Adjacent to each relatedness test category a Roman numeral is included, within brackets, corresponding to one of the questions above in order to make clear the reason for the analysis.

4.4.7.1 Comparison of Relatedness of Queue Jumpers to Nest Mates (across Queue Jumping Nests Only)

Table 4.10 shows the intranidal relatedness values of each queue jumper to its queue jumped nestmates and age corresponding to rank nestmates. When a queue jumper was able to jump over more than one wasp, the average relatedness of the queue jumper to the queue jumped individuals is listed. Due to the normality of each relatedness distribution (see Table 4.11) and low level of variance between distributions (Levene $_{(0.05)} = 0.418$) a One Way Anova was used to examine the significance of these results.

There was no significant difference between the relatedness pairings: queue jumper to queue jumped (I), queue jumper to age corresponding to rank (II), queue jumped to age corresponding to rank (II) and queue jumpers to age corresponding to rank plus queue jumped (II) (One Way Anova $_{(0.05)(7, 7, 8, 8)}$ $F = 0.637$, $p > 0.5$).

Table 4.10 Intranidal Relatedness between Queue Jumpers (QJ), Queue Jumped (QJD) and Age Corresponding to Rank members (NQJ). Roman numerals within brackets correspond to the hypothesis they are testing (See Section 4.4.7).

Site	Nest	QJ to QJD (I)	QJ to NQJ (II)	QJD to NQJ (II)	QJ + NQJ to QJD (II)
2	105	0.803	0.803	0.701	0.721
4	16	0.630	0.444	0.291	0.392
4	26	0.116	0.052	0.590	0.415
4	29	•	•	0.476	0.476
4	102	0.813	0.766	0.747	0.757
4	103	0.637	0.819	0.723	0.692
5	103	0.424	0.136	0.178	0.209
5	118	0.452	0.451	0.770	0.729

Table 4.11 Mean Relatedness of Different Queue Jumper Pairings.

Queue Jumper Pairing	n	Mean Relatedness	Shapiro-Wilk Test of Normality
QJ to QJD	7	0.55	0.446
QJ to NQJ	7	0.50	0.195
QJD to NQJ	8	0.56	0.137
QJ + NQJ to QJD	8	0.55	0.181

4.4.7.2 Comparison of Relatedness of Queue Jumpers to Nest Mates (Across all Nests)

The degree of relatedness between queue jumping and corresponding queue jumped wasps was compared with controls, which consisted of relatedness values between ranks 1 and 2 on all nests where queue jumping did not occur. Ranks 1 and 2 were chosen as controls because these ranks are present upon all of the nests and can therefore provide a larger sized sample (see Figure 4-1).

There was no significant difference in relatedness between queue jumpers and those they jumped and between ranks 1 and 2 of the controls where no queue jumping had taken place ($t_{(0.05)(2)(19,8)}$ = -0.033, $p > 0.5$). Equal variance was assumed with Levene $_{(0.05)}$ = 3.528, $p > 0.5$.

4.4.8 Relatedness of Queue Jumpers to fellow nestmates using *Kinship*

Using *Kinship* it is possible to see the most likely relationship that may exist between each of the individuals upon a queue-jumping nest (see Chapter 3 for more details). The results here show that in 5 of 7 nests in which queue jumping took place, the queue jumper was more likely to be a sister to the wasp that it queue-jumped than an aunt or niece (see Appendix 3). In 1 of the 7 nests the queue jumper relationship was more uncertain and it could have been either a sister or aunt to the queue jumped. Finally, in 1of the 7 nests the queue jumper was likely to have been unrelated to the queue-jumped individual (see Table 4.12).

The size of the sibships in which queue jumpers existed does not seem to be important in predicting queue jumping behaviour as sizes varied from 0 to 5 individuals (see Appendix 3).

Table 4.12 Most likely Relationships that exist between Queue Jumpers and those they Jumped (*when using a Primary hypothesis of Sister - Sister and a Null hypothesis of Aunt – Niece at the $p < 0.05$ level).

Site	Nest	Queue Jumper (QJ)	Queue Jumped (QJD)	Most likely relationship of QJ to QJD*	Most Likely relationship using Census Data
2	105	RBrDGO	ODGRO	Sister	Sister
4	16	YYRW	ODGPW	Sister	Sister
4	15	YWWW	PROW	Sister	Sister
5	103	WPOLB	DGYOR	Not Sisters	Unrelated
			YRBrLB	Not Sisters	Unrelated
4	103	DGOWW	YYDGW	Sister	Sister
			RWBrW	Sister	Sister
4	29	UM	WYOY	?	?
			YWOW	?	?
5	118	RYDGR	PWOLB	Sister	Sister
			YDGRLB	Sister	Sister
4	26	PDGPW	DGYRW	Not Sisters	Sister
			OYBrW	Not Sisters	Sister
			RRPW	Not Sisters	Sister

4.4.9 Are Queue Jumpers Significantly Different in Body Size to their Nestmates?

A t-test was carried out to identify whether queue jumpers differ significantly in size from their nestmates. There was no significant difference in wing length, and therefore body size, between individuals ($t_{(0.05)\,(2)\,(7,\,12)} = -1.054$, $p > 0.1$) (See Table 4.13). Equal variances were not assumed.

Table 4.13 Wing cell length of queue jumping and queue jumped individuals. * If more than one wasp was jumped over in the queue the average of their wing cell lengths was taken.

Queue Jumper ID	Wing Cell Length (mm)	Queue Jumped ID	Wing Cell Length (mm)*
RBrDGO	4.40	ODGRO	4.16
YYRW	4.43	ODGPW	4.51
YWWW	4.22	PROW	4.11
DGOWW	4.14	RWBrW	4.28
		YYDGW	
WPOLB	4.35	DGYOR	4.14
		YRBrLB	
RYDGR	4.42	PWOLB	4.08
		YDGRLB	
PDGPW	4.14	DGYRW	4.27
		OYBrW	
		RRPW	

4.4.10 Behaviour of Queue Jumpers after they inherit Dominance (2002)

Figure 4-6 to Figure 4-9 illustrate the behaviours of queue jumpers and their nestmates. It was only possible to look at behaviour after the queue jump to dominance had taken place due to the difficulty of predicting queue jumping and the intensive nature of the behavioural observations.

In 2002, four behaviours were focused upon, namely aggressive behaviour received and initiated, the number of foraging returns that an individual receives from returning nestmates and the number of cell inspections that are carried out. These behaviours are the most common and are easily noted. Queue jumping was only found upon four nests and therefore statistical tests would be severely limited in their power to identify any difference in behaviour between individuals on queue-jumping and non-queue-jumping nests. Each queue-jumping nest was compared with a control nest (see Section 4.3.3.1) of a similar group size. Behaviour depends, to some extent, upon rank as subordinates carry out more foraging and dominants have been noted to be more aggressive (Samuel 1987; pers obs.) Therefore behaviour was compared between individuals of the same rank upon both nests.

Overall, the results show that queue jumpers tend to be more active than their non-queue jumping counterparts in both initiation and reception of aggression. Such aggression was directed towards rank 2 individuals more frequently than any other ranks. However it is not possible to determine whether this behaviour made it possible to queue jump or whether it is a result of defending the dominant position once the queue jump has taken place.

4.4.11 Can Queue Jumping be predicted?

Overall, seventy nests were censused over a period of six months to identify queue jumping individuals (see Section 4.3.3). Therefore, census data could be compiled upon the foraging effort of each queue-jumping individual; their nestmates and their corresponding control nests (see Figure 4-10 to Figure 4-17). In analysing these census data it may be possible to find whether queue jumping individuals exhibit any particular kind of behaviour before they inherit dominance.

Control Nest 8 (Site 8) versus Queue Jumping Nest 8A (Site 8) (see Figure 4-10 and Figure 4-11).

Both nests have a clear age corresponding to rank relationship at the beginning of the investigation. However, when the dominant LOWY dies upon Nest 8A, age 3 BBRB appears to make an immediate bid for dominance. In control nest 8 the age corresponding to rank behaviour continues after the death of the dominant with a smooth transition to dominance by the previous second ranked individual LWBB.

Control Nest 8A (Site 4) versus Queue Jumping Nest 9 (Site 4) (see Figure 4-12 and Figure 4-13)

A clear Dominant is established at the beginning of the investigation in both of the nests. There is an obvious difference in foraging effort between age 2 YDWW and age 3 RWWW on nest 8A before the dominant OPRW dies. When OPRW dies YDWW establishes her dominance immediately because she hardly ever leaves the nest. In contrast, age 1T WDYW (T corresponds to a tied age) and age 4 LWOW (nest 9) display a similar foraging effort before the dominant ROPW dies. LWOW then takes over as Dominant immediately. Age 1T WDYW is away from the nest frequently, for approximately a month, before she returns and remains regularly upon the nest.

Control Nest 6 (Site 2) versus Queue Jumping Nest 7 (Site 2) (see Figure 4-14 and Figure 4-15)

Both nests fail to establish a Dominant earlier in the investigation. When the dominant OWWD upon Control Nest 6 dies, the previous rank 2 LPWD starts to spend less time foraging ready to establish Dominance. On queue Jumping Nest 7 a Dominant is not established until the oldest individual PiORD dies, yet she did not hold a dominant position. When PiORD dies they then establish a gerontocratic queue with the oldest individual PYWD inheriting dominance.

Control Nest 11 (Site 5) versus Queue Jumping Nest 61 (Site 5) (see Figure 4-16 and Figure 4-17)

Both nests have a clear dominant at the start of the investigation, PiBrWO on Nest 11 and DRRO on Nest 61. Unfortunately, the observations ended close to the time of DRRO's death so that the foraging effort of her nestmates could not be tracked for a sufficient amount of time. However, before the dominant's death age 4 ROYO forages substantially less than age 1T OWDO. The behavioural observations taken after the death of DRRO show that ROYO was aggressive towards OWDO, yet such aggression was not returned by this older individual. ROYO also carries out substantially more cell inspections. This behaviour is typical of a Dominant individual therefore it is likely that a queue-jumping event has occurred.

In three of the four queue-jumping nests (nest 8a, site 8; nest 9, site 4 and nest 61, site 5), the queue-jumping individual tends to forage less as the time of the Dominant's death approaches. Earlier in the investigations their behaviour does not seem to be abnormal i.e. they exhibit a suitable amount of foraging effort for their low rank. Yet, approximately one month before the Dominant dies the queue jumper forages less and either matches the foraging effort of the second oldest individual or even performs less foraging than this individual. The time of the queue jump on these nests could even be said to have occurred at this time i.e. they have queue jumped to the second rank. On the remaining

nest 7 (Site 2) the situation before the initial dominant's death is more chaotic and it is therefore hard to reach any conclusion as to how queue jumping occurred on this nest.

Figure 4-6 Number of Aggressive Acts Initiated by Individuals on Queue Jumping Nests and their corresponding Control Nests (the first digit of the Nest number refers to its Site; Black bars are Queue Jumpers, Hatched bars are Queue Jumped and Clear bars are age = Rank individuals)

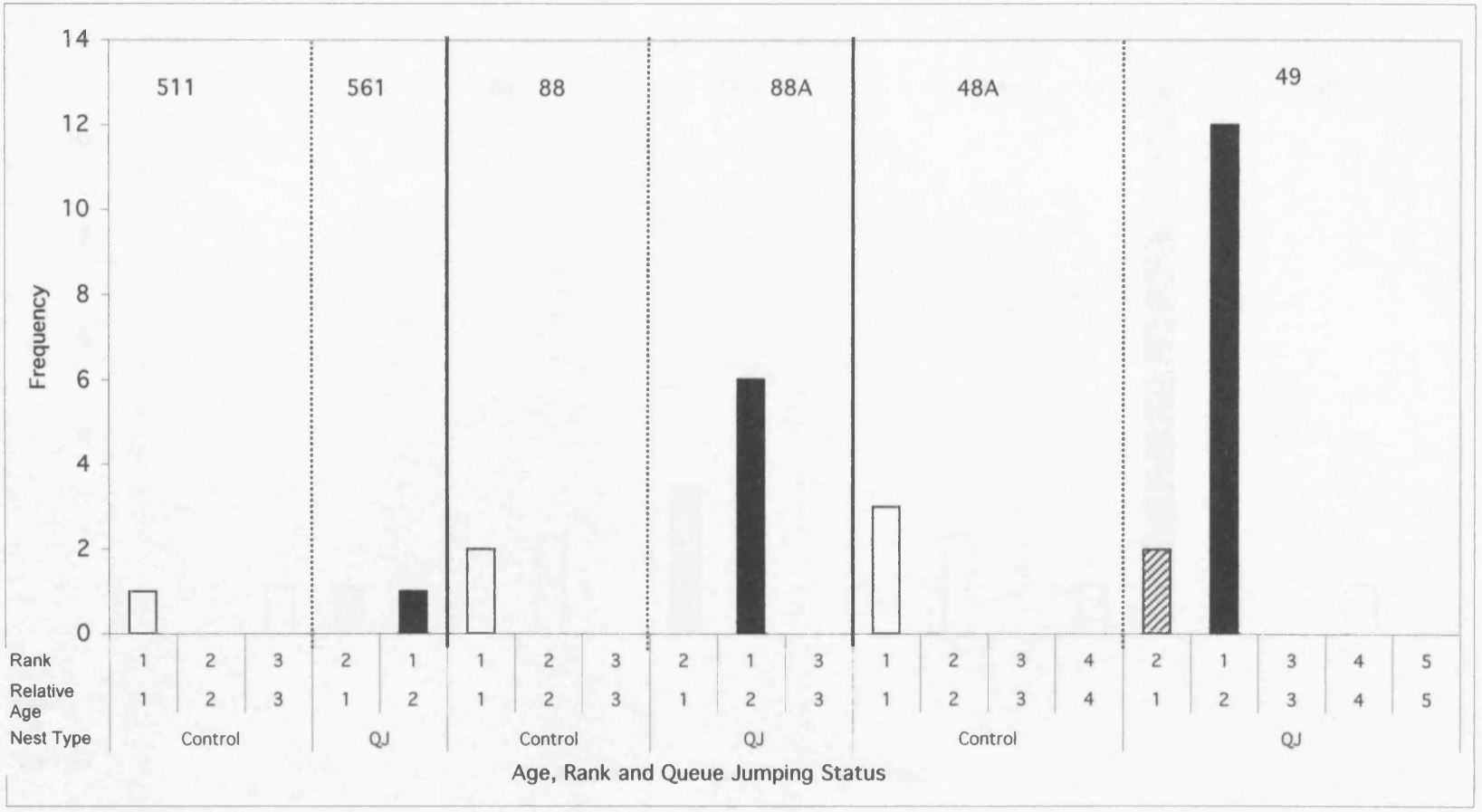


Figure 4-7 Number of Aggressive Acts Received by Individuals on Queue Jumping Nests and their corresponding Control Nests (the first digit of the Nest number refers to its Site; Black bars are Queue Jumpers, Hatched bars are Queue Jumped and Clear bars are age = Rank individuals)

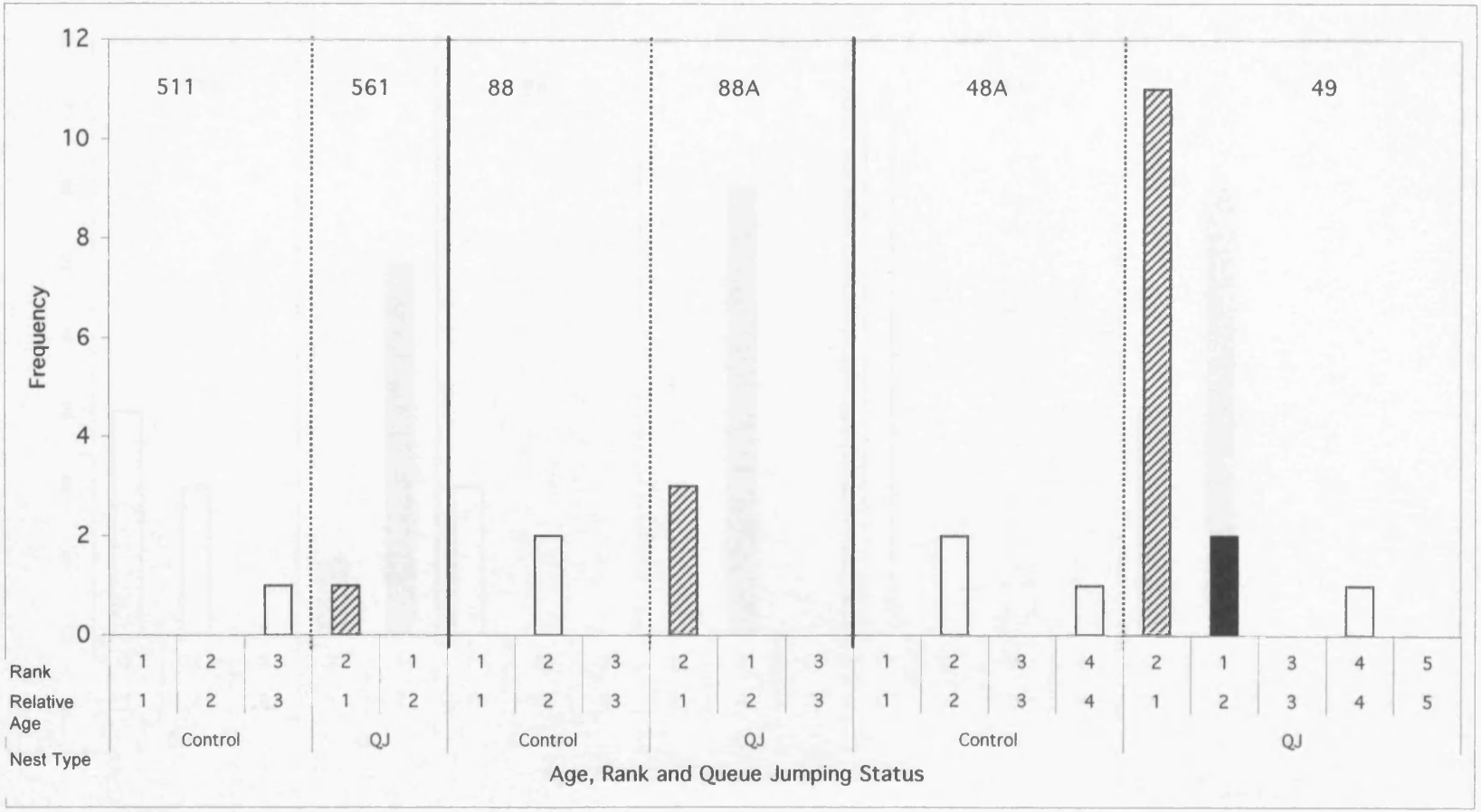


Figure 4-8 Number of Foraging Returns Met and Received by Individuals on Queue Jumping Nests and their corresponding Control Nests (the first digit of the Nest number refers to its Site; Black bars are Queue Jumpers, Hatched bars are Queue Jumped and Clear bars are age = Rank individuals)

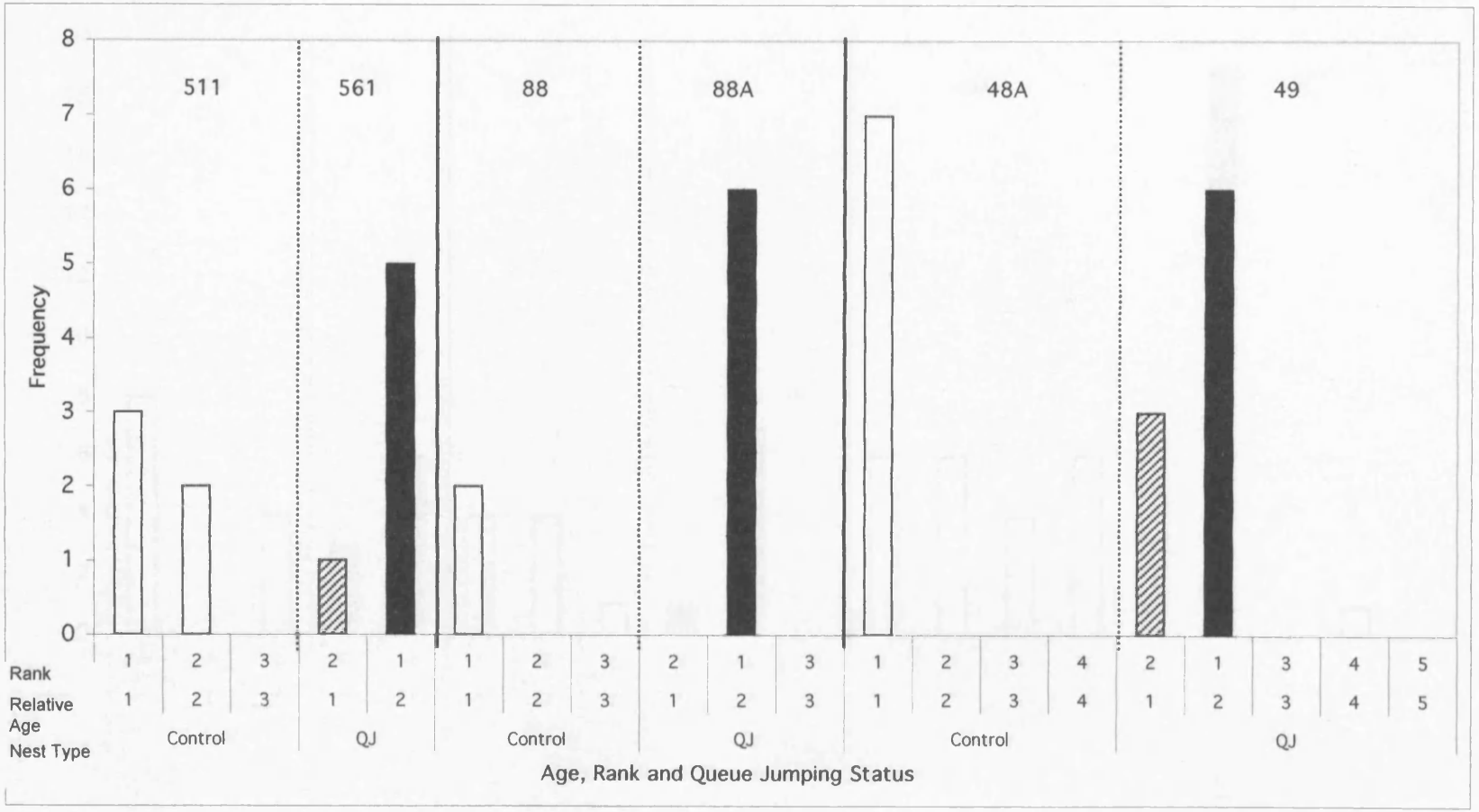


Figure 4-9 Number of Cell Inspections and/or Feedings by Individuals on Queue Jumping Nests and their corresponding Control Nests (the first digit of the Nest number refers to its Site; Black bars are Queue Jumpers, Hatched bars are Queue Jumped and Clear bars are age = Rank individuals)

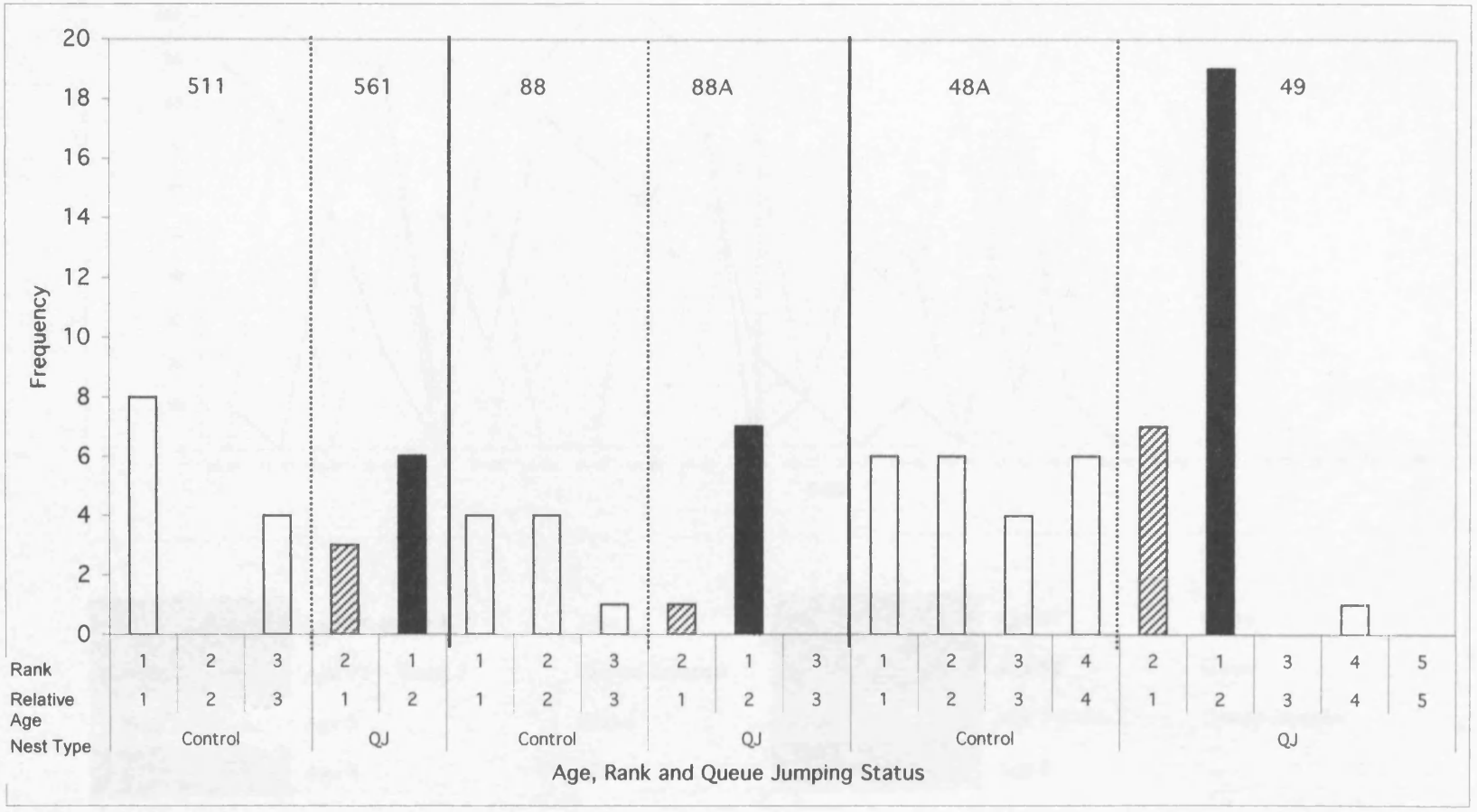
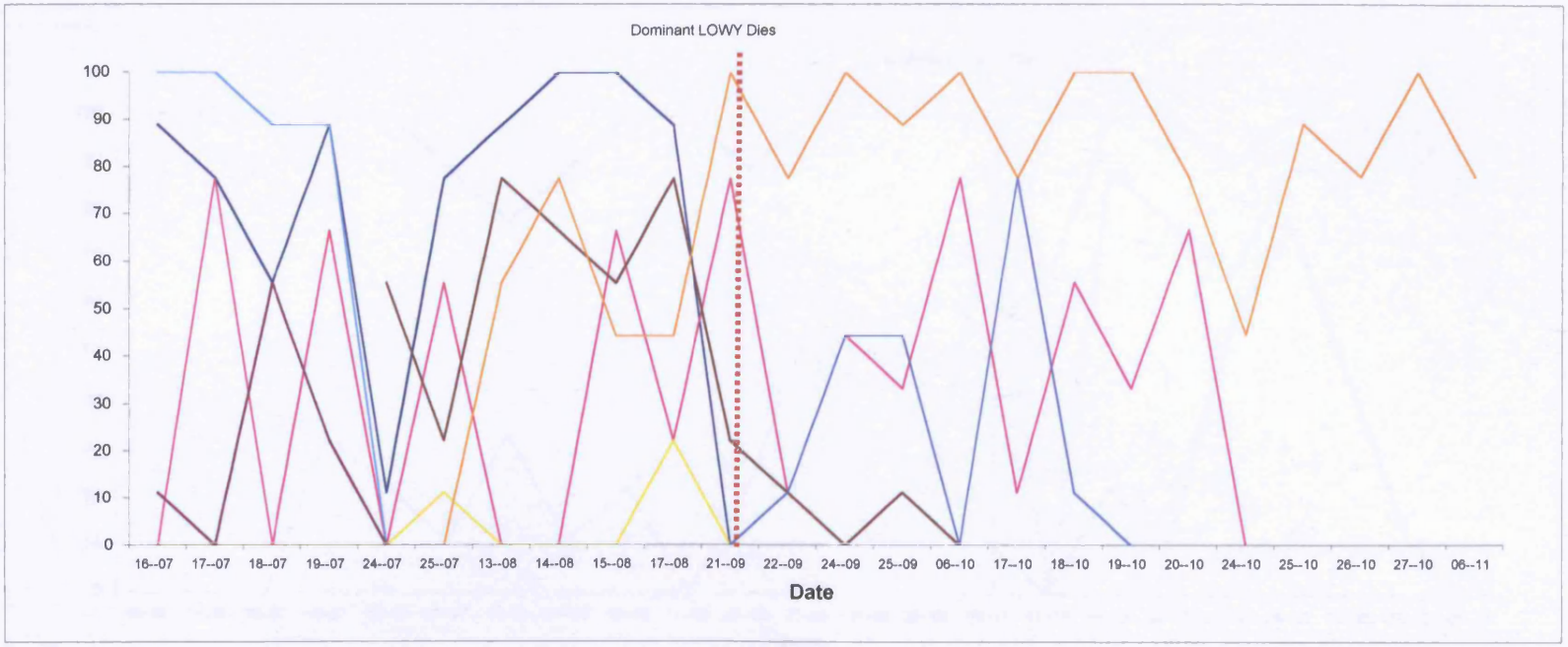
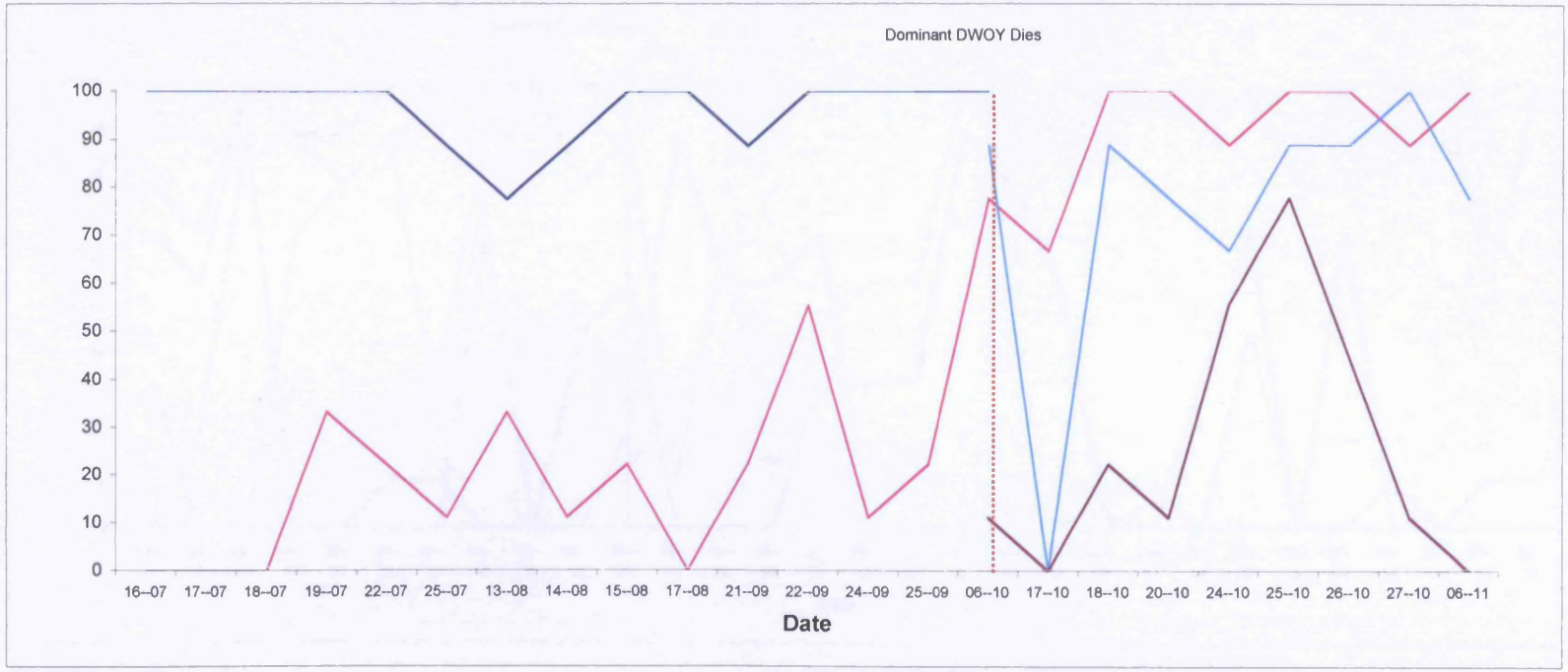


Figure 4-10 Percentage of Time on the Nest within Queue Jumping Nest 8A (Site 8)



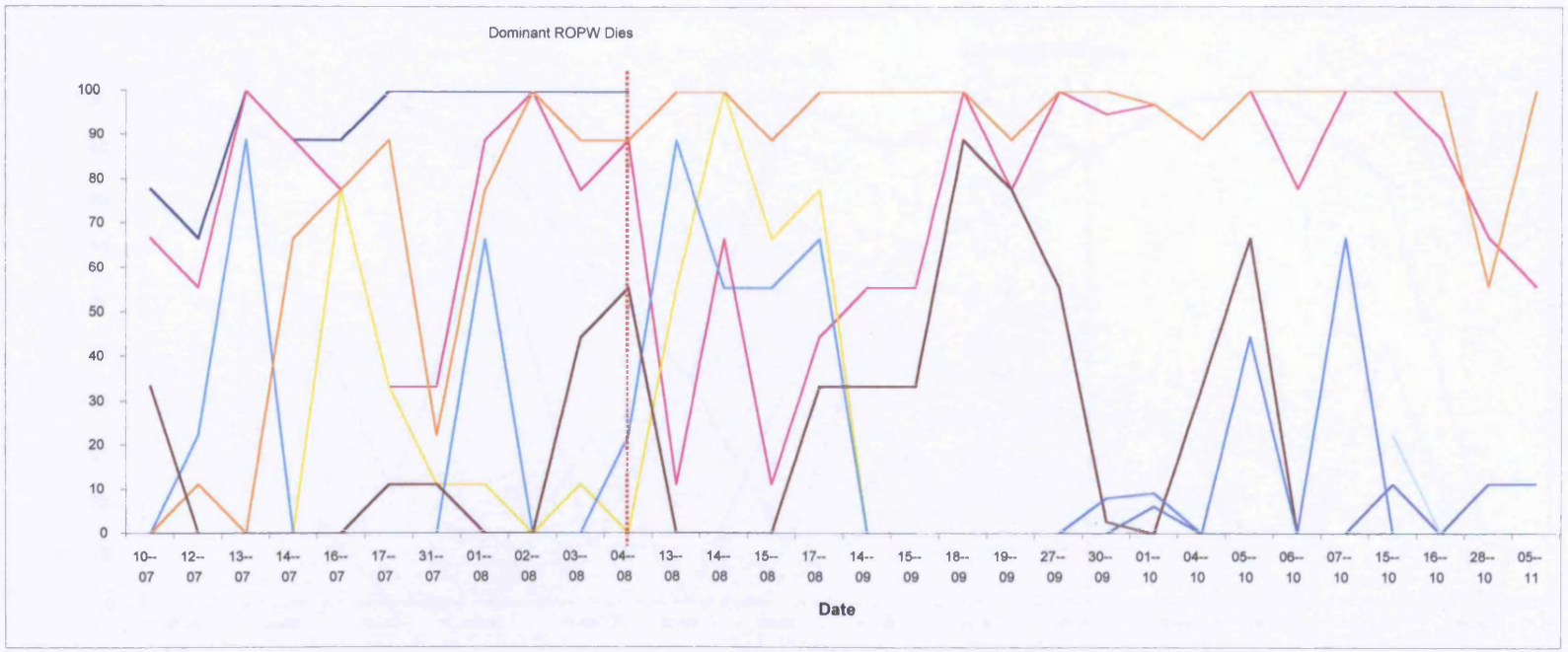
LOWY	Age 1T Rank 1	Dies	WLWB	Age 5T	Gone
OLRB	Age 1T ~ Rank 3	Queue Jumped	RPPB	Age 5T	Gone
PPLB	Age 3	Gone	BBRB	Age 7 Rank 2	Queue Jumper
RDYB	Age 4	Gone	WOL	Age 8	

Figure 4-11 Percentage of Time on the Nest within Control Nest 8 (Site 8)



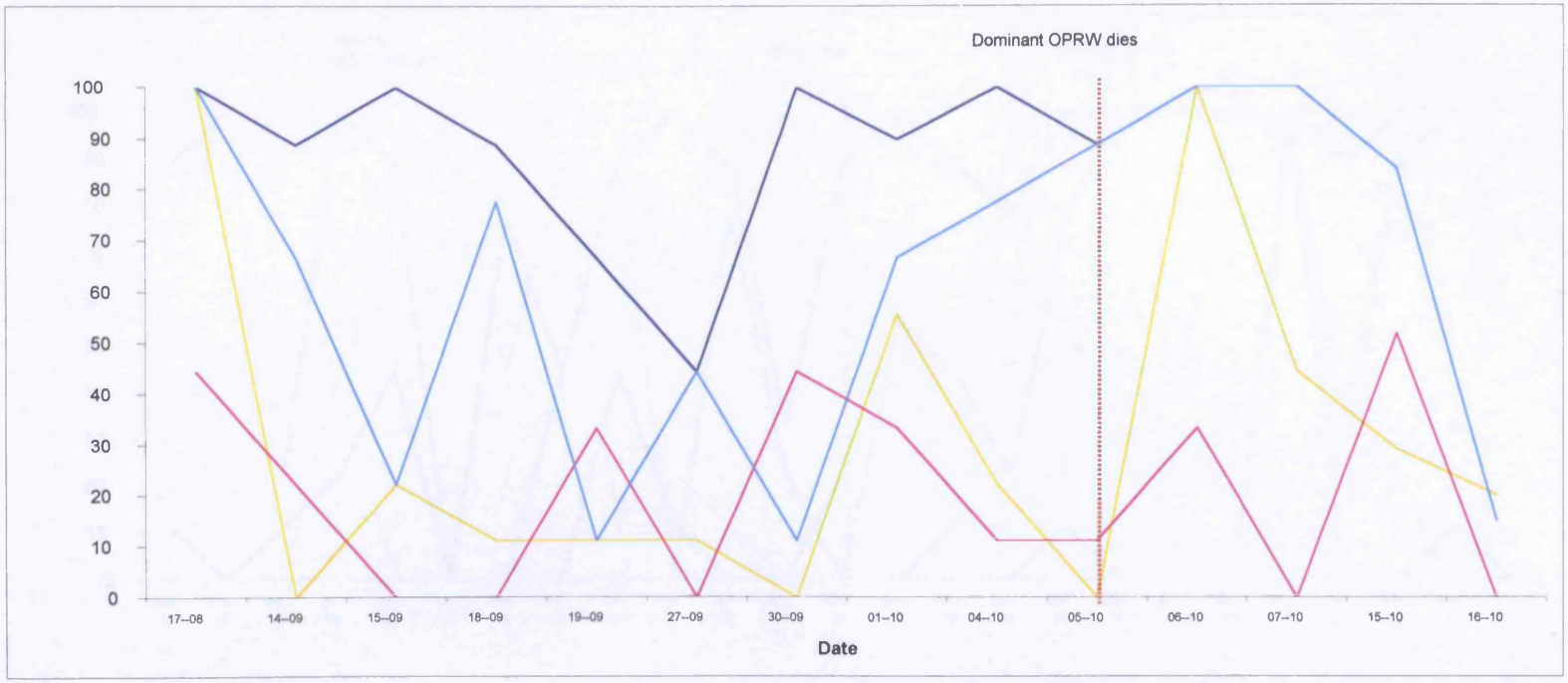
DWOY	Age 1 Rank 1	Dies
LWBB	Age 2 Rank 2	
OBPB	Age 3	
RRPB	Age 4	

Figure 4-12 Percentage of Time on the Nest within Queue Jumping Nest 9 (Site 4)



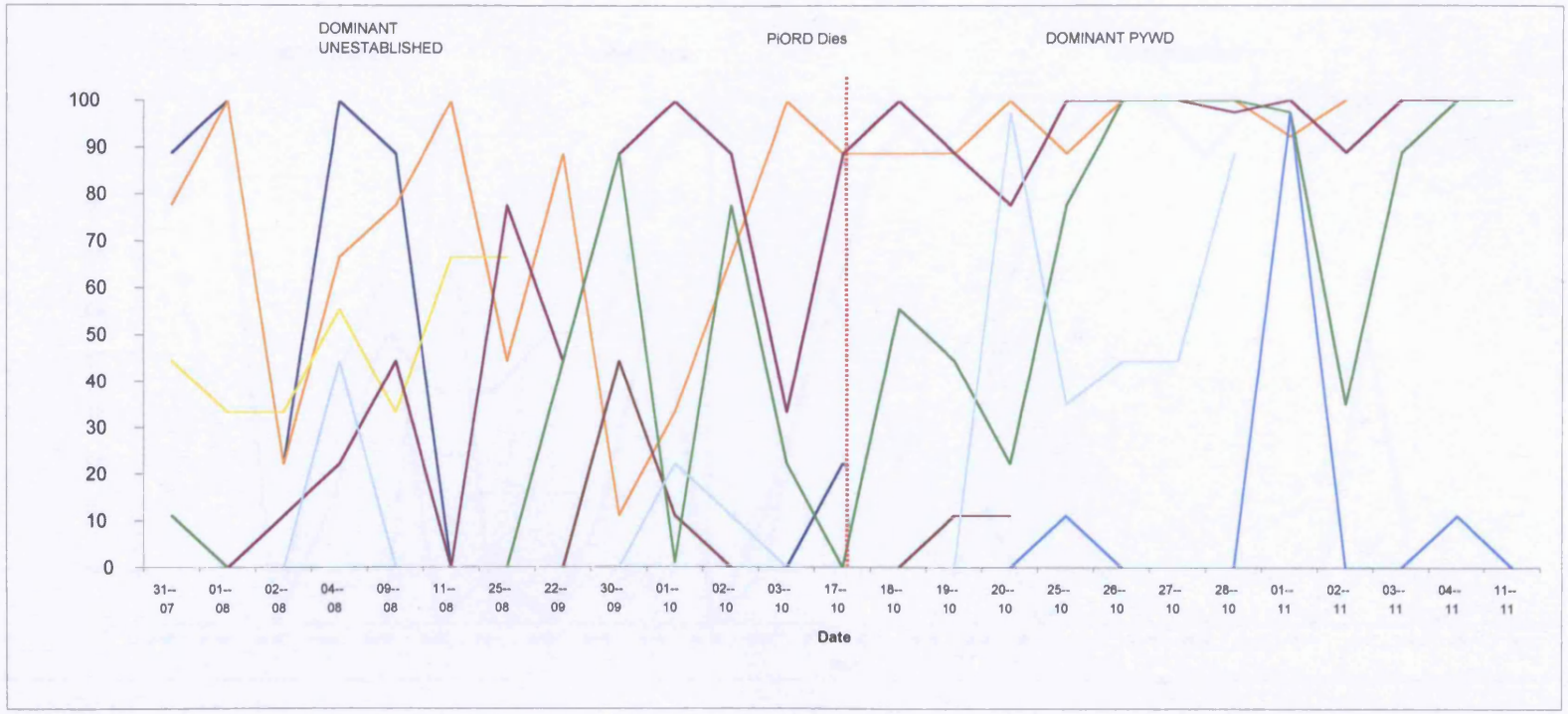
ROPW	Age 1T Rank 1	Dies	OYLW	Age 5
WDYW	Age 1T	Queue Jumped	LOR	Age 6T
YWWY	Age 1T	Gone	OLP	Age 6T
LWOW	Age 4 Rank 2	Queue Jumper		

Figure 4-13 Percentage of Time on the Nest within Control Nest 8A (Site 4)



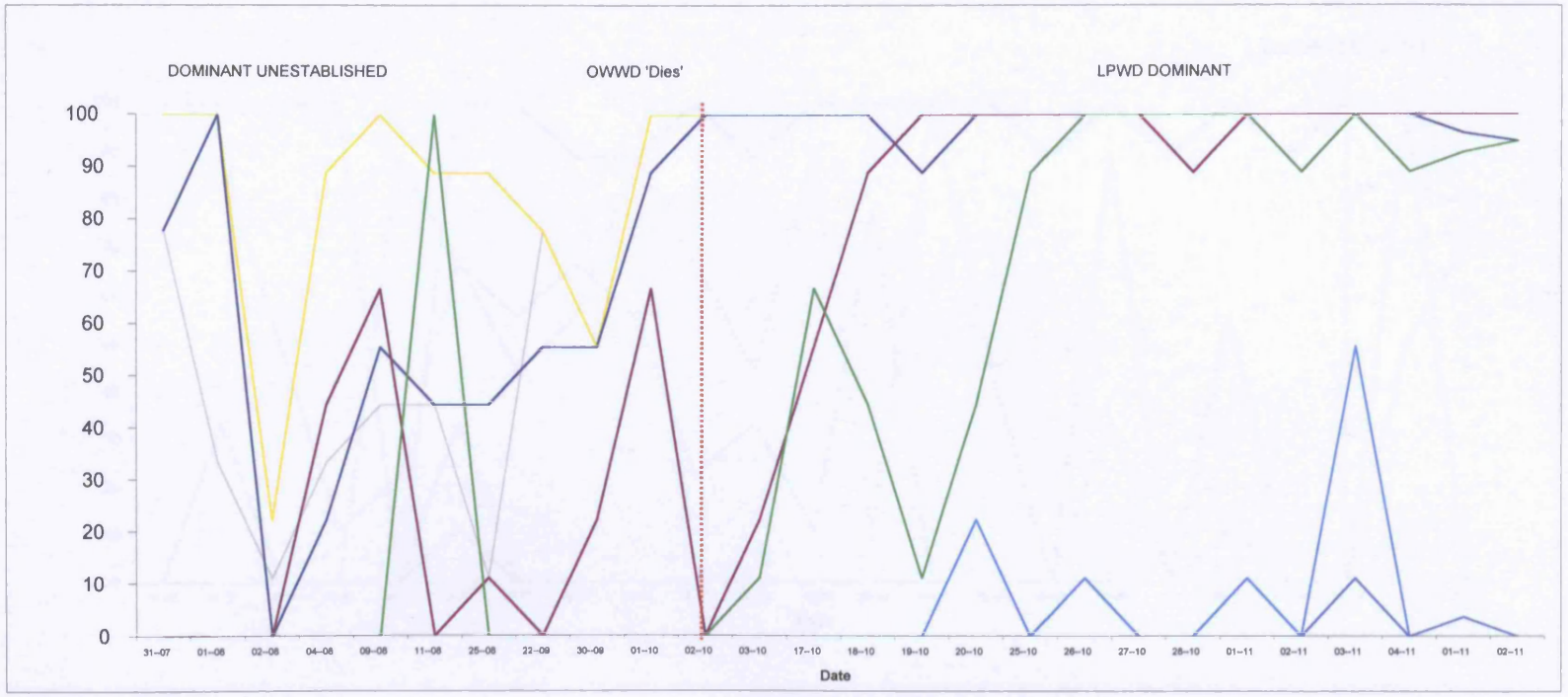
OPRW	Age 1 Rank 1	Dies
YDWW	Age 2 Rank 2	
RWWW	Age 3	
RLLW	Age 4	

Figure 4-14 Percentage of Time on the Nest within Queue Jumping Nest 7 (Site 2)



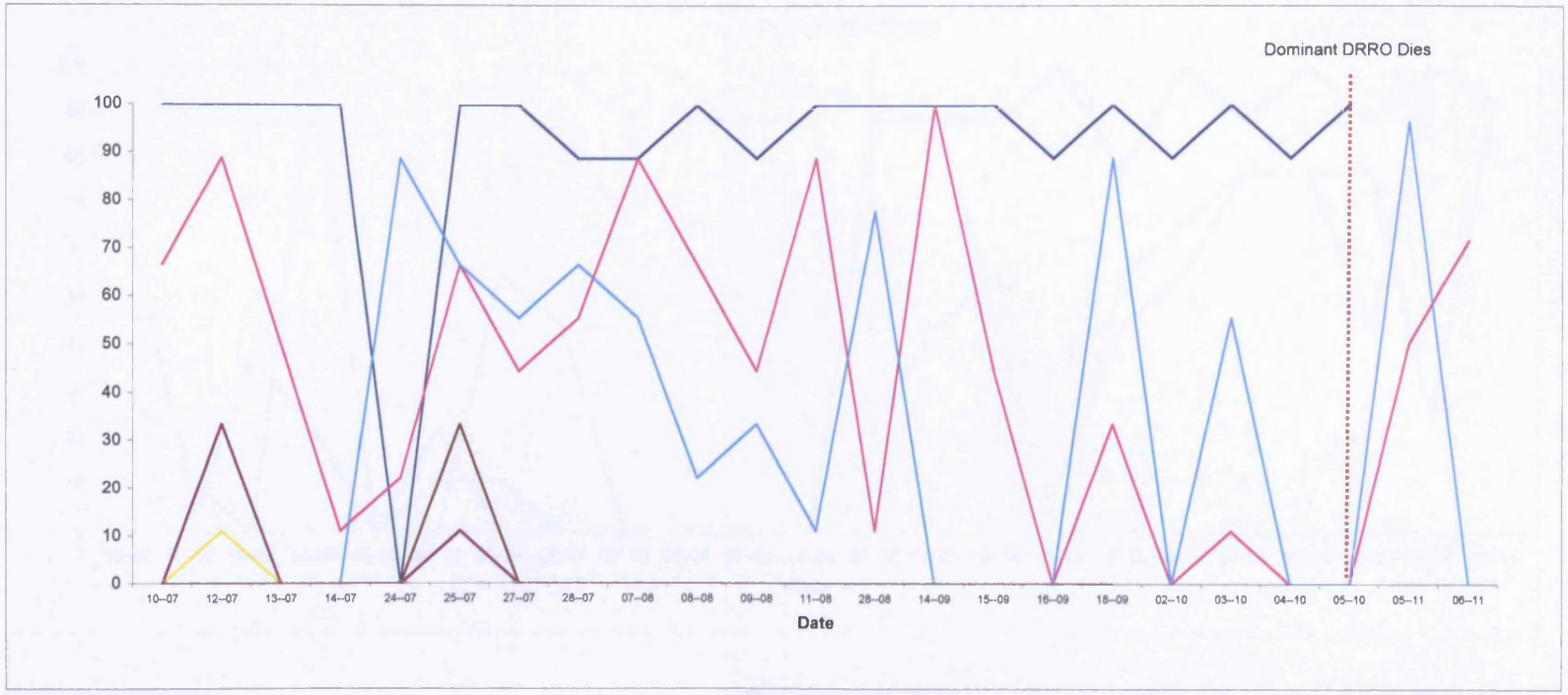
PiORD	Age 1	Queue Jumped	DOOD	Age 5	Gone
PYWD	Age 2 Rank 1	Queue Jumper	PLWD	Age 6	
YYRD	Age 3	Gone	PRL	Age 7T	
OLBD	Age 4		ROR	Age 7T	

Figure 4-15 Percentage of Time on the Nest within Control Nest 6 (Site 2)



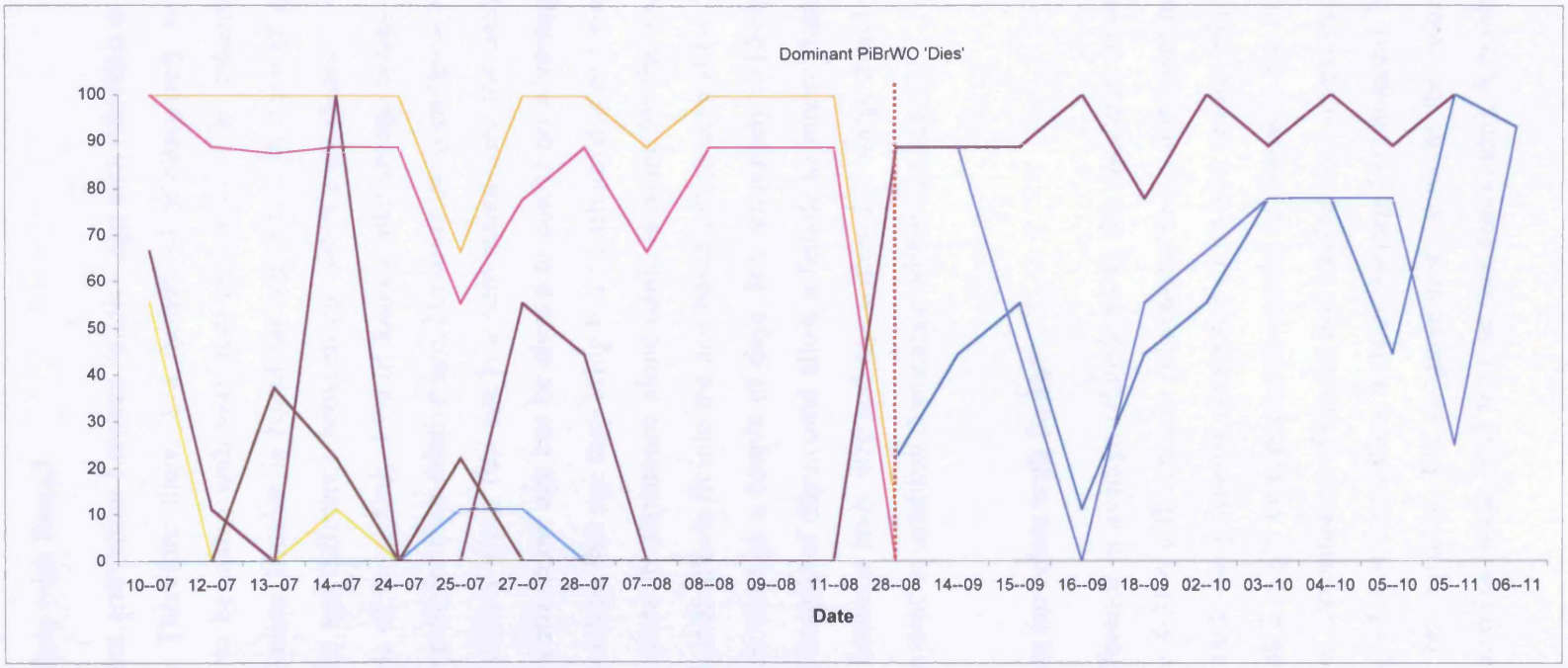
OWWD	Age 1T	Dies	PWBD	Age 5
LPWD	Age 1T Rank 1		PBB	Age 6T
WYRD	Age 1T	Gone	ROP	Age 6T
BOOD	Age 4			

Figure 4-16 Percentage of Time on the Nest within Queue Jumping Nest 61 (Site 5)



DRRO	Age 1T Rank 1	Dies	WYLO	Age 5	Gone
OWDO	Age 1T	Queue Jumped	RRWO	Age 6	Gone
PPWO	Age 3	Gone			
ROYO	Age 4	Queue Jumper			

Figure 4-17 Percentage of Time on the Nest within Control Nest 11 (Site 5)



PiBrWO	Age 1 Rank 1		OLPO	Age 5 Rank 5
ROPO	Age 2	Gone	RPPO	Age 6
RDOO	Age 3	Gone	WRBO	Age 7
LRLO	Age 4	Gone	RRDGO	Age 8

4.5 Discussion

4.5.1 How does Age correlate with Rank?

There was a highly significant correlation between relative age and inheritance rank in the nests (see Figure 4-2). Therefore there is a system of gerontocracy within *L. flavolineata,* which appears to be stable with very few exceptions. The inheritance of dominance within *L. flavolineata* females is based on seniority and interestingly this seems to hold true even if the age difference between the oldest individuals, at ranks 2 and 3, is as small as a couple of days. Only 13% of known relative age wasps did not hold the 'correct' inheritance rank for their relative age. However the correlation between age and rank does not conclusively prove that age is a convention used for establishing dominance within *L. flavolineata* unless age can be shown to confer no advantage to an individual. The one clear advantage that age may bring to an individual is experience yet it seems unlikely that differences in experience alone could account for the age-based inheritance observed. Indeed, individuals fit into the age-based queue even if the interval between their emergence is as short as a couple of days. It is extremely unlikely that a difference in experience of a couple of days could allow a female to inherit dominance. There is also no correlation between body size and age within this wasp; therefore it is likely that age is a convention used to establish dominance within this species.

4.5.2 How does Relatedness correlate with Rank?

There was no significant difference in average relatedness to the dominant between the subordinate ranks (see Figure 4-3) A difference in relatedness to the dominant might be expected if each subordinate rank was primarily composed of its own unique relationship class to the dominant e.g. rank 2 might be expected to consist of sisters to the dominant and rank 3 of nieces. However, examination of census and kinship data reveal that this is not the case. At the beginning of nest foundation, a straightforward relationship between rank and relatedness might be expected, but as generations overlap the relationship becomes more complex (see also Gadagkar 1993 for a similar discussion). A combination

becomes more complex (see also Gadagkar 1993 for a similar discussion). A combination of differential survivorship between individuals and the addition of joining, unrelated individuals might lead to the situation revealed by these results, i.e. ranks of mixed kinship with the dominant within each rank.

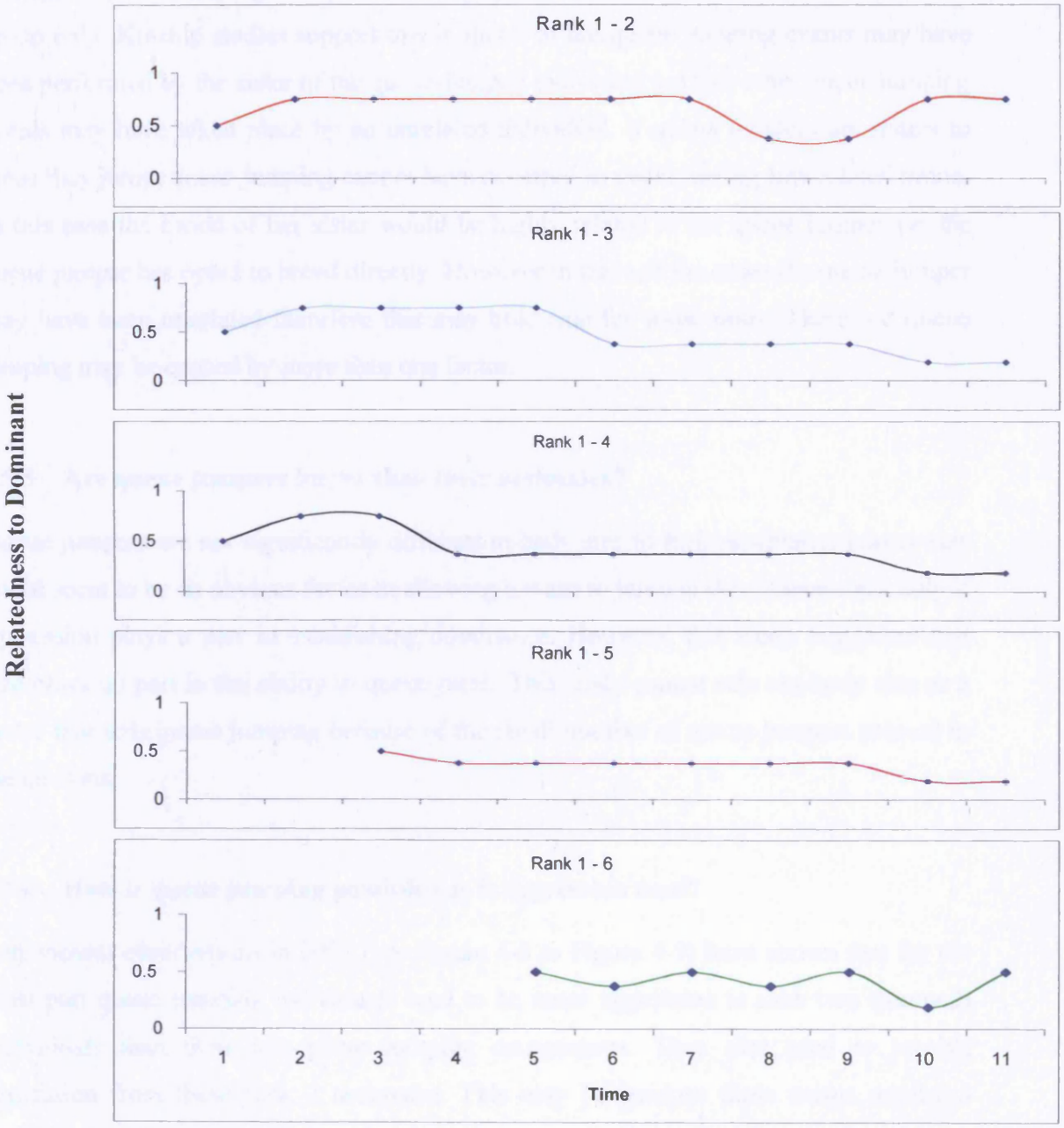
4.5.3 Relatedness to the Dominant from different ranks within a theoretical *L. flavolineata* nest

Using standard, theoretical, coefficients of relatedness for relationships between individuals, within a haplodiploid colony, a model colony can be built through which one can look at the relatedness of different ranks to a dominant individual over time. This model helps to illustrate that differential survivorship can affect the relatedness between ranks. This makes it almost impossible to predict relatedness to the dominant based on rank (see Appendix 4). In this model, only differential survivorship of subordinates has been included, with many other events such as joining and premature dominant mortality (i.e. death due to predation or disease) excluded for simplicity. These events could confound any clear relationship between rank and relatedness to an even greater extent.

From this model, it is clear that, as the nest ages; the relationship between rank and relatedness becomes a very complex one. In the beginning there is a pattern that emerges for ranks 2, 3 and 4 in which relatedness to the dominant rises from 0.5 to 0.75 then falls to 0.375 within the first three generations (see Figure 4-18). This is to be expected as the first generation produced will consist of daughters to the foundress, one of which may inherit the dominant position, in which case the rest of the nestmates will be her sisters before her own offspring emerge. Relatedness to the dominant of some of the nestmates may then fall sharply as the next sister inherits the dominant position. The daughters of the last dominant will be related to the new dominant, i.e. her sister, by 0.375 as they are her nieces. Therefore it is not difficult to see how the above pattern emerges. However, it is clear that as generations progress such a pattern disappears, leaving a complex

relatedness structure. Thus, the relatedness of a subordinate rank to the dominant heavily depends upon the age of the nest colony.

Figure 4-18 Relatedness to the Dominant of Subordinate Ranks over Time (Ranks Separate)



4.5.4 Are queue jumpers related to their nestmates?

The overall intranidal relatedness values of each category, 'queue jumper', 'queue jumped' and 'age corresponds to rank' show no significant difference between groups. Therefore queue jumping is unlikely to take place by individuals from one relatedness group only. Kinship studies support this in that 5 of the queue jumping events may have been performed by the sister of the queue-jumped individual yet two other queue jumping events may have taken place by an unrelated individual. If queue jumpers are sisters to those they jump, queue jumping cannot have occurred to avoid rearing low related brood. In this case the brood of her sister would be highly related to the queue jumper yet the queue jumper has opted to breed directly. However in three of the cases the queue jumper may have been unrelated therefore this may hold true for some nests. Therefore queue jumping may be caused by more than one factor.

4.5.5 Are queue jumpers larger than their nestmates?

Queue jumpers are not significantly different in body size to their nestmates. Larger size might seem to be an obvious factor in allowing a wasp to jump to dominance especially if aggression plays a part in establishing dominance. However, this study suggested that size plays no part in the ability to queue jump. This study cannot rule out body size as a factor that aids queue jumping because of the small number of queue jumpers present in the analysis.

4.5.6 How is queue jumping possible e.g. is aggression used?

Behavioural observations in 2002 (see Figure 4-6 to Figure 4-9) have shown that for the most part queue jumping individuals tend to be more aggressive to rank two (jumped) individuals than their non-queue jumping counterparts. They also tend to receive aggression from these rank 2 nestmates. This may be because these wasps require a higher level of aggression to remain as dominant due to their queue jumping behaviour. However, at the present time it is difficult to establish when such aggression was used by

queue jumping individuals i.e. whether it was used to queue jump or merely to maintain their dominance. In Nest 9, Site 4, levels of aggression after the queue jump took place were particularly high compared with the other nests. The queue-jumping dominant also seemed to be particularly vigilant in cell inspection. The foraging effort data show that the queue-jumped rank 2 individual tends to remain on the nest more frequently than rank two individuals on other nests. Therefore there may be some competition for dominance. The over-zealous cell inspection by the dominant may be performed to check that the rank 2 has not laid any of her own eggs. However, this would depend on *L. flavolineata* possessing the ability to recognise relatedness to brood, which has yet to be proved.

4.5.7 Can 'queue jumping' behaviour be predicted e.g. from foraging effort or other behaviour?

Figure 4-10 to Figure 4-17 show that it is very difficult to predict whether a wasp will queue jump from its foraging effort. On two of the nests, 6 and 7 at Site 2, it is difficult to see how any gerontocratic queue could emerge from a group that initially appears to have no ranking order at all. Yet in both instances, as soon as the oldest individual dies, a gerontocratic queue is immediately established. However there does seem to be a general trend in queue jumping nests for the queue jumper to remain increasingly upon the nest as the dominant's death approaches. It may be possible that there are some cues present when a dominant is close to death. If this is the case, an opportunist wasp may be able to use this to be present upon the nest at the crucial point of dominant turnover. Such cues may include signs of illness or fatigue. However it is impossible to identify any such signs from foraging data collected here, and they may be evident only to nestmates themselves rather than to any outside observer.

One factor that queue jumpers tend to have in common in the 2001 data set (in which experimental removals took place) is that they are age five individuals. In the 2002 data set where inheritance of queue jumpers occurred naturally, they are all age two individuals. It is possible that performing removals upon the nest in an accelerated

fashion, compared to the natural situation in which dominant turnover is much slower, may have created some artificial queue jumping behaviour. However it is unclear why this should affect mainly age five individuals. The fact that queue jumping occurred naturally within the 2002 data set indicates that such cheating behaviour is not merely an artefact of artificial dominant removal.

It is evident from the results collected here that queue jumping occurs within *L. flavolineata* nests. However the mechanism by which this is possible is still ambiguous. Due to the small number of queue jumpers, this analysis cannot rule out body size or relatedness as factors in the ability to queue-jump. However, it seems likely that queue jumping is an opportunistic act, which occurs close to the point of the present Dominant's death and may be initiated by certain cues as to when this death may occur.

4.6 Conclusion

L. flavolineata has been shown here to have a strict age-based queue. The convention of dominance based on seniority is obeyed even when the interval between hatching individuals is as small as a couple of days. Such a rigid convention has been previously unproven in this as well as many other species assumed to observe the age-based rule.

There was no relationship between rank and relatedness to the dominant. *L. flavolineata* lives in an aseasonal, tropical environment and therefore can build and found nests throughout the year. Because of this, each nest lineage or pedigree has the potential to continue for a number of years. The study carried out here focused upon a number of nests, each of which may have been at a different stage in the nes'ts 'lifecycle' e.g. some nest colonies may have been recently founded whereas some 'families' may have existed upon a nest for a longer length of time. The relationship between rank and relatedness may depend greatly upon the age of the wasp's lineage (see Section 4.5.2). At the start of a nest's foundation the relationship between rank and relatedness to the dominant may be a simple one. However, as the nest ages, each rank may become composed of a number of different relatives of the dominant. This may be the reason that there was no significant difference in relatedness between any of the ranks and the dominant.

A clear relationship between rank and relatedness to the dominant may perhaps be seen in some temperate wasp species. The annual nest foundation shown in such species may produce a relationship between rank and relatedness similar to that seen in the first few generations of the theoretical *L. flavolineata* nest examined above (see Section 4.5.3) i.e. higher ranks composed of sisters to the dominant and lower ranks composed of her daughters. This, however, would depend upon a sole foundress contributing to each of the nests. If more than one female founded each nest, the relatedness structure may be as complex as those seen in *L. flavolineata.* This work illustrates the importance of factors

such as the number of foundresses and number of generations upon the nest in understanding intranidal relatedness within these organisms.

4.6.1 Can Queue Jumping be considered as Cheating Behaviour?

There may be two mechanisms by which dominance is attained in *L. flavolineata*. One may be gerontocracy and the other may yet be unknown. If this is the case, the queue jumping individuals detailed in this chapter may not be cheating but merely incorporating a second, subsidiary criterion for attaining dominance such as "use relative age unless the rank above is parasitised". It is almost impossible to test whether this may be the case as the convention in question may only be apparent to the wasp's fellow nestmates. However, queue jumping was rare in both years and it therefore seems unlikely that so few individuals possess an alternative mechanism to attain dominance. The previous foraging effort of queue jumping individuals before they achieve dominance in 2002 also indicates that the queue jump seems to be opportunistic. The 'cheating' individuals often carry out foraging effort that is appropriate for their rank at the beginning of the investigation. Their foraging effort then reduces shortly before the dominant's death. Surely, if these individuals were able to achieve dominance through the possession of another trait than age, queue jumpers would achieve dominance as soon as possible after their emergence. Queue jumping dominants also give and receive heightened amounts of aggression compared to 'normal' dominants thus suggesting that their queue jump is not merely part of another convention but is actually cheating behaviour.

5 Rank and Foraging Effort

5.1 Introduction

Variation in foraging effort between individuals is common to many cooperatively breeding and eusocial animals yet the reasons for this are unclear (Schmid-Hempel 1990; Reeve 1992; Heinsohn & Cockburn 1994; Heinsohn & Legge 1999; Clutton-Brock *et al.* 2000). Helping within cooperatively breeding and eusocial groups has been shown to provide indirect benefits through the increased production of relatives (Heinsohn & Legge 1999; see Section 1.2.4.3). Therefore, it has remained largely unclear why some individuals work to their full capacities and yet others do not. Two possible explanations suggested, to date, for such variation in helping effort concern differential relatedness of subordinates to the dominant, and variance between subordinates in the cost of helping. These explanations are detailed below:

1. Differential Relatedness to the Dominant

(See Hamilton 1964a; Grafen 1984; Kokko *et al.* 2001, 2002).

The most common explanation for variation in foraging effort, until recently, was based on variation in genetic relatedness (Hamilton 1964a). In this explanation, Emlen & Wrege (1988) and Emlen (1991) proposed that closer relatives to the dominant provide greater levels of help as these individuals stand to gain the greatest increase in their indirect fitness. An example, which was used to support this idea, came from White-fronted bee-eaters *(Merops bullockoides)*, cooperative breeders in which half of all nesting attempts are helped by non-breeders. Here, kinship was a significant predictor of whether an individual would help a breeding pair (Emlen & Wrege 1988). However, Clutton-Brock *et al.* (2000) found no such pattern in their study of helping effort within Meerkats (see Section 5.1.1). The failure to find such a pattern may be due to differences

in the cost of helping between group members or indeed the inability to detect variation in relatedness between group members.

2. Differential Costs of Helping

Costs of helping for individuals can be numerous, from weight loss to risk of predation (Helms Cahan *et al.* 2002; Schmid-Hempel & Wolf 1988; Nielsen 2001). Studies upon the cost of helping have mainly focused upon behaviours such as foraging or brood incubation (for examples see Table 5.1). It is clear that helping within groups is costly, yet the degree of cost may vary. For example, weaker members of a group could suffer greater costs through helping than stronger members.

The fact that some individuals do not work to their full capacity suggests that there is a trade-off between the amount of investment put into current helping and future fitness (Cant & Field 2001). In the case of *L. flavolineata*, the greater the amount of effort put into current helping, the greater the risk of jeopardising the chance of inheriting a dominant position. As a female *L. flavolineata* progresses in its age-based hierarchy the costs of helping are accelerated. This is because the probability of inheriting the dominant position is higher when nearer the front of the queue. Therefore, the costs of helping may be greater to higher ranks than to lower ranks, which could lead to foraging effort varying between the ranks (see also Cockburn 1998; Heinsohn & Legge 1999).

Table 5.1 Costs of Helping within different groups

Species	Cost of Helping	Reference
Lamprologus richardi	Reduced Growth	(Taborsky 1984)
Marmota marmota	Loss of Mass	(Arnold 1990)
Corcorax melanorhamphos	Loss of Mass	(Heinsohn & Cockburn 1994)
Suricata suricatta.	Loss of Mass	(Clutton-Brock *et al.* 1999, 2000.
Polistes dominulus	Mortality	(Reeve 1991; (Cant & Field 2001)
Polybia occidentalis	Mortality	(O' Donnell & Jeanne 1992)
Apis mellifera	Mortality (predation*)	(Schmid-Hempel & Wolf 1988)

*The effect of foraging effort upon honeybee lifespan was clearly illustrated by Schmid-Hempel & Wolf (1988). They were able to show that the relationship between lifespan and foraging effort follows a threshold pattern within this species. If workload was reduced, this would not increase lifespan, yet if it was increased above the average it would decrease lifespan. The cause of this reduced life span was purported to be predation.

5.1.1 Past studies on variation in helping effort

A number of previous studies have been carried out upon cooperatively breeding groups and eusocial organisms to establish whether group members exhibit any variation in foraging effort. Some of these studies are detailed below:

The White-Winged Chough (*Corcorax melanorhamphos*) is a cooperatively breeding bird that relies so heavily upon helpers it has never been seen to breed successfully in their absence (Heinsohn & Cockburn 1994). Helping, within this species, mainly takes the form of incubation and can be costly in terms of mass loss. Heinsohn & Cockburn (1994) found that the greatest costs of helping were carried by the youngest of the helpers, which are poor at foraging and consequently have to spend the most time foraging in order to feed themselves. Helping effort was shown to vary with group size in these younger helpers. In small groups younger members incubated brood as much as the older members yet in larger groups they rarely performed any incubation at all. It was concluded that this reduction in helping effort was due to the unwillingness of young birds to partake in a behaviour that is so costly to them when the behaviour can be performed by older members, which are unlikely to suffer the same costs.

A similar case to the White-Winged Chough was studied by Clutton-Brock *et al.* (1999). The study focused upon the cooperatively breeding meerkat (*Suricata suricatta*) in which babysitting is a particularly energy costly behaviour with babysitters losing up to 1% of their body weight over a 12-hour shift. Clutton-Brock *et al.* (2000) found that age and nutritional condition affected the contribution made to babysitting whereas no correlation existed between difference in contributions to babysitting and differences in relatedness to brood. They therefore suggested that helping effort was likely to be influenced by the energetic costs of helping rather than relatedness. Helping in banded mongooses (*Mungos mungo*) also takes the form of babysitting and bears similar costs to that seen in Meerkats (Rood 1974, 1983; Cant 2000; Cant *et al.* 2001; De Luca & Ginsberg 2001). Pups from several mothers, are usually born on the same day and are kept underground for the first

few weeks of their life. One or more adult individuals care for these pups, whilst the rest of the group forages (Rood 1974). Just as in suricates, Cant (2003) suggested that the propensity to babysit within this species might depend on an individual's energetic or nutritional state.

From these three studies it is clear that the amount of helping effort exerted, in the form of incubation or babysitting, is likely to depend upon the energetic cost to the individual. Indeed Clutton-Brock *et al.* (2000) found that the proportion of babysitting contributed does not depend upon relatedness to the brood, thus supporting the costs of helping hypothesis.

5.1.2 Optimum levels of help

Clearly, from the dominant's perspective, the greater the amount of helping effort she receives from her subordinates, the greater the benefit to her brood. However, if there are costs entailed by helping, from the subordinate's perspective there must be a trade-off between these costs and the benefits that could be achieved through prolonged survivorship i.e. achievement of direct fitness. This is because prolonged survivorship allows an individual to near the front of the social queue and thus increases the probability of inheritance (Field *et al* 1999; Shreeves & Field 2002). Hence, the level of helping should be adjusted accordingly to ensure maximum fitness (Helms Cahan *et al.* 2002). The optimum levels of help that should be provided by a subordinate have been discussed by Cant and Field (2001), in their kin-selection model:

5.1.2.1 **The Kin-Selection Model** (Cant and Field 2001)

A kin selection model was developed to account for variation in helping effort. The model considers a subordinate in a stable group where her investment in brood belonging to the current dominant is at a cost to her potential future reproduction. $w(h)$ denotes the expected future direct fitness of a subordinate as a function of the amount of help (h). k

(h) denotes the increase in indirect fitness of the dominant when the subordinate supplies help at level h. The inclusive fitness payoffs to the subordinate and dominant, as a function of h can then be written:

$W_{sub}(h) = w(h) + r\,k(h)$

and

$W_{dom}(h) = k(h) + rw(h)$

W_{sub} = inclusive fitness payoff to the subordinate.

W_{dom} = inclusive fitness payoff to the dominant

r = the coefficient of relatedness between the dominant and subordinate.

Helping is assumed to be costly (see Section 5.1) therefore $w(h)$ is a decreasing function of h. Thus, let

$w(h) = w_0(1 - c)$.

w_0 = the expected future direct fitness of a subordinate who stays in the group but does not help
c = the cost of helping.

Cant and Field (2001) also assumed that increasing individual investment in the current brood leads to a diminishing benefit in production. Therefore, in their model $k(h)$ is a positive decelerating function of h:

$k(h) = b(1 - e^{-qh})$

q = a parameter of the speed at which the marginal benefits of help diminish

b = the asymptotic value of the benefit conferred by the subordinate.

The optimum level of help for a subordinate to provide can then be given by substituting the expressions for w (h) and k (h) into the initial equation W_{sub} $(h) = w$ $(h) + r$ k (h). These are maximised with respect to h. This substitution results in the following two equations:

1) Optimum levels of help from a Subordinate's perspective:

Cant and Field (2001) predicted that the optimum level of help that a subordinate should provide (if uninfluenced by the dominant) is given by Equation 5-1.

Equation 5-1. The optimum level of help for a subordinate to provide from its own perspective

$$\hat{h}_{sub} = \frac{1}{q} \ln\left(\frac{bqr}{cw_0}\right)$$

2) Optimum levels of subordinate help from a Dominant's perspective:

If the dominant has full control over helping behaviour within its group, the optimum level of help that a subordinate should provide can be given by Equation 5-2.

Equation 5-2. The optimum level of help for a subordinate to provide from a dominant's perspective

$$\hat{h}_{dom} = \frac{1}{q} \ln\left(\frac{\acute{b}q'}{rcw_0}\right)\Big)$$

If both subordinate and dominant exercise equal control over levels of helping, the amount of effort provided by a subordinate should be halfway between $h^{\wedge}_{sub}$ and $h^{\wedge}_{dom}$

5.1.2.2 The predictions of Cant & Field's (2001) model

One important result that can be drawn from this model is that an increase in the future fitness of the subordinate, w_0, can lead to a decrease in optimum levels of help from both a subordinate and dominant's perspective, $h^{\wedge}_{sub}$ and $h^{\wedge}_{dom}$. Therefore, as a subordinate's expected future fitness increases she should work less hard. If the model is extended to multiplayer queues (see Cant & Field 2001) then, for a given group size, the optimum levels of helping effort from both the subordinate and dominant's perspective are lower for higher-ranking subordinates. Thus individuals at the top of a social queue, near to inheriting dominance, should work less hard. The model also predicts that subordinates of a given rank should provide less helping effort in larger groups because the fitness benefit from inheriting a larger, more productive group is higher. Therefore, an individual should try to maximise its chance of inheriting a large group through providing less helping effort, thus mimimising its energy expenditure and exposure to predation.

In conclusion, two predictions can be made from the Cant and Field (2001) model:

1) **Helping effort will be lower in higher-ranking subordinates,** as they are closer to inheriting the dominant position.

2) **Subordinates, of a given rank, should work less hard in larger groups** because the pay-off from inheriting a large group is greater.

Cant and Field (2001) tested their kin-selection model upon a population of *Polistes dominulus*. Helping behaviour in *P. dominulus* takes the form of foraging by subordinates, in which they catch insects to feed to the dominant's brood. Foraging is a costly behaviour, presumably becasue it leaves subordinates vulnerable to attack from

predators, and because it can be heavily energy consuming. Indeed, Cant and Field (2001) found that wasps that spend more time foraging do suffer higher mortality (Cant & Field 2001). The pattern of foraging effort by *P. dominulus* subordinates agreed with Cant and Field's (2001) prediction. First, lower ranked subordinates did spend more time off the nest than higher-ranking subordinates. Second, subordinates of a given rank foraged less in larger groups.

5.1.3 The importance of *L. flavolineata* in testing Cant & Field's (2001) model

An important result of these models is that an increase in the subordinate's future fitness leads to a decrease in helping effort, regardless of whether the dominant or subordinate is in control of helping behaviour. In order for this to be tested, a system is needed whereby future fitness varies among subordinates independently of relatedness to the dominant or the costs and benefits of helping.

L. flavolineata provides an ideal system upon which to test Cant and Field's (2001) model. There is an age-based queue to inherit the dominant position (see Chapter 2 and 4) so that the expected direct fitness of a female varies as a consequence of her position within the queue, as in *P. dominulus*. An extra, useful feature of *L. flavolineata* however, is that rank is independent of factors such as relatedness to the dominant (as relatedness does not vary with rank; see Sections 4.4.1 and 4.4.2). Therefore, if a strong correlation between rank and helping effort can be found in this species, this will provide valuable evidence to support the model. *L. flavolineata* also displays a variety of group sizes, varying from 2 to 9 females (pers obs,), which may be used to test Cant and Field's (2001) model.

5.2 Aims of this Chapter

1) **To test the predictions of Cant and Field's (2001) Kin Selection Model by determining whether foraging effort correlates with either:**

    a) **Rank**
    b) **Group Size**
    c) **Body Size or**
    d) **Relatedness to the dominant**

2) **To determine whether foraging effort predicts foraging success. For example, does the amount of time off the nest correspond to how successful an individual is at finding food? Is *L. flavolineata* an efficient forager? If foraging success is low this suggests that foraging in *L. flavolineata* is a very costly behaviour, as greater amounts of time must be spent looking for food.**

5.3 Methods

5.3.1 Variation in Foraging Effort Investigation

Fourty-seven nests from sites 2, 4 and 5 were used in the experiment (see Figure 2-3 and Table 5.2). Each nest was labelled and all wasps upon the nests at the start of the experiment were marked to denote their individual identity and unknown age status (see Figure 2-4 & Figure 2-5). Each nest was then brood mapped and censused every two days to identify newly emerged individuals (see Section 2.1.2.for full details of experimental preparation). In this way the age of each newly emerged wasp could be established.

Table 5.2 Sample Sizes within each site for the variation in Foraging effort analysis

Site	Number of Nests
2	18
4	12
5	26

5.3.1.1 Determination of Foraging Effort

Rapid nest censusing was used to determine the foraging effort of each individual. Such censusing took place between 07:00 and 11:00, as it is the optimal foraging time for *L. flavolineata* (Samuel 1987; Sumner 1999; pers obs.). Censuses were conducted at half-hourly intervals throughout the morning, noting the identity of each wasp present upon each nest. The foraging effort of each of the wasps can be measured according to the proportion of half hour time blocks that a wasp is absent. A census interval of 30 minutes is suitable as it allows adequate time for each of the nests in a site to be censused as well as providing a reasonably independent census. Each rapid nest census was repeated over four consecutive days in order to build up a clear idea of foraging effort between each individual (the census was conducted on consecutive days to ensure that environmental

variance and changes in group composition had a minimal influence upon foraging behaviour).

5.3.1.2 Determination of Rank

In Chapter 4 (see Section 4.4.1) a highly significant, positive relationship was found between Relative Age and Inheritance Rank (see Table 4.3 and Figure 4-2). Therefore the relative age of a wasp is a good predictor of its inheritance rank and can be used to infer the rank of each individual in this investigation. Additionally, rank was confirmed for a subsample of females through order of inheritance (i.e. the age-based inheritance investigation in Chapter 4; see Section 4.3.1). Thus, the first step in determining rank was to identify the relative age of each individual.

5.3.1.2.1 Establishing Rank through Relative Age

The relative age of each of the wasps was obtained through frequent censusing of hatched pupal caps and unmarked individuals over a period of four months. The date of emergence was assigned to each wasp and censusing visits were repeated frequently (every two days) to ensure that each hatching event was recorded and could be pinpointed down to a short time interval. Complications arose when more than one wasp emerged between censuses; in such a case the relative age of these individuals was 'tied'. Such 'tied' individuals are excluded from the analysis.

5.3.1.2.2 Confirming Rank through Order of Inheritance

As discussed in Chapter 4 (see Section 4.3.1.3), the most effective way of determining rank is to look at the order in which the wasp inherits the dominant position. Yet, the lifespan of a dominant can be too long to wait for it to inherit naturally. Therefore, the accession to dominance for many of the females was accelerated by removal of the

accession to dominance for many of the females was accelerated by removal of the dominant. Through sequential removal of each dominant, the inheritance rank of each individual could be determined (see Table 4.2). There was a minimum interval of one week between removals to ensure that there was adequate time for the new dominant to begin to develop her ovaries and exhibit convincing dominant behaviour.

5.3.1.3 Group Size

Most nests within study sites 2, 4 and 5 were included within the investigation in order to maximise sample sizes within each group size category. The group size of these *L. flavolineata* nests averaged 2 to 6 females and therefore group sizes outside of this range are limited (see Table 5.3).

Table 5.3 Number of Nests with each Group Size within the investigation

Group Size	Number of Nests
2	9
3	17
4	9
5	7
6	12
7	0
8	1
9	1

5.3.1.4 Wing Measurement

The body size of each wasp was deduced from measurement of the right forewing (see Section 2.2.1). Wing cell measurements have been shown to correlate closely with overall body size within this species (Sumner 1999).

5.3.1.5 Microsatellite Analysis

A more detailed description of microsatellite analysis can be found in Chapter 2: General Methods. The DNA was extracted from each of the adult females using a simple salt protocol. PCR analysis was used to amplify three microsatellites in the sample DNA strands; these were K18, I3 and LF25, previously identified by Sumner (1999) (see also Sumner & Field 2001) which have been shown as highly polymorphic in this species. The PCR products were then separated using Polyacrylamide Gel Electrophoresis (PAGE) (see Chapter 2). Each allelic score was entered into the software programs *Relatedness* (Queller & Goodnight 1989) and *Kinship* (Goodnight & Queller 1999) in order to determine the likely relationship between subordinates and their respective dominant. The results of the analysis can be seen within Chapter 3. *Kinship* calculated a log-likelihood ratio of the probability that each pair of individuals' genotypes were more likely to arise from being sisters ($r = 0.75$; the primary hypothesis) than from being cousins ($r = 0.375$; the null hypothesis).

5.3.1.6 Statistical analysis

Each variable that could affect the proportion of time an individual spends off the nest had to be considered in the statistical analysis. Dominant individuals were excluded from the analysis as they generally remain upon the nest at all times in order to lay eggs and would therefore bias the results if they were included.

Due to the binomial nature of the y variable "proportion of time off the nest", an arcsine transformation was used. A generalised linear model was generated, using the statistical package "R", incorporating all measured variables that might affect time off the nest i.e. study site, group size, number of brood, date of birth and rank. Step-wise removal of the variable that explained the smallest proportion of the total variance was performed until any further removals resulted in a significant increase in residual deviance as assessed using F-tests.

5.3.2 Monitoring Foraging Success

Two sites were chosen for intensive foraging observation, site 1 and site 7 (see Figure 2-1). These sites were chosen as they contained a high density of nests within one confined area, and therefore many nests could easily be observed at any one time. Each of the wasps present at the beginning of the investigation was marked. However, a limited amount of time could be dedicated to this project, due to the intensive nature of the main foraging effort study (see Section 5.3.1), so that the age and rank of each wasp was not identified. Overall, twelve 12-hour intensive censuses were carried out, each from 07:00 to 19:00, during similar weather conditions. During this time, the length of each trip off the nest was recorded as well as the type of forage wasps returned to the nest. One difficulty experienced was that nectar and water were impossible to distinguish from one another (the implications of this are discussed in Section 5.5).

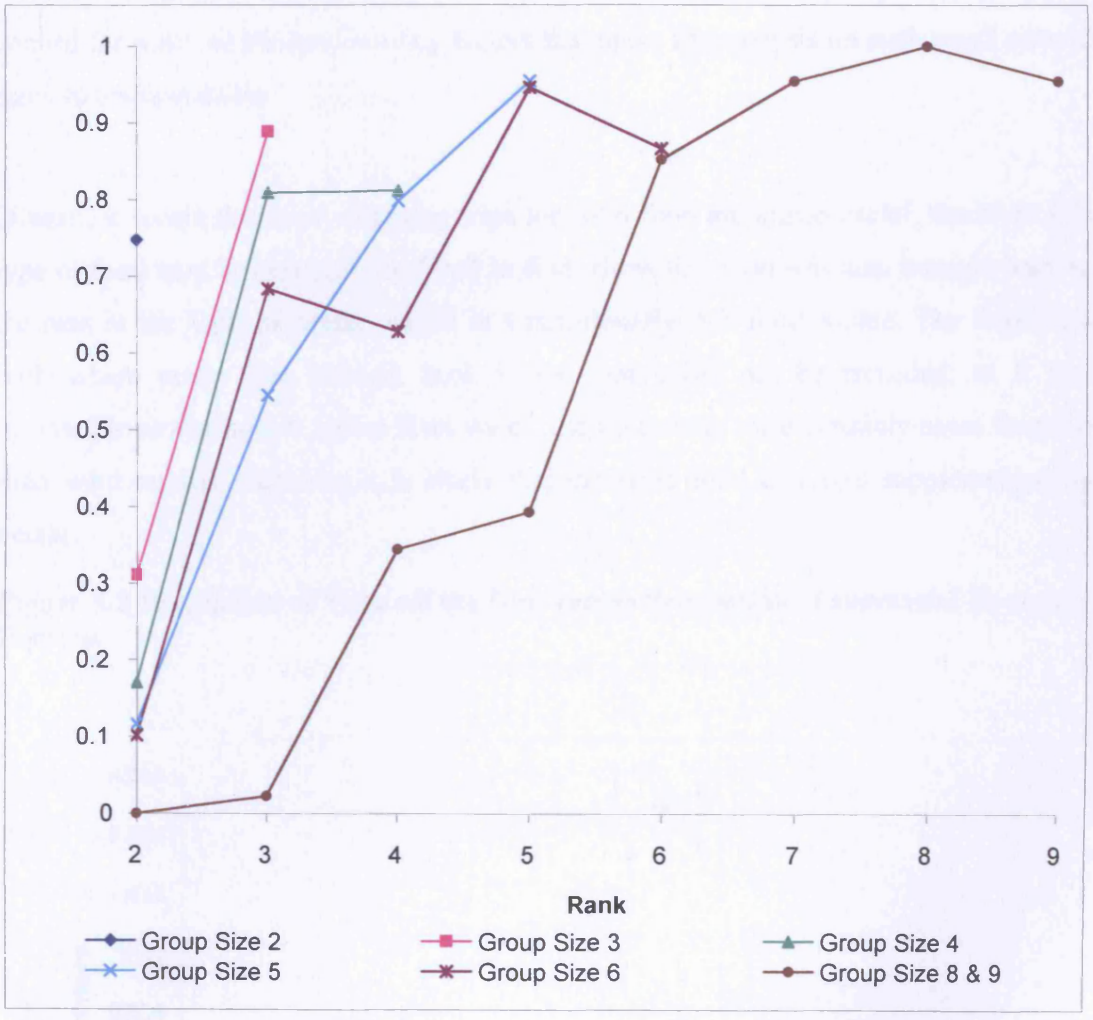
5.4 Results

5.4.1 Factors affecting Proportion of time off the Nest

This investigation was used to elucidate the main factors that affect foraging effort within *L. flavolineata* (see Aim 1 Section 5.2). The final or 'minimal adequate model', from which no further x-variables could be removed without causing a significant increase in residual deviance, included only rank, group size and date of birth (all $p < 0.001$). Rank was the variable that explained the most variance in the proportion of time off the nest (27%) with group size explaining a further 22%. Thus, rank and group size play a large part in determining the proportion of time that an individual spends off the nest (see Figure 5-1).

From Figure 5-1 it is clear that within all group sizes, foraging effort increases with decreasing rank, the greatest difference in foraging effort between consecutive ranks being that between ranks 2 and 3 (excluding group size 8 and 9 where sample sizes are inadequate). At a given rank, foraging effort decreases with increasing group size, with the most distinct differences occurring at ranks 2 and 3.

Figure 5-1 The Proportion of time that each Rank spends off the nest according to the size of its Group. For sample sizes, see Table 5.2.

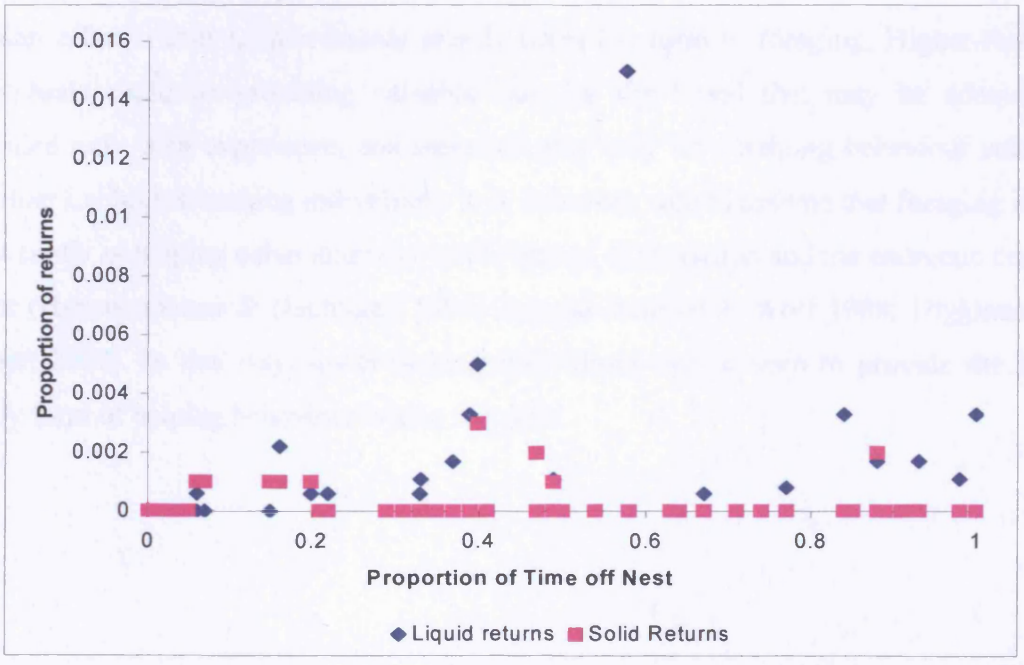


5.4.2 Foraging Effort and Foraging Success

The frequency of successful foraging returns made by each individual was very low (see Figure 5-2) thus making any type of analysis unreliable. Therefore, if the investigation were to be repeated, the age and rank of each individual should ideally be identified to control for many of the confounding factors that make the analysis on such small sample sizes so undependable.

Overall, it seems that many foraging trips for solid food are unsuccessful, therefore this type of food may be especially difficult to find. However, food was also brought back to the nest in the form of nectar, which is a nutritionally rich food source. The frequency with which nectar was brought back to the nest could not be recorded, as it was impossible to distinguish nectar from water. Liquid returns were certainly more frequent than solid returns; therefore it is likely that the solid food diet was supplemented by nectar.

Figure 5-2 Proportion of Time off the Nest versus Proportion of successful Foraging Returns



5.5 Discussion

5.5.1 Rank and Group Size affect Foraging Effort

It is clear from this analysis that the amount of foraging effort performed depends primarily upon rank and group size. Both body size and relatedness to the dominant had no significant effect upon foraging effort. As rank decreases, the amount of foraging effort increases. As group size increases, the amount of foraging effort within a particular rank decreases, so that high-ranking individuals within large groups carry out smaller amounts of foraging.

1.1.1.1 **Support for the Kin Selection Model** (Cant and Field 2001)

As rank decreases within *L. flavolineata*, foraging effort increases. This agrees with the first prediction of Cant and Field (2001) in that foraging effort, as a form of helping effort, is higher in low ranking subordinates (see Section 5.1.2.2). Cant and Field (2001) predicted that this would be the case because an individual should work less hard the closer it is to inheriting dominance. Higher ranks in *L. flavolineata* are at the front of the social queue and consequently closer to inheriting the dominant position. Therefore, the analysis provides good support for their model. However, it should not be assumed that helping effort within *L. flavolineata* merely takes the form of foraging. Higher-ranking individuals could be providing valuable care for the brood that may be adequately provided only with experience, and therefore may only be a helping behaviour suitable for older i.e. higher-ranking individuals. It is, however, safe to assume that foraging is the most costly of helping behaviours due to the hazard of predation and the energetic cost of flight (Hanauerthieser & Nachtigall 1995; Schmid-Hempel & Wolf 1988; Higginson & Gilbert 2004). In this way, lower-ranking individuals can be seen to provide the most costly form of helping behaviour within the nest.

Relatedness to the dominant was shown not to be a factor influencing foraging effort, thus refuting the differential relatedness to the dominant hypothesis. This may be because *L. flavolineata* cannot estimate individual relatedness (see Section 1.2.10.), so that helping effort cannot be directed towards more closely related relatives. Some species that do possess such kin recognition abilities may direct their helping effort towards more closely related kin.

Cant and Field's (2001) model also predicts that subordinates of a given rank should work less hard in larger groups (see Section 5.1.2.2), because the pay-off from inheriting a large group is greater. This prediction is also supported by the analysis as foraging effort decreased, within a given rank, as group size increased (see Figure 5-2). The results presented here follow the predictions of Cant and Fields (2001) Model so closely that it seems unlikely that any other factor could produce such a result.

5.5.2 Other Factors that may lead to Variation in Foraging Effort with Rank and Group Size

Previous Foraging Costs may affect the Propensity to Stay upon the Nest when Group Size Increases. If a female emerges onto a two female nest, generally this new individual joins the bottom of the social queue (see Section 4.4.1). This would allow the second ranking female to spend more time upon the nest as she is no longer responsible for all of the foraging. Indeed, such a female may have used so much of her energy when she was the sole forager that she is no longer capable of partaking in as much foraging. However, if the second ranking wasp emerged at approximately the same time as the third ranking wasp this explanation is no longer applicable.

Lack of Experience of Younger Members may affect Foraging Effort. One of the reasons that foraging effort may increase with decreasing rank is due to the lack of experience of younger, lower-ranking members. Their high foraging rates may be due to their inexperience rather than provision of greater helping effort. However, foraging effort would only decrease with increasing rank if higher ranking, more successful individuals did not carry out further foraging trips after they returned with prey. The foraging data suggest that this is not the case as some individuals made several successful foraging returns on the same day.

Deteriorating Condition due to Ageing may affect Foraging Effort. Higher-ranking individuals in *L. flavolineata* are also the oldest of nest members. They may therefore be in the worst condition and stay upon the nest to reserve the last of their energies or because they have no other choice because of their poor state. Indeed, age was found to be a significant factor in the foraging effort analysis. Thus within a given rank and group size the oldest of nest members forage less, which supports the idea that perhaps these older nest members are less capable of providing as much foraging effort as younger individuals. The costs of foraging within *L. flavolineata* may accelerate as an individual ages as it may become more susceptible to predation. Additionally, foraging within this species is likely to cause wing wear and therefore flight efficiency may decrease as an individual ages.

In larger groups higher ranks spend more time upon the nest and could coerce lower ranks into providing more foraging effort. A proportional increase in older individuals, which could force lower ranks off the nest, may partly explain the increase in foraging effort of lower ranks within larger groups. As group size increases, higher ranks spend more time upon the nest and could feasibly force lower ranks to perform more foraging.

5.5.3 Foraging Success

The foraging success of the *L. flavolineata* studied in this investigation appears to be very low. Indeed, many foraging trips appeared to be unsuccessful, therefore their food may be very difficult to find or catch. If this is the case, foraging within this species may be particularly costly. Solid food was scarce, and it is therefore likely that this was supplemented with nectar. However, the frequency of nectar returns could not be recorded, as it was impossible to distinguish nectar returns from water returns. Further study of *L. flavolineata's* foraging success should be carried out to look at the foraging success of each rank, as older more experienced individuals may be more successful foragers than younger individuals.

5.6 Conclusion

It may be concluded that the predictions of Cant and Field's (2001) Kin Selection Model have been met within this analysis. As rank increases within *L.flavolineata,* foraging effort decreases. As group size increases foraging effort decreases at a given rank. Relatedness to the dominant had no influence upon foraging effort, and therefore the most common explanation that differential relatedness to the dominant influences helping effort is not applicable in this species. However, it is unclear whether relatedness fails to influence helping effort because *L. flavolineata* lacks relatedness discrimination or because relatedness estimates for individual pairs of wasps have large standard errors. Perhaps if relatedness could be measured accurately some effect may have been found.

Other factors have been put forward in this discussion that may account for some of the variation in foraging effort yet the results fit the predictions of the Kin selection model so well that costs of helping seem likely to play a large role in determining foraging effort in *L. flavolineata*. Age was found to have a significant affect upon foraging effort, with older individuals, within a given rank and group size, foraging to a lesser extent than younger individuals. Such a result indicates that the effect of ageing also plays a large part in determining foraging effort. As an individual ages the costs of foraging may increase as an older individual may be slower to respond to threats from predation and flight might be particularly energy costly as an older individual's wings are more likely to be worn and are therefore less efficient. Therefore future studies into foraging effort within this species could focus upon the effect of ageing upon *L. flavolineata's* physiology and flight efficiency.

6 Summary

In this chapter I will briefly summarise my main findings using the framework of the initial aims of this thesis.

6.1.1 The Genetic Structure of *L. flavolineata* colonies

The intranidal relatedness between adult females on the nests in this investigation averaged 0.45; the most frequent values being 0.5 and 0.6. Relatedness differed significantly from the theoretical value of 0.75 (for haplodiploid sisters), because many of the nests were composed of two sib groups. The number of sib groups, within a nest, was found to exert the greatest influence upon intranidal relatedness rather than group size, which had no influence. Group size also had no influence upon the number of sibships that existed within a nest as even large groups could consist of just one sib group. Group persistence and consequently Dominant turnover is likely to have the greatest effect upon the number of sibgroups. As each female inherits dominance, a new sib ship is produced. Therefore the younger individuals in the nest are united within one sib group whereas the older individuals are united within their own sibgroup. The relatedness between the two groups decreases the overall relatedness of the nest.

The existence of multiple sib groups within nests was found to be a more frequent factor in decreasing intranidal relatedness than joining events from unrelated wasps. Joining events were rare and consequently had little impact upon the average intranidal relatedness of the colony, contradicting the previous hypothesis of Strassmann *et al.* (1994). Indeed the intranidal relatedness results presented here do not differ significantly from those of Sumner (1999), implying that Strassmann *et al.*'s (1994) estimate of relatedness was certainly too low. Joining events did occur, but not at the frequency that was suggested by Strassmann *et al.* (1994) in order to account for their low intranidal relatedness values. In 2001 there were eight joining events, yet there seems to be no common factor that allows an individual to join a nest. Certainly, group size and

intranidal relatedness had no effect upon the ability to join a nest. Indeed, the relatedness data for the few joining nests that were collected suggest that the nests joined consisted of a single sibship so that intranidal relatedness was relatively high. However, joining events were rare and the sample size collected was small so the conclusions that can be reached are inevitably limited. I suggest that in most cases joining events were probably opportunistic, with individuals choosing to join any nest that would admit them to the nest. Joiners did tend to be able to join at a rank higher than that they left behind on their original nest; hence this may have influenced their decision to join a different nest as this allowed them to increase in rank in a very short space of time.

Behavioural observations during this study have shown that nests are generally reluctant to let any individual from the outside population join the nest. Joining individuals have been shown to contribute helping effort to the nest, which may therefore be used to placate nest mates into letting them stay. However, subordinate nestmates should be reluctant to admit unrelated joining individuals to the nest if there is any chance that the joiner will inherit dominance before them. Most joiners were able to inherit dominance. However it would be interesting to see if such dominants are eventually usurped from their position by younger nest members

6.1.2 Inheritance of Dominance within *L. flavolineata* colonies

Age was found to correlate with rank therefore an age- based queue does exist within *L. flavolineata.* Indeed, age determines rank even if the age interval between individuals is as small as a couple of days. I suggest that age is a convention used by *L. flavolineata* to assign dominance rather than a mere correlate of a factor such as experience. However, queue jumping was detected within the study population. This study suggested that body size might not determine the ability of a wasp to be able to queue jump although a small sample size was used in the analysis. One important point to note is that, due to the small sample sizes of queue jumpers, only very strong effects of factors such as body size and relatedness could be detected.

If a larger body size did allow a wasp to queue jump, this would have important consequences for the age-based queue, as it would mean that any larger individual would be able to push their way up the queue to dominance so that the age-based convention would be unstable.

Queue jumpers were commonly sisters of those that they queue jumped yet in one instance the individual was likely to be unrelated. Queue jumping is difficult to predict, although one pattern that seems to emerge is that queue jumpers start to forage less up to one month before the dominant dies. This might indicate that the queue jumpers can detect the imminent death of a dominant individual at around this point, and therefore adjust their foraging effort so that they are present when the dominant dies. In doing so it may be easier for the queue jumper to establish itself as Dominant with the aid of slightly heightened aggression towards the second rank individual that should have inherited dominance. This pattern of adjusted foraging effort suggests that queue jumping is a cheating behaviour, rather than the possession of an alternative criterion for inheriting dominance.

6.1.3 Rank and Foraging Effort

The fact that relatedness does not correlate with rank makes *L. flavolineata* a suitable species for testing the effect of rank upon foraging effort. In this species the amount of foraging effort undertaken by a wasp depended primarily upon rank and group size. The higher the rank of the wasp the less foraging effort it undertakes. Cant and Field (2001) predicted that this would be the case within social queues, as the cost of helping effort increases the nearer an individual is to inheriting dominance. A high-ranking individual may be unlikely to risk the chance of inheriting direct fitness in order to increase its indirect fitness through large amounts of foraging. The observed pattern of foraging effort meets the predictions of Cant and Field's (2001) kin selection model since the relatedness study has shown that there is no correlation between a subordinate's

relatedness to the dominant and the foraging effort it exerts. My results support the costs of helping theory rather than the relatedness to the dominant hypothesis.

There are other factors that may have caused such a foraging pattern to emerge. For example, ageing may cause an individual to carry out less foraging. Indeed, age was shown to be a significant factor in determining foraging effort but it did not have as strong an influence as rank or group size. Reduced foraging effort due to ageing also fails to explain why group size should have such a strong influence within rank. As group size increases, the amount of foraging effort, within a particular rank, decreases. Cant and Field (2001) suggested that this would be the case as higher ranks within large groups stand to inherit a particularly valuable, productive nest. Group size has a greater influence upon foraging effort so that the pattern shown is unlikely to be due solely to the effects of ageing. *L. flavolineata* appears to be an inefficient forager when considering solid returns. Such returns commonly consisted of ants, which therefore seem to be particularly difficult to acquire. Liquid returns were also infrequent making analysis impossible.

6.1.4 Suggestions for further studies within *L. flavolineata*

This study was successful in fulfilling many of its objectives. For example I have shown that there is a system of gerontocracy within *L. flavolineata*, in which queue jumping may sometimes occur and in which foraging effort decreases as individuals increase in rank. There are some questions that remain unanswered and could provide the focus of future work. One such question is the effect of ageing upon *L. flavolineata* in terms of physiological factors such as wing tear and the affects this has upon foraging. Indeed, foraging success within *L. flavolineata* proved very difficult to study due to their low rate of returns. If such a study upon foraging success was repeated it would be vital to identify every confounding factor such as rank and age in order to make statistical analysis possible.

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Appendix 1

Sequential removals carried out in 2001

	Individual present upon the nest at the start of the investigation. This period denotes the maximum development time of an individual providing that it was newly emerged at the start of the experiment.
	Individual emerges during the investigation. This period denotes the maximum development time of this individual, from egg to newly emerged adult.
	Individual has joined from another nest. This period denotes the maximum development time of an individual providing that it is newly emerged.
XXXX	Individual Collected for Microsatellite Analysis
XXXX	Individual not Collected
•	Individual Present at the start of the investigation
E	Individual emerges during the investigation. Date of emergence or the time interval in which it occurred is given in brackets
†	Individual Removed
	*** Sister Likelihood using KINSHIP
	** Sister Likelihood using KINSHIP
	* Sister Likelihood using KINSHIP

Sequential Removals From Nests 2001

Nest	Date	01/01-24/01	25/01-24/02	25/02-24/03	25/03-24/04	25/04-25/05	26/05-25/06	26/06-25/07	26/07-27/08	28/08-30/08	31/08-06/09	09/09-11/09	12/09-13/09	14/09-16/09	End
	Days (Cumulative)					31	61	91	124	126	133	137	138	140	
	Inheritance Rank														
2111	1					PRRO	PRRO	PRRO	PRRO	PRRO	PRRO	†			
	2					•						BrWOO	†		
	3					•							OWRO	†	
	4						E (25/06)							YDGDGO	†
								E (10/07)						DGOPO	
27	1					DGOOO	DGOOO	DGOOO	DGOOO	DGOOO	DGOOO	†			
	2					•						YYDG	†		
	3					•							RWDG		
							E(13/06)							DGDGRO	†
26	1					• (N27)	RYDGO J (01/	RYDGO	RYDGO	RYDGO	RYDGO	†			
	2					•						OPRO	†		
	3					E (25/05-01/06)							YYDGO	†	
							E (21/06)							YPRO	
								E (30/06)						DGRPO	
2105	1					BrYPO	BrYPO	BrYPO	BrYPO	BrYPO	BrYPO	BrYPO	†		
	2					•							DGWBrO	†	
	3							E (16/07-30/07)						YRYO	†
	4									E (29/08)					RBrDGO
								E (16/07-30/07)							ODGRO
215	1					PDGDGO	PDGDGO	PDGDGO	PDGDGO	PDGDGO	PDGDGO	†			
	2							E (10/07-19/07)				DGROO	†		
	3							E (10/07-19/07)					WYDGO	†	
	4								E (13/08)					DGBrOO	
217	1					WROO	WROO	WROO	WROO	WROO	WROO	†			
	2					•						RDGRO	†		
	3					E (30/04-15/05)							ORRO	†	

Nest	Date Days (Cumulative) Inheritance Rank	01/01-24/01	25/01-24/02	25/02-24/03	25/03-24/04	25/04-25/05 31	26/05-25/06 61	26/06-25/07 91	26/07-27/08 124	28/08-30/08 126	31/08-06/09 133	09/09-11/09 137	12/09-13/09 138	14/09-16/09 140	End
220	1					•				DGPYO	DGPYO	DGPYO	†		
	2							E (13/07-19/07)					WPWO	†	
2112	1					OBrWO	OBrWO	OBrWO	OBrWO	OBrWO	OBrWO	†			
	2					•						YDGPO	†		
	3							E (13/07)					YRDGO	†	
	4							E (13/07)						ODGWO	
223	1					WRPO	WRPO	WRPO	WRPO	WRPO	†				
	2					•					DGYDGO				
							E (13/06)				ORPO				
225	1					RYOO	RYOO	RYOO	RYOO	RYOO	RYOO	RYOO	RYOO	†	
	2					•								OWWO	
									E (10/08)					YOBrO	
227	1						E (25/06)		DGWYO	DGWYO	DGWYO	†			
	2				N229			E (18/07 N229)	J (10/07-13/07)			PDGWO	†		
	3							E 07/07-16/07)					RWRO	†	
	4								E (18/08)					BrYBrO	
									E (23/08-25/08)					DGPOO	
											E (06/09)			WPBrO	
229	1					•	DGDGWO	DGDGWO	DGDGWO	DGDGWO	†				
	2						E (25/06-27/06)				WOPO	WOPO	WOPO	WOPO	
									E (02/08)					YYWO	

Nest	Date Days (Cumulative) Inheritance Rank	01/01-24/01	25/01-24/02	25/02-24/03	25/03-24/04	25/04-25/05 31	26/05-25/06 61	26/06-25/07 91	26/07-27/08 124	28/08-30/08 126	31/08-06/09 133	09/09-11/09 137	12/09-13/09 138	14/09-16/09 140	End
2109	1					PBrRO	PBrRO	PBrRO	PBrRO	PBrRO	PBrRO	†			
	2					•						YWDG			
						•						ORPO			
								E (12/07)				PPPO			
45	1					E (13/05)	RPWW	RPWW	RPWW	RPWW	†				
	2					•					PPPW	†			
	3							E (02/07-22/07)				PYOW	†		
	4							E (02/07-22/07)					RWWW		
								E (01/07-04/07)					WRDGW		
									E (19/08-29/08)				BrOYW		
416	1					WLBLBW	WLBLBW	WLBLBW	WLBLBW	WLBLBW	WLBLBW	†			
	2								E (19/08)			YYRW	†		
	3							E (28/06)					ODGPW	†	
	4								E (19/08-26/08)					DGWDGW	
419	1						E (27/05-30/05)			RYPW	RYPW	†			
	2						E (27/05-30/05)					OOOW			
4102	1					•	DGPPW	DGPPW	DGPPW	DGPPW	DGPPW	DGPPW	†		
	2						E (16/06-28/06)						YWWW	†	
	3						E (27/05-12/06)							DGWBrW	
							E (16/06-28/06)							DGDGPW	
								E (12/07-22/07)						DGODGW	
4103	1					•				LBRWW	LBRWW	†			
	2						E (19/06)					DGOWW	†		
	3						E (27/05-16/06)						YYDGW	†	
	4						E (27/05-16/06)							RWBrW	

Nest	Date Days (Cumulative) Inheritance Rank	01/01-24/01	25/01-24/02	25/02-24/03	25/03-24/04	25/04-25/05 31	26/05-25/06 61	26/06-25/07 91	26/07-27/08 124	28/08-30/08 126	31/08-06/09 133	09/09-11/09 137	12/09-13/09 138	14/09-16/09 140	End
4104	1					DGWYW	DGWYW	DGWYW	DGWYW	DGWYW	†				
	2					•					PRDGW	†			
	3					•						LBOLBW	LBOLBW	†	
								E(13/06-28/06)						RWDGW	
													E (10/09)	YR	
423	1					PPDGW	PPDGW	PPDGW	PPDGW	PPDGW	†				
	2					•					PYPW				
					N428			E (10/07) J (15/07)			RORW				
						•					OWPW				
424	1					•			WOWW	WOWW	†				
	2						E (04/06-05/06)				WYDGW				
							E (22/06)				PPOW				
425	1					WPPW	WPPW	WPPW	WPPW	WPPW	WPPW	†			
	2							E (25/06-22/07)				PRDGW	†		
									E (27/07-16/08)				WWYW		
											E (27/07-26/08)		YYBrW		
426	1					•			PDGOW	PDGOW	G				
	2						E (20/06-22/06)				PDGPW	†			
	3						E (13/06-19/06)					OYBrW			
428	1					DGPRW	DGPRW	DGPRW	DGPRW	DGPRW	DGPRW	†			
	2				N423			E (06/07) J (15/07)				DGROW	†		
	3								E (18/08)				OYDGW	OYDGW	
											E (02/09-04/09)			WWBrW	

Nest	Date	01/01-24/01	25/01-24/02	25/02-24/03	25/03-24/04	25/04-25/05	26/05-25/06	26/06-25/07	26/07-27/08	28/08-30/08	31/08-06/09	09/09-11/09	12/09-13/09	14/09-16/09	End
	Days (Cumulative)					31	61	91	124	126	133	137	138	140	
	Inheritance Rank														
429	1					•	RYYW	RYYW	RYYW	RYYW	RYYW	†			
						•					QWOW	G			
	2										E (29/08-06/09)	UM	†		
	3						E (22/05)						WYOW		
							E (25/06)						YWOW		
5103	1					•				PRLB	PRLB	†			
	2		N5102				•					WPOLB (J 09/	†		
	3								E (17/08)				DGYOR		
									E (27/08)				YRBrLB		
5105	1					E (09/05-20/05)			ORYLB	ORYLB	ORYLB	†			
	2					E (09/05-21/05)						WRDGLB	†		
	3						E (04/06-07/06)						DGWBrLB	DGWBrLB	†
											E (17/07-20/08)				RWBrR
												E (17/07-01/09)			ODGRR
												E (17/07-01/09)			DGYDGR
5106	1		N5138		J (20/04)	OORLB	OORLB	OORLB	OORLB	OORLB	OORLB	†			
	2		N5117				J (17/06)					RYOLB			
							E (17/06)					DGYRLB			
										E (20/08)		WWBrLB			
5112	1					PDGDGLB	PDGDGLB	PDGDGLB	PDGDGLB	PDGDGLB	PDGDGLB	†			
	2								E (14/07-17/07)			WWOR	†		
	3								E (14/07-17/07)				WOOR		
												E	UM		
5113	1					•			DGDGDGLB	DGDGDGLB	DGDGDGLB	†			
	2					•						OYDGLB	†		
	3					E (23/05-26/05)							RYYLB	†	
						E (23/05-26/05)								WWOLB	
												E (11/09)		DGWWR	

Nest	Date Days (Cumulative) Inheritance Rank	01/01-24/01	25/01-24/02	25/02-24/03	25/03-24/04	25/04-25/05 31	26/05-25/06 61	26/06-25/07 91	26/07-27/08 124	28/08-30/08 126	31/08-06/09 133	09/09-11/09 137	12/09-13/09 138	14/09-16/09 140	End
5114	1					PLBRLB	PLBRLB	PLBRLB	PLBRLB	PLBRLB	PLBRLB	†			
								E (26/06)			BrRYLB	†			
	2		N169 or N122									DG_OR (J 09/09)			
5115	1					•	YOYLB	YOYLB	YOYLB	YOYLB	†				
	2					E (18/05)					WPWLB				
								E (02/07/11/07)			WPRLB				
5118	1					E (09/05)			DGYBrLB	DGYBrLB	DGYBrLB	DGYBrLB	†		
	2								E (20/08)				RYDGR	†	
	3							E (05/07-08/07)						YDGRLB	
								E (05/07-08/07)						PWOLB	
												E (11/09)		UM	
5123	1					RLBYLB	RLBYLB	RLBYLB	RLBYLB	RLBYLB	†				
	2						E (17/06)				RODGLB	RODGLB	RODGLB	RODGLB	RODGLB
									E (04/08)						RYRR
									E (26/08)						YDGBrLB
									E (26/08)						OBrDGLB
519	1					BrBrRLB	BrBrRLB	BrBrRLB	BrBrRLB	BrBrRLB	BrBrRLB	†			
	2					•						RPBrLB	†		
	3						E (17/06)						WODGLB		
										E (30/08)			YDGOLB		
516	1					LBYYLB	LBYYLB	LBYYLB	LBYYLB	LBYYLB	†				
	2					•					WOYLB	WOYLB	WOYLB	WOYLB	WOYLB
			N5130			E (13/05 N130) J (17/05)									OWOLB
										E (29/08)					UM

Nest	Date	01/01-24/01	25/01-24/02	25/02-24/03	25/03-24/04	25/04-25/05	26/05-25/06	26/06-25/07	26/07-27/08	28/08-30/08	31/08-06/09	09/09-11/09	12/09-13/09	14/09-16/09	End
	Days (Cumulative)					31	61	91	124	126	133	137	138	140	
	Inheritance Rank														
523	1					OWWLB	OWWLB	OWWLB	OWWLB	OWWLB	†				
	2						E (11/06-14/06)				OWBrLB	OWBrLB	OWBrLB	OWBrLB	OWBrLB
								E (11/07-17/07)							RDGOR
											E (04/09)				UM
5137	1					YRLBLB	YRLBLB	YRLBLB	YRLBLB	YRLBLB	†				
	2						E (07/06)				BrRRLB	BrRRLB	BrRRLB	BrRRLB	BrRRLB
								E (05/07)							WPOLB
											E (06/09)				UM
5150	1					WLBDGLB	WLBDGLB	WLBDGLB	WLBDGLB	WLBDGLB	†				
	2					•					PBrOLB	PBrOLB	PBrOLB	PBrOLB	PBrOLB
										E (11/08)					RRRR
5160	1					•			YYRLB	YYRLB	YYRLB	†			
	2		N5138			J (05/05)						BrRPLB	†		
	3								E (04/08)				RYOR		
										E (29/08)			WBrBrR		

Appendix 2

Solutions used in DNA Extraction

1. GRINDING BUFFER

1M	NaCl	800µl
1M	Fresh Sucrose	1600µl
1M	Tris-HCL	800µl
0.5M	EDTA	800µl
20%	SDS	20µl
	ddH_20	3980µl

*1M Fresh Sucrose = 4.28g Sucrose dissolved in 12.5ml ddH_20. Use for up to 1 week.

2. 10X TBE

dH_20	To make solution up to 1L
Tris	103g
Boric Acid	55g
EDTA	9.3g

pH 8.3 – change pH with large amouts of 10N NaOH if necessary (See pH meter)

3. 1X TBE

10X TBE	100ml
dH_20	900ml

4. UREA/TBE

dH_20	To make solution up to 1022.4ml
10X TBE	120ml
Urea	576g

Reagents used in DNA Extraction

1. DNA

The DNA extraction needs to be diluted before it is used in the PCR. A 1:10 dilution often works well.

DNA extraction	2µl
ddH_20	18µl

2. d/G/A/TTP

dATP	50µl
dGTP	50µl
dTTP	50µl
ddH_20	850µl

3. dCTP

dCTP	15µl
ddH_20	485µl

4. Primer Mix

Forward Primer	2.5µl
Reverse Primer	2.5µl
ddH_20	95µl

DNA Extraction for Microsatellite Analysis

1. Collect **Dry Ice** and switch on water bath (65°C).

   Select samples from freezer and place in ice. No more than 20 samples should be selected as it is important to keep them frozen.

2. Label a tube for each sample and pipette into these **150µl Grinding Buffer**.

3. Wash scalpel and forceps with dH_20 followed by **Ethanol**.

4. Cut thorax in half. Place half in its allocated tube of grinding buffer and the other half, back in its original. Wash instruments and glass plate thoroughly as in step 3.

5. Repeat step 4 for all samples ensuring that no cross-contamination occurs.

6. Wash micropestle with dH_20 followed by ethanol. Grind sample with micropestle.

7. Repeat step 6 for all samples ensuring that no cross-contamination occurs.

8. Spin briefly to collect parts at bottom of tube.

9. Add **150µl Grinding Buffer.**

   Mix well and incubate at 65°C for **30 minutes.**

10. While tubes are still warm add **43µl 8M KAc**.

    Mix well and tap tubes to bring contents to the bottom.

    Incubate on **Wet Ice** for **30 minutes** to precipitate salt and SDS.

11. Centrifuge at 14,000xg for **15 minutes**.

    Label a new set of 1.5ml tubes.

12. Transfer supernatants to new tubes.

13. Add **250µl of Ice Cold Ethanol**.

Mix and place tubes in –80°C freezer for at least 1 hour **OR** in –20°C freezer overnight.

14. Remove from freezer and centrifuge at 14,000xg for **15 minutes**.
15. Remove supernatant and allow pellet to dry (about **15 minutes**)
16. Resuspend pellet in **25µl of ddH_20.**
17. Assess success of extractions by running **2µl** on an agarose gel.

Assessing the Success of DNA Extractions

To Make Agarose Gel:

1. Tape the ends of the small gel rig with autoclave tape (this will withstand the heat of hot agarose).
2. Make a 1% agarose gel by placing **0.4g of agarose** in a small conical flask.
3. Add a solution of **1 X TBE** (See Solutions).
4. Cover the top with cling film and pierce once.
5. Place in microwave for about **1 minute** on **MEDIUM** heat. Check constantly for signs of over heating. The solution is ready as soon as it becomes clear.
6. **CAREFULLY** add **2μl of Ethidium Bromide** and stir.
7. Put 2 x 12 lane moulds in the gel rig and pour in the gel solution. Leave to set (about **30 minutes**).
8. Remove the tape and lane moulds from the gel rig. Place in the gel rig tank.
9. Cover the gel with **1 X TBE** solution by about **2mm**.

Loading the Gel:

1. Take a square of **'Nescofilm'**
2. Pipette **2µl of Loading Buffer** at intervals along the film.
3. Pipette **2µl of each extraction** onto each spot of loading buffer ensuring no cross-contamination occurs.
4. Pipette each extraction (total **4µl**) into each of the wells. The pipette tip should be just under the surface of the buffer, not pushed into the gel.
5. Place the lid upon the rig ensuring that the leads are connected correctly. Run the gel at 100V.

Polymerase Chain Reaction (PCR)

1. Take the required DNA extractions from the –80°C freezer.
2. Label 0.5 ml tubes with sample numbers.
3. Pipette 2µl of diluted DNA in to each of the designated tubes. Take care not to cross-contaminate samples.
4. Place the tubes in to the fridge whilst the PCR mixture is being prepared.
5. In a 1.5ml tube-mix together the NH_4, dNTPs, $MgCl_2$, Primer Mix and ddH_20. Place this tube in the fridge.
6. Collect together the Auto pipette, Pasteur pipette, Mineral oil and 5-40µl pipette and place on a tray.
7. Take the PCR mixture from the fridge and add the Taq. Return the Taq to the fridge as quickly as possible to stop degradation. Taq is an enzyme, which will start working as soon as it is added to the mixture so the rest of the procedure must be done swiftly.
8. Take the samples from the fridge and place on the tray with the pipettes, oil and PCR mixture and proceed to the hot lab.
9. Pipette the required amount of radiolabel into the PCR mixture. Dispose of the tip in the Perspex bin.
10. Ensure that all of the sample tubes are open.
11. Using the auto pipette – draw the PCR mixture up by pressing the button underneath the pipette.
12. The auto-pipette is pre-set to deliver 8µl so this does not need to be altered.

13. Add mixture to each of the tubes by simply pressing the underneath button once over each of the tubes.

14. The auto pipette is set so that it needs to be reset after every 12 tubes i.e. each row. Always hold the pipette tip over the mixture tube before pressing the reset button. Dispose of the tip in the Perspex bin.

15. Using the Pasteur pipette, add 2 drops of mineral oil to each of the tubes.

16. Cap tubes and load PCR machine.

PCR Calculation Sheet

Reagent	Stock Conc.	ul/tube	Primer:	Primer:	Primer:
DNA	1:10	2	********	********	********
NH4 Buffer	10x	1			
d/G/A/TTP	5mM	0.6	0		
dCTP	0.3mM	0.2	0		
$MgCl_2$	25mM	0.6	0		
Primer Mix	2.5uM	2	0		
ddH_2O		3.25	0		
Taq	5U/ul	0.05	0		
32P dCTP	1: 80	0.3	0		

Making PAGE Gel

1. Wash plates thoroughly with detergent and water.
2. Thoroughly rinse plates.
3. Clean plates with water followed by ethanol using tissue.
4. Repeat Step 3.
5. Take the **Back plate** to the **Hot Lab** and place in the Fume cupboard.
6. Pour a small amount (about **5ml**) of **Silanization Solution** onto the plate and spread it evenly using some tissue. Leave the plate for 5 minutes. Ensure that the fume cupboard is switched on as this solution produces a lot of fumes.
7. Whilst waiting for the back plate, wipe ethanol over the side spacers. Retrieve the Back plate.
8. Place the **side spacers** in position on the Back plate.
9. Lower Front plate onto spacers and fix to Back plate with side clamps.
10. Place a strip of chromatography paper into the pouring base and 2 smaller strips overlapping the edge of the base on each end (to soak up excess solution).
11. Seal the bottom of the gel rig by making **50ml** of gel mixture and pouring into the base. The mixture is made as follows:

    42.5ml Urea/TBE

    7.4ml Acrylamide

    500µl Ammonium Persulphate

    30µl Temed

12. Securely clamp the base in a vertical position and leave to set for about 30 minutes.

13. When the base is set – position gel rig on bench so that the buffer chamber is uppermost. Then raise the top end of the rig and position it so that it is at approx. 30 degrees to the horizontal plane. (See diagram 1).

14. Make 100ml of gel mixture as follows:

    85ml Urea/TBE

    14.8ml Acrylamide

    500µl Ammonium Persulphate

    30µl Temed

15. Slowly pour mixture into the space between the 2 plates.

16. Once the gel space is full, slide the comb (rear edge first) 4mm into the top of the gel and clamp in position with 3 large bulldog clips.

17. Leave gel to set overnight.

Running a PAGE Gel

1. Make 2 L of **1X TBE** by adding 200ml of 10X TBE to 1800ml dH_20. Microwave on high for **7 minutes.**
2. Flush the inverted comb of the gel with water and carefully remove it. Wipe away any excess gel left behind.
3. Remove the pouring base and place the gel into the running stand (in the Hot Lab). Clamp in place.
4. Pour the pre-heated 1X TBE buffer into a large measuring cylinder and then pour between the plates filling the rig to **within 0.5cm of the very top**. Pour **400ml of buffer into the base**. The remaining 300ml (approx.) should be put to one side for topping up.

Running a PAGE Gel

1. Carefully connect the rig to the power supply. Switch the rig on at the side. Select **PROGRAM**, then **RUN**, Programme number **1**. If running 2 gels select program number 2. The power readings should eventually reach 90W constant, 2200V, 100mA.
2. Whilst the gel is warming up. Remove the samples from the fridge or PCR machine **(Remember to wear 2 pairs of gloves)**. Ensure that the area is set up for radioactive work (See PCR sheet).

3. Add **4µl** of **loading buffer** to each sample. Ensure the pipette tip is applied to the very bottom of the tube and each tip is changed between samples. Dispose of tips in the Perspex tip bin.
4. When the gel reaches 45-50°C incubate the samples in the PCR machine at 90°C for 3 minutes. On PCR machine 1 this is program 5, on PCR machine 2 this is program 4. Ensure that ladder (in freezer compartment) is incubated along with the samples. For 1 gel you should need 1 tube of AGT and 1 tube of C.
5. Whilst the tubes are incubating, switch off the gel rig power supply and squirt buffer between the plates using a syringe. This should flush out excess urea. Carefully remove excess urea stuck between the plates by teasing out with the urea comb. Take care not to damage the gel itself.
6. Carefully slide the loading comb between the plates so that the teeth just penetrate the gel evenly over its surface. Clamp the comb in place with 3 small bulldog clips.
7. After incubation, arrange the tubes into their loading order and place behind Perspex screen. Move the screen alongside the gel.
8. Working from left to right flush out the first 7 wells of gel with 200µl pipette. If urea is left in the wells it can stop the samples running properly. Load 3-4µl of the samples in to each well according to loading order. The same tip can be used each time as long as it is flushed properly before the next loading.
9. Repeat Step 12 until loading is complete.
10. Run gel until the upper most blue buffer line reaches the desired end point (depends on the size of the loci you are looking at).

11. Disconnect power supply, remove rig from running base and carefully pour buffer down sink.
12. Lie rig down flat with front plate uppermost.
13. Carefully separate the two plates by sliding a knife point between them at the base of the rig. The gel should stick to the non-siliconized front plate).
14. Lie the front plate down with gel facing upwards.
15. Place chromatography paper over the gel and smooth down gradually.
16. Peel the paper from the plate carefully (the gel will be stuck to it).
17. Cover the gel with cling film.
18. Trim the gel to cassette size – put all waste in to the Perspex solid waste bin.
19. Place the gel in to the drier (1^{st} floor). Ensure that you surround the glass flasks with ice. Dry for 2 hours at 80°C.
20. Place the dry gel in film cassette and tape in to place (with masking tape).
21. In the dark room (5^{th} floor), ensuring that only the red light is on at all times, place the emulsion side of a film sheet on to the dry gel. To identify the emulsion side, locate the notch on the top of the sheet. When the notch is situated in the top right corner, the emulsion side is facing up. This is the side, which should be in contact with the gel. So when laid down on the gel it will be in the top left corner.
22. Ensure that the cassette and film sheets are entirely covered before putting the normal light on.
23. Place the cassette in the -80°C freezer for exposure. The exposure time will vary depending on the age of the isotope. If only a week old, overnight exposure will

suffice. Intensity plates may be needed if the isotope is old or the DNA is weak. Try to avoid using intensity plates as they lessen resolution.

24. Remove cassette from freezer and thaw for 20 minutes.

25. Develop the gel using automatic developer

Appendix 3

Table 0.1 Relatedness Between All Ranks

Site	Nest	Rank 1 to 2	Rank 1 to 3	Rank 1 to 4	Rank 2 to 3	Rank 2 to 4	Rank 3 to 4
2	6	0.226	0.127		0.563		
2	7	1.000	0.603	0.139	0.603	0.139	0.139
2	15	0.386	0.602	0.602	0.801	1.000	0.801
2	17	0.792					
2	20	1.000	0.551		0.551		
2	27	0.862	- 0.175	1.000	- 0.175	0.861	- 0.114
2	105	1.000	0.546	0.803	0.395	0.607	1.000
2	109	0.412					
2	111	0.550	0.698	0.541	0.849	0.083	0.312
2	112	- 0.008	0.596	0.606	- 0.009	0.015	0.606
4	5	0.625	- 0.007		- 0.208		
4	16	0.021	0.629	0.594	0.259	- 0.014	0.595
4	19	0.805					
4	25	0.817	0.633		0.817		
4	26				- 0.012		
4	28	0.416	0.430	0.448	0.715	0.172	0.448
4	29	0.573		0.381		0.382	
4	102	0.456	0.626		1.000		
4	103	0.637	0.637	0.814	1.000	0.443	0.443
4	104	0.813	0.590		0.386		
5	19	0.201	0.072		0.629		
5	103	- 0.092	0.424	0.477	- 0.152	- 0.107	0.302
5	105	0.814	0.813		1.000		
5	112	0.679	0.679		1.000		
5	113	0.729	0.134		0.134		
5	114	0.101					
5	118	0.596	0.452	1.000	0.452	0.612	0.456
5	160	- 0.071	0.574		- 0.066		

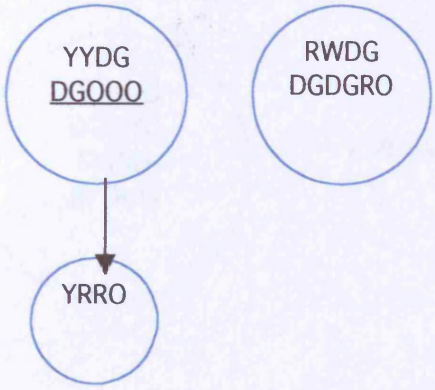
Figure 0-1 Sib groups and pedigrees inferred from *Kinship* and Census Data.

Key: Blue circles indicate a kin group where both Kinship and Census data agree; red circles indicate an individual which appears to be unrelated as it did not emerge upon the nest on which it appears and is unrelated according to kinship data; grey circles indicate that the relationship of the individual to the rest of the nest is unclear from *Kinship* and Census data yet census data indicates that the individual was born upon the nest.

Underlined individuals are those inferred from census data to be the most likely mother of those individuals in the sibship placed in the circle beneath her on the diagram (indicated by an arrow). Individuals, within a sib group, known to have joined the nest are highlighted in green.

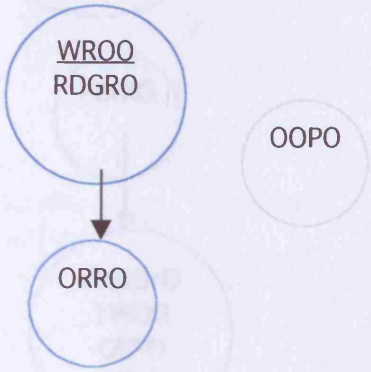
Site 2 Nest 6

Site 2 Nest 7



Site 2 Nest 15

Site 2 Nest 17



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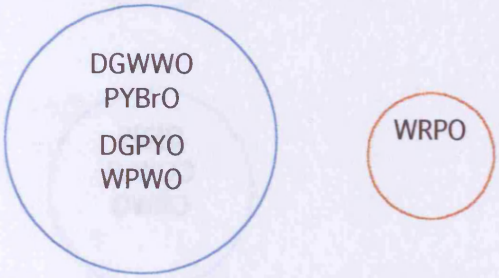

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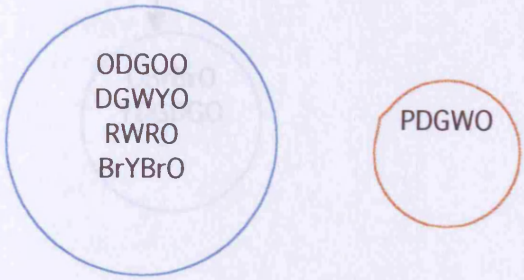

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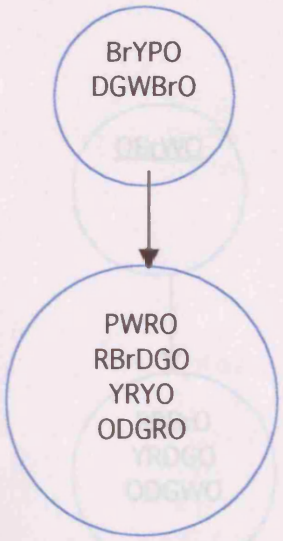
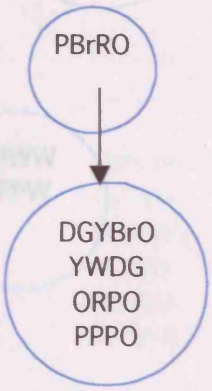
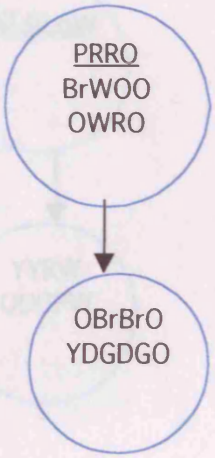


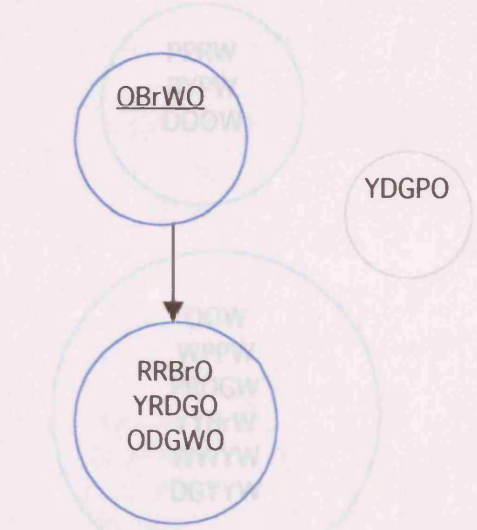
Site 2 Nest 109



Site 2 Nest 111

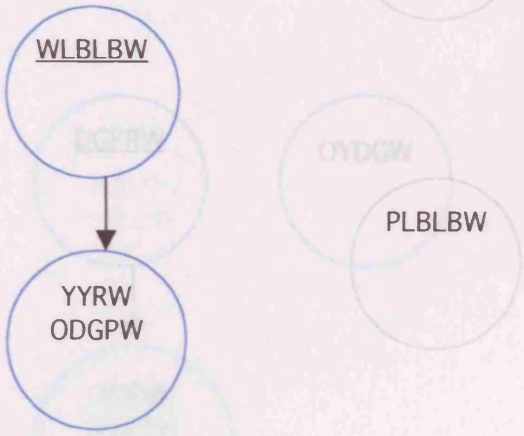


Site 2 Nest 112



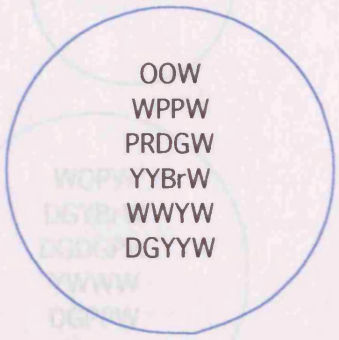
Site 4 Nest 5

Site 4 Nest 16

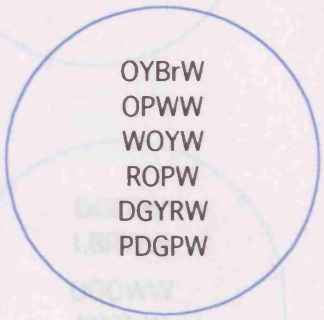


Site 4 Nest 19

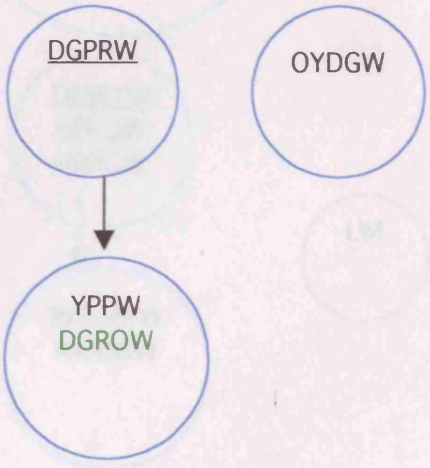
Site 4 Nest 25



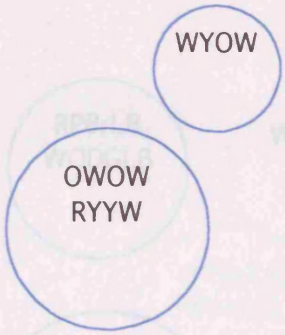
Site 4 Nest 26



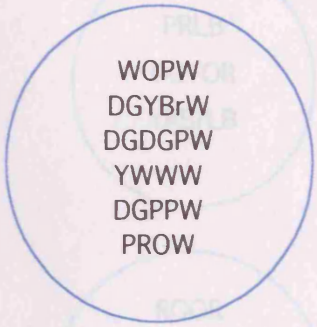
Site 4 Nest 28



Site 4 Nest 29

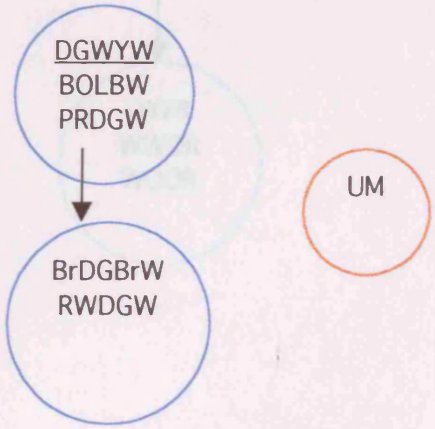


Site 4 Nest 102



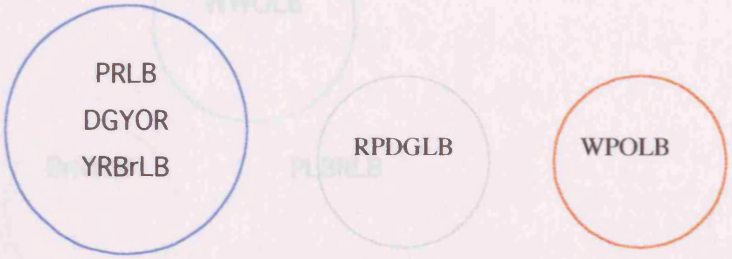
Site 4 Nest 103

Site 4 Nest 104

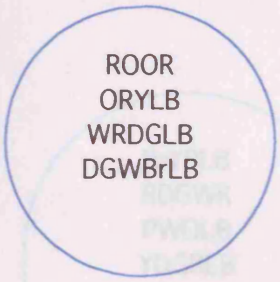


Site 5 Nest 19

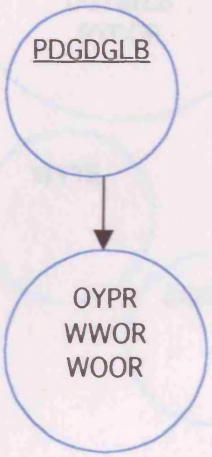
Site 5 Nest 103



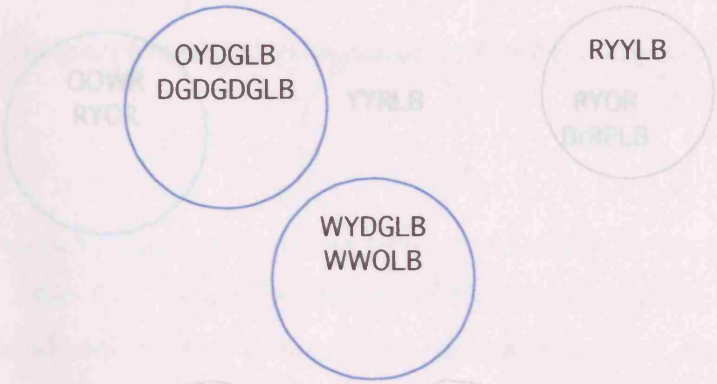
Site 5 Nest 105



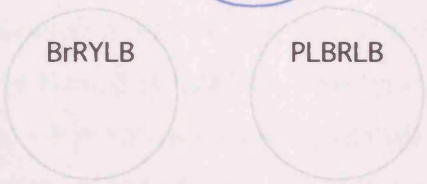
Site 5 Nest 112



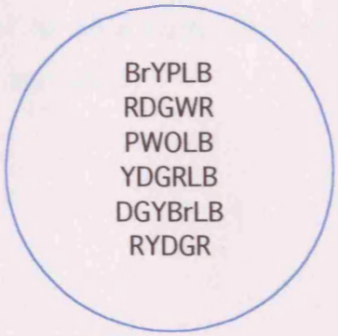
Site 5 Nest 113



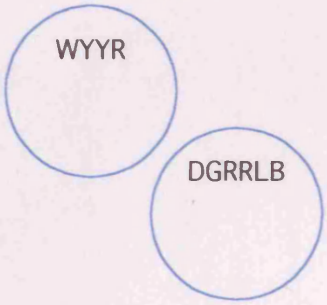
Site 5 Nest 114



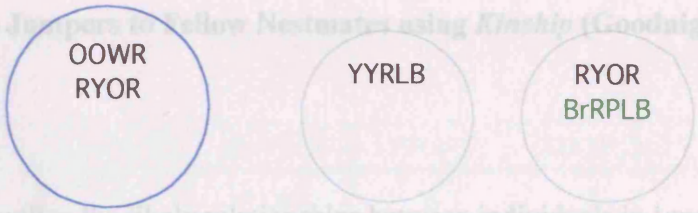
Site 5 Nest 118



Site 5 Nest 126



Site 5 Nest 160



Relatedness of Queue Jumpers to Fellow Nestmates using *Kinship* (Goodnight and Queller, 1999)

The following tables' outline the likely relationships between individuals in 'queue jumping' nests (see Table 0.2 to Table 0.8). 'Queue Jumping' individuals are highlighted in grey and 'queue jumped' individuals are outlined in bold. Each pairing was tested against a null hypothesis of an aunt – niece relationship (rm = 0.375, rp = 0) and a primary hypothesis of sibship (rm = 0.5, rp = 1) using the *Kinship* programme. *Kinship* significance flags are given on the left hand side of the tables. These values were used together with census data to infer the most likely relationship between individuals, e.g. wasps that emerged at a similar time and had high significance values using *Kinship* were deemed to be sisters. These inferences are given on the right hand side of the table. Each inference states the relationship of the side column individual to the top row individual.

Key:
S = Sister
M = Mother
D = Daughter
A = Aunt
N = Niece
U = Unrelated
X = Non – significant

Table 0.2 Kinship in Queue Jumper Nest 105, Site 2

	PWRO	RBrDGO	BrYPO	YRYO	**ODGRO**	DGWBrO
PWRO	-	S	M or A	S	**S**	S or N
RBrDGO	***	-	M or A	S	**S**	N
BrYPO	X	*	-	D or N	**M or A**	S
YRYO	**	***	X	-	**S**	A
ODGRO	**	***	*	**	-	**A**
DGWBrO	*	*	**	X	*	-

Number of females in queue jumper sibship = 3.

In Summary:

Left Hand Side of the Table:

	PWRO	RBrDGO	BrYPO	YRYO	**ODGRO**	DGWBrO
PWRO						
RBrDGO	***					
BrYPO	X	*				
YRYO	**	***	X			
ODGRO	**	***	*	**		
DGWBrO	*	*	**	X	*	

If a primary hypothesis of sibship is used against a null hypothesis of an aunt – niece relationship then:

Relationships of:

RBrDGO (Queue Jumper) to PWRO, ***, very likely to be sibs.

BrYPO to PWRO, X, likely to be a more distant relationship than Aunt – Niece.

BrYPO to RBrDGO (Queue Jumper), *, could be sibs.

YRYO to PWRO, **, likely sibs.

YRYO to RBrDGO (Queue Jumper), ***, very likely to be sibs.

YRYO to BrYPO, X, likely to be a more distant relationship than Aunt – Niece.

ODGRO (Queue Jumped) to PWRO, **, likely sibs.

ODGRO (Queue Jumped) to RBrDGO (Queue Jumper), ***, very likely to be sibs.

ODGRO (Queue Jumped) to BrYPO, *, could be sibs.

ODGRO (Queue Jumped) to YRYO, **, likely sibs.

DGWBrO to PWRO, *, could be sibs.

DGWBrO to RBrDGO (Queue Jumper), *, could be sibs.

DGWBrO to BrYPO, **,likely sibs.

DGWBrO to YRYO, X, likely to be a more distant relationship than Aunt – Niece.

DGWBrO to ODGRO, *, could be sibs.

Right Hand Side of the Table:

	PWRO	RBrDGO	BrYPO	YRYO	**ODGRO**	DGWBrO
PWRO		S	M or A	S	**S**	S or N
RBrDGO			M or A	S	**S**	N
BrYPO				D or N	**M or A**	S
YRYO					**S**	A
ODGRO						**A**
DGWBrO						

Relationships of:

PWRO to RBrDGO (Queue Jumper), S, very likely to be sibs.

PWRO to BrYPO, M or A, could be BrYPO's Mother or Aunt.

PWRO to YRYO, S, very likely to be sibs.

PWRO to ODGRO (Queue Jumped), S, very likely to be sibs.

PWRO to DGWBrO, S or N, PWRO could be the Sister or Niece of DGWBrO.

RBrDGO (Queue Jumper) to BrYPO, M or A, could be BrYPO's Mother or Aunt.

RBrDGO (Queue Jumper) to YRYO, S, very likely to be sibs.

RBrDGO (Queue Jumper) to ODGRO (Queue Jumped), S, very likely to be sibs.

RBrDGO (Queue Jumper) to DGWBrO, N, RBrDGO could be the niece of DGWBrO.

BrYPO to YRYO, D or N, BrYPO could be the Daughter or Niece of YRYO.

BrYPO to ODGRO (Queue Jumped), M or A, BrYPO could be the Mother or Aunt of ODGRO.

BrYPO to DGWBrO, S, very likely to be sibs.

YRYO to ODGRO (Queue Jumped), S, very likely to be sibs.

YRYO to DGWBrO, A, YRYO could be the Aunt of DGWBrO.

ODGRO (Queue Jumped) to DGWBrO, A, ODGRO could be the Aunt of DGWBrO.

Table 0.3 Kinship in Queue Jumper Nest 16, Site 4

	PLBLBW	WLBLBW	YYRW	**ODGPW**
PLBLBW	-	U	U	**U**
WLBLBW	X	-	D or N	**N**
YYRW	X	*	-	**S**
ODGPW	**X**	**X**	**	**-**

Number of females in queue jumper sibship = 1

Table 0.4 Kinship in Queue Jumper Nest 26, Site 4

	ROPW	**DGYRW**	**OYBrW**	OPWW	WOYW	PDGPW
ROPW	-		**C**	**C**	**C**	**U**
DGYRW	**X**	-	**N**	**N**	**N**	**U**
OYBrW	**X**	*	-	**S**	**S**	**U**
OPWW	**	*	***	-	S	U
WOYW	**	*	***	***	-	U
PDGPW	**X**	**X**	**X**	X	X	-

Number of females in queue jumper sibship = 0

Table 0.5 Kinship in Queue Jumper Nest 102, Site 4

	WOPW	DGPPW	DGYBrW	**PROW**	DGDGPW	YWWW
WOPW	-	A	S	**A**	A	S
DGPPW	X	-	N	**S**	S	S or N
DGYBrW	***	*	-	**A**	A	S
PROW	*	***	**X**	-	**S**	**S or N**
DGDGPW	X	***	*	***	-	S or N
YWWW	***	**	***	***	**	-

Number of females in queue jumper sibship = 2 – 5

Table 0.6 Kinship in Queue Jumper Nest T, Site 4

	DGPRW	LBRWW	DGOWW	YYDGW	RWBrW
DGPRW	-	S	S	**S**	**S**
LBRWW	**	-	S	**S**	**S**
DGOWW	***	**	-	**S**	**S**
YYDGW	*	***	*	-	**S**
RWBrW	***	***	***	**	-

Number of females in queue jumper sibship = 4

Table 0.7 Kinship in Queue Jumper Nest 103, Site 5

	RPDGLB	PRLB	WPOLB	DGYOR	YRBrLB
RPDGLB	-	U	U	U	U
PRLB	X	-	U	D or N	D or N
WPOLB	X	X	-	U	U
DGYOR	X	*	X	-	S or C
YRBrLB	X	**	X	**	-

Number of females in queue jumper sibship = 0

Table 0.8 Kinship in Queue Jumper Nest 118, Site 5

	BrYPLB	DGYBrLB	RDGWR	RYDGR	PWOLB	YDGRLB
BrYPLB	-	S	S	S or C	S	S
DGYBrLB	*	-	S	S or C	S	S
RDGWR	**	**	-	S or C	S	S
RYDGR	X	**	*	-	S	S
PWOLB	**	***	X	**	-	S
YDGRLB	*	***	**	**	***	-

Number of females in queue jumper sibship = 2 - 5