

**Conservation genetics and ecology of the endangered Black Bog
Ant, *Formica picea***

by

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Summary

In this study I aimed to investigate the genetic and ecological factors that may influence the distribution and conservation of the Black Bog Ant, *Formica picea*, within the UK, where it is classed as an endangered species. All main UK *F. picea* sites known at the start of this study (Cors Goch Llanllwch, Carmarthenshire; Rhossili Down, West Glamorgan; Hartland Moor, Dorset; the New Forest, Hampshire) were investigated. The first study of its kind of a UK ant species, this investigation involved measuring micro-geographic ecological parameters and using both microsatellite and mitochondrial DNA markers for genetic analyses.

High percentage cover of Bell Heather (*Erica tetralix*) and Deer Grass (*Trichophorum cespitosum*) in the immediate vicinity of the nest and a low density of Heather (*Calluna vulgaris*) in the local area occupied by *F. picea* appear to contribute to favourable habitat. Water table level may be the most influential determinant of *F. picea* nest distribution, however, as an unoccupied area appearing favourable in terms of *E. tetralix* and *T. cespitosum* cover revealed significantly lower relative humidity and vegetation height in 2003.

Colonies of all studied populations were highly polygynous, containing multiple reproducing queens per colony, and two populations revealed significant levels of inbreeding. Isolation by distance was detected in two populations, suggesting nest reproduction by budding and poor dispersal. All populations were genetically differentiated from one another, with the exception of the physically closest pair. Within Cors Goch Llanllwch a degree of social polymorphism was identified, with two sectors showing differing levels of polygyny and polydomy (multiple nests per colony). Aggression between non-nestmates was absent in this population, suggesting a largely unicolonial organisation.

Despite relatively large population sizes conferred by polygyny, UK populations of *F. picea* are at risk due to ecological specificity, inbreeding and limited dispersal. Within this study I make suggestions for conservation management including detailed ecological analyses and colony relocation.

Dedication

This thesis is dedicated to Claire Rees, for support, patience and listening to the words “It’s nearly done now” for eight long months

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CHAPTER 1

Introduction

The importance of ants

Insects are an extremely important and diverse group of organisms. Out of approximately 10 million total species on the planet, it is estimated that insects comprise an astonishing 80% (Wheeler 1990). However, being such a large and integral part of the planet's biodiversity does not mean that insects are without need of conservation (Samways 2005). Despite estimates that only <1% of insect species are threatened by extinction (Baillie and Groombridge 1996; McKinney 1999), it has been suggested that our poor understanding of the group could make this a vast underestimate (May et al. 1995), with the true value being as high as one quarter (McKinney 1999). In well studied regions, such as the UK, it has been shown that insects have higher rates of threat than many other taxa, including mammals (McKinney 1999). This trend is probably continued within the ants (Hymenoptera: Formicidae). Out of approximately 11000 species currently known, only 148 species appear in the IUCN Red List (IUCN 2004). However, within the UK, where ants are relatively well studied, out of approximately 40 species present, four are classed as Endangered on the GB Red List and nine are UK BAP (Biodiversity Action Plan) Priority Species (www.ukbap.org.uk). Seven of these nine are of the genus *Formica*.

Due to their huge diversity, it is unsurprising that insects have the ability to greatly effect the ecosystems in which they reside (Samways 2005). The ants are no exception, performing a host of ecosystem services (Hölldobler and

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Wilson 1990). Ants can occur in extremely high densities (Abbott 2005). Numerical dominance can make ants both an important food source (Samways 2005) and key predators of other invertebrates (Floren et al. 2002; Oliveras et al. 2005), sometimes overwhelming prey over 100 times their own biomass (Abbott 2005). In some ecosystems, especially grasslands and deserts, collecting seeds for food makes ants important dispersal agents for plants (Hölldobler and Wilson 1990). Some species have such a large effect on the physical composition of their environment that they are known as ecosystem engineers. Ants can move a large amount of soil due to the excavation of their nests. This in turn can lead to an increased flow of nutrients deep into the soil (Wagner et al. 2004; Dostal et al. 2005). The diversity of ant species and the different ecosystems they inhabit has also led to them being used as bioindicators of environmental characteristics (de Bruyn 1999; van Hamburg et al. 2004).

Genetic and social structure in ants

Ant colonies are often characterised by the number of resident reproductive queens. They are typically termed either monogynous (single reproductive queen) or polygynous (multiple reproductive queens). A monogynous colony comprising a singly mated queen and her offspring is often seen as the prototype for ant colony social structure, being the most simple. Multiple mating (polyandry) and polygyny increase the complexity and genetic diversity of colonies, and decrease the intra-colony relatedness (Queller 1993). Polygyny can be either primary or secondary (Hölldobler and Wilson 1977). In primary polygyny, multiple queens (a foundress association) found a colony together and polygyny is immediately established (Bourke and Franks 1995). However, in most cases primary polygyny is unstable and execution of all but one queen after foundation results in a monogynous colony (Herbers 1993). Far more common is secondary polygyny, where daughter queens are recruited into the natal nest when the cost of independent colony foundation

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is high (Bourke and Heinze 1994; Heinze and Keller 2000). Although variable queen number is a feature of social insect biology in general (Herbers 1993), the ants are a group in which queen number is particularly variable (Ross and Carpenter 1991). The genus *Formica* is no exception, showing both inter- and intra-specific social polymorphism. Populations of *Formica* ants range from monogynous through to highly polygynous, although no species has been found to be obligately monogynous (Sundström et al. 2005). Species such as *F. exsecta* (Seppä et al. 2004), *F. cinerea* (Lindstrom et al. 1996) and *F. truncorum* (Sundström 1993) exhibit separate monogynous and polygynous populations. It has recently been shown that individual colonies within a single population of *F. selysi* can vary between monogyny and high levels of polygyny (Chapuisat et al. 2004).

The number of queens within a colony has a great effect on the social and genetic structure of populations (Hölldobler and Wilson 1990). Monogyny is often associated with a nuptial flight, followed by independent colony foundation, leading to little or no intra-population genetic structuring. Conversely, queens from polygynous colonies are notable for a lack of nuptial flight and, instead, exhibit recruitment of new queens back into the natal nest. When new colonies are established by polygynous queens, it is often through a process termed budding (Rosengren and Pamilo 1983). In this case a newly mated queen, accompanied by a number of workers, will disperse a very short distance from the natal nest and found a new nest in the vicinity. As this founding queen is likely to be the daughter of a queen from the natal nest, nest budding leads to a genetic structure within populations where nests are genetically more related to physically closer nests. This genetic structure, termed isolation by distance, is exhibited in populations of species such as *F. paralugubris* (Chapuisat et al. 1997) and *F. lugubris* (Gyllenstrand and Seppä 2003). After foundation, exchange of individuals between the bud nest and the natal nest may cease, leading to two separate (albeit related) colonies. However, in some cases individuals may continue to be exchanged freely

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between nests, creating what is essentially a single colony with multiple nests, termed polydomy (Hölldobler and Wilson 1990). This exchange of individuals often results in low nestmate worker relatedness. Very high levels of polygyny and polydomy can sometimes lead to a phenomenon termed unicoloniality. In unicolonial populations relatedness between nestmate workers is close to zero and individuals can be exchanged between separate nests due to a lack of aggression between non-nest mates (Hölldobler and Wilson 1990; Bourke and Franks 1995). Completely free exchange of individuals between nests results in a lack of genetic structure within the population, as seen in a population of *F. truncorum* (Elias et al. 2005). However, in another unicolonial population of the species *F. paralugubris*, genetic structure is retained due to nest budding and poor dispersal (Chapuisat et al. 1997; Chapuisat and Keller 1999). Advantages conferred by polydomy include increased foraging efficiency and reduced risk of predation and stochastic damage to a colony (Rosengren and Pamilo 1983).

Factors affecting the persistence of ant populations

Several factors intrinsically linked to the social organisation of the ants can have adverse effects on the persistence of their populations (Gyllenstrand and Seppa 2003). In most species workers are sterile, drastically reducing the effective population size compared to its biomass. In a monogynous population, the effective population size is close to the number of colonies. Polygynous populations, having a larger number of reproductive queens, have a larger population size, although the exact value depends on the proportion of reproduction shared by each queen (reproductive skew). Haplo-diploidy itself reduces population size, as males only carry half the genetic material of a diploid female (Pamilo et al. 1997). This reduces the genetic variation of populations and makes them more sensitive to the effects of inbreeding depression (Chapman and Bourke 2001), which in turn can increase the risk of extinction (Saccheri et al. 1998). One more aspect of ant

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biology, together with small population size and sensitivity to inbreeding, adversely affects populations. In Hymenoptera with complementary sex determination, such as (as far as is known) occurs in the majority of ants, sex is determined at a single locus (Cook 1993). Homozygosity at this locus leads to the production of diploid males (instead of diploid females) which are usually sterile, therefore contributing nothing to the colony and reducing population growth rate due to fewer females being produced. Mating of genetically-related individuals increases the chance that the sex locus will be homozygous and that the genetic load of diploid males will be imposed upon the colony. Furthermore, this may cause a feedback loop whereby the reduced population growth rate caused by diploid male production leads to lower population size which, in turn, leads to genetic drift reducing variation at the sex determination locus and the production of more diploid males (Zayed and Packer 2005; Hedrick et al. 2006).

***Formica picea* ecology**

The Black Bog Ant, *Formica picea* Nylander, 1846 (formerly referred to as *F. candida* Smith, 1878 and *F. transcaucasica* Nasonov, 1889), a species recently shown to be distinct from the morphologically similar *F. candida* found in Central Asian mountains (Seifert 2004), is a Palaearctic species confined mainly to bog and wet heathland habitat. These habitats are declining in Europe and it seems likely that this will affect the distribution of *F. picea*, which is of conservation concern in both France (Falk 1991) and Germany (Orledge and Smith 1999). Within the UK it is estimated that 80% of lowland heath (including wet heath) and 94% of raised bog habitats have been lost over the last two centuries (www.ukbap.org.uk). *Formica picea* is currently known from just five UK sites. It is included both on the GB Red List as Endangered (Falk 1991) and as a BAP Priority Species. Due to the conservation status of *F. picea*, the last decade has seen agencies such as the Countryside Council for Wales, Wildlife Trust of West Wales and English

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Nature surveying it in some detail. The ensuing reports, although unpublished, have provided important information about the ecology of *F. picea*.

Formica picea is a ground-nesting species of the sub-genus *Serviformica*. Although *F. picea* nests are approximately 30 cm underground (Fowles and Hurford 1996), during the spring and summer months workers construct chambers above ground in which to incubate the brood, termed solaria (North 1998b). The micro-habitat surrounding each solarium is likely to be an important factor in the ants' choice of nest-site. Pieces of dead vegetation, such as Purple Moor Grass (*Molinia caerulea*) and *Sphagnum* moss, from the immediate vicinity of the nest are used to construct the solarium (Fowles and Hurford 1996). Thermal properties of the solaria are thought to be extremely important for brood development as an increase in temperature and relative humidity within solaria has been shown to correlate significantly with an increased number of solaria found to contain brood (North 1998a).

Another factor thought to affect distribution of *F. picea* nests is the level of ground saturation. Despite being a specialist of bog and wet heathland habitat, it has been suggested that extremely high levels of ground saturation and a year-round high water table are not essential for *F. picea* (Orledge et al. 1998). It is possible that where *F. picea* inhabits extremely wet areas it avoids competition with other ant species. Despite a lack of knowledge concerning the exact levels of ground saturation required by *F. picea*, it is known that waterlogged habitat can be tolerated. Within the UK, nests have been found surrounded by water and in extremely wet habitat. Outside the UK, when Seifert (2004) opened a solarium of *F. picea*, workers were observed actively escaping under the water's surface by climbing down the stems of *Sphagnum* moss. A study of the ant *F. uralensis*, a closely related species that shares the habitat of *F. picea* throughout much of its range, showed that this species can tolerate submersion for longer than species

inhabiting dry ground (Gyllenberg and Rosengren 1984). It is likely that *F. picea* shares this ability (North 1998a). *Formica picea* nests are patchily distributed within sites of occurrence (North 2000; Abbott 2001; Orledge and Lucas 2002). It has been suggested that this distribution could be due to each patch arising from a single founding nest (North 2000). This assumes colony reproduction by budding (Hölldobler and Wilson 1990). Genetic data from continental European populations suggesting restricted dispersal is consistent with this theory (Pamilo 1983). However, it is also possible that the patchy distribution of nests within UK *F. picea* populations is due to specific ecological requirements of the ant and a patchy distribution of suitable habitat.

Project aims

This project is the first integrated study of the conservation genetics and ecology of any UK ant species. I aimed to investigate the genetic and ecological factors that might influence the distribution and conservation of the Black Bog Ant, *Formica picea*, within the UK. Given that *F. picea* is an endangered species with apparently fragmented and isolated populations, studies of *F. picea* should also contribute to our knowledge of the biology of social insects with restricted ranges. This knowledge may have an applied aspect concerning the conservation of *F. picea*, as it could be used to help inform UK BAP strategies and site managers.

The use of polymorphic microsatellite markers in this study allowed a detailed investigation of colony and population genetic and social structure, including intra-nest relatedness, inbreeding and population genetic differentiation. Emphasis was given to the relationship between genetic and spatial structure. Using maternally inherited mtDNA markers in tandem with microsatellite markers, I also aimed to partition gene flow by sex, examining the dispersal and colonisation ability of *F. picea*. Although the genus *Formica* is relatively

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well studied, the only genetic studies previously conducted on this species investigated Finnish populations, using relatively invariable allozyme markers, over 20 years ago (Pamilo 1982a; Pamilo 1982b; Pamilo 1983). This study is the first to investigate the social and genetic structure of *F. picea* within the UK.

Intra-population distribution of *F. picea* nests is often patchy, possibly caused by a similarly patchy distribution of suitable habitat. To increase our knowledge of the micro-environmental conditions favourable to *F. picea*, this study examined ecological parameters in the immediate vicinity of the nest that may affect nest site distribution within populations. The use of Cors Goch Llanllwch, Carmarthenshire, an un-grazed lowland bog rarely visited by the public, as the study site, allowed the marking and revisiting of nests so that parameters could be measured repeatedly from the same subset of nests. This also enabled phenology to be investigated throughout both seasons. Due to the endangered status of *F. picea* within the UK, nests could not be sampled exhaustively and sexuals could not be sampled at all. This placed some limitations on the study, as social and genetic structure had to be inferred solely from worker genotypes and information about *F. picea* phenology had to be gathered without damaging the nests.

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Ecological requirements of the endangered Black Bog Ant (*Formica picea*)

Abstract

The Black Bog Ant, *Formica picea* Nylander, 1846 is an endangered species within the UK, with only five known sites of occurrence. Habitat loss is often cited as the main reason for its rarity. I studied the micro-geographic ecological parameters that may affect the distribution of *F. picea* in a lowland raised bog at Cors Goch Llanllwch, Carmarthenshire, between May and August in 2003 and 2004. Measurements of multiple ecological parameters were taken at nest sites, non-nest sites within an area inhabited by *F. picea* (inhabited area controls) and non-nest sites within an area not inhabited by *F. picea* (uninhabited area controls). Results suggest that a greater percentage cover of Bell Heather (*Erica tetralix*), Deer Grass (*Trichophorum cespitosum*), and *Hypnum* moss and greater vegetation diversity are favourable parameters for *F. picea*, as values for these parameters were significantly greater at nest sites compared with inhabited area controls. Percentage cover of Heather (*Calluna vulgaris*) and Bog Myrtle (*Myrica gale*), and degree of vegetational shading were significantly greater at inhabited area controls compared to nest sites, suggesting that these parameters are unfavourable to *F. picea*. Results from the comparison of inhabited area controls with uninhabited area controls suggested that the northern section of the bog, uninhabited by *F. picea*, was favourable in terms of most ecological parameters measured. In 2003, however, nest-level relative humidity was significantly lower in uninhabited area controls compared to inhabited area controls, suggesting that nests may have been absent from the northern,

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uninhabited, area of the bog due to low humidity. This may have been caused by a higher water table in the southern section of the bog (personal observation). Although this relationship was not observed in 2004, it is possible that heavy rainfall in this year may have homogenised the two sections in terms of relative humidity.

Introduction

Species that rely exclusively on specific habitat will often face more threats to their persistence than more generalist species, especially if that habitat is scarce. Bog and wet heathland are declining habitats in Europe, but are of great conservation importance due to the many rare species that inhabit them. Within the UK, 80% of lowland heath, including wet heath, and 94% of raised bog habitats have been lost since the beginning of the 19th century (www.ukbap.org.uk). This is mainly owing to commercial excavation of peat, agriculture and afforestation. The Black Bog Ant, *Formica picea* Nylander, 1846 is a Palearctic species confined mainly to bog and wet heathland habitat. There has been much confusion over its taxonomy, but recent genetic (Goropashnaya 2003) and morphometric (Seifert 2004) data have revealed two distinct species. The Black Bog Ant, *F. picea*, is distributed across Europe, the Caucasus and the West Siberian lowland, whereas the species found in Central Asian mountains, formerly treated as the same species, has now been re-described as *F. candida* Smith, 1978 (Seifert 2004).

Within the UK, *F. picea* is currently known from just five sites, two in Wales and three in England (Figure 2.1). It is included in the GB Red List as endangered (Falk 1991) and is a Biodiversity Action Plan (BAP) priority species (www.ukbap.org.uk). Owing to the decline of its habitat, *F. picea* is also of conservation concern in both France (Falk 1991) and Germany (Orledge and Smith 1999). For these reasons, agencies such as the

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Countryside Council for Wales, Wildlife Trust of West Wales and English Nature have monitored *F. picea* in some detail, providing much needed insight into its ecology.

F. picea is a ground nesting species, its nests being approximately 30 cm below the surface of the bog (Fowles and Hurford 1996). In spring and summer, however, workers construct chambers called solaria in which to incubate brood (North 1998b). These solaria are constructed from pieces of dead vegetation, such as Purple Moor Grass (*Molinia caerulea*) and *Sphagnum* moss (Fowles and Hurford 1996). Optimum conditions within these solaria are thought to be highly specific, significant positive correlations having been found between the number of solaria containing brood and both internal temperature and relative humidity of solaria (North 1998a). Ground saturation also seems linked to the presence of nests, although evidence appears to suggest that complete saturation is not required (Orledge et al. 1998). However, nests have been found surrounded by water, and outside the UK, Seifert (2004), upon breaching a solarium, even noticed workers actively escaping under the water's surface. Studies on the ant *F. uralensis*, which shares habitat with *F. picea* in continental Europe, have shown that this species can tolerate submersion for longer than species inhabiting dry ground (Gyllenberg and Rosengren 1984). This is a trait believed to be shared by *F. picea* (North 1998a). Workers are individual foragers (Else 1997) and known sources of food include other arthropods, nectar collected from Heather (*Calluna vulgaris*) and honeydew collected from aphids (North 1998a; Orledge and Smith 1999). The aphids are usually situated on the grass *M. caerulea* and are sometimes enclosed by the ants in a cylindrical structure made from vegetation fragments, similar to the material used in the ants' solaria. Throughout its UK range, *F. picea* demonstrates a patchy distribution within sites (North 2000; Abbott 2001; Orledge and Lucas 2002). It has been suggested that this pattern could arise from the each patch of nests expanding from a single nest (North 2000), implying philopatric foundation of

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new nests. In this situation daughter queens, accompanied by workers from a natal nest, found a new nest a short distance away (Hölldobler and Wilson 1990). This correlates with genetic evidence from continental European (Pamilo 1983) and UK (this study, Chapters 3 and 4) populations suggesting that *F. picea* has limited dispersal. However, the patchy distribution of *F. picea* within populations may also be due to similarly patchy distribution of suitable habitat.

To add to our knowledge of the micro-environmental conditions favourable to *F. picea*, this study aimed to measure ecological parameters, in the immediate vicinity of the nest, which may affect the distribution of *F. picea* nests within sites. The study site, Cors Goch Llanllwch, Carmarthenshire, is lowland raised bog, visited very little by the public and is un-grazed. This meant that individual nests could be marked and revisited; hence relevant parameters could be measured on multiple occasions from the same subset of nests. This site therefore offered an ideal situation in which to conduct a detailed, micro-environmental study into the parameters affecting the distribution of this scarce and endangered species. Very little work has been published concerning the ecological requirements of *Formica* species. Brian (1964) studied the distribution of five ant species (including one *Formica* species) on Hartland Moor, Dorset, but the author himself states that the analyses are more concerned with description than statistical inference. More recently, Mabelis and Chardon (2005) have investigated the fragmentation of *F. picea* habitat in The Netherlands, but here the definition of suitable habitat is pre-defined and no attempt is made to measure ecological parameters in the vicinity of nests. This investigation is also on a much larger scale than the present study, examining between-site differences rather than correlates of nest occurrence or non-occurrence within sites. Given the lack of published work on ecological requirements of *Formica* species, especially on a small scale, this study may provide a unique insight into how micro-geographic

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ecological parameters can affect the distribution of an ant species within its habitat.

Methods

Establishment of nest-sampling transects

To locate and sample *F. picea* nests at Cors Goch Llanllwch, four transects were established in May 2003 (Transects 1-4; Figure 2.2). Transects 1-3 were established to sample the area of bog broadly inhabited by *F. picea*. During 1995, previous researchers established a permanent *F. picea* monitoring grid (15 m by 30 m, Figure 2.3) at Cors Goch Llanllwch (Fowles and Hurford 1996) enclosing a location rich in *F. picea* nests identified in 1993 (Franks 1993). The number of *F. picea* nests in this grid has been counted almost annually since its establishment (Orledge 2002). To minimise disturbance to the grid, and to sample additional areas of the bog, it was therefore decided that Transects 1-3 should not pass through the grid, but should start in close proximity to it. To establish Transect 1, I therefore selected the first nest encountered just outside the western border of the grid. The next nest was discovered by walking 10 m north and searching for the nearest nest. This was repeated until no more nests were found. Transects 2 and 3 were established so as to cross or abut Transect 1. Nests were located using the same method but for these transects I walked west from either the drainage ditch dividing the bog (Transect 2) or the southern end of Transect 1 (Transect 3). (The southern section of the bog is divided into two parts (Figure 2.3). A drainage ditch running north-south separates the western section, which supports *F. picea*, from the eastern section, which is densely covered with the heather *C. vulgaris* and does not support *F. picea*). Extension of Transects 1-3 was halted when 20 *F. picea* nests had been located on them. For all three transects, the midpoint between each pair of adjacent nests was then selected to provide a set of sites, not occupied by *F. picea* nests but

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within the broad area occupied by them, from which measurements could be taken for comparison with those taken from the nest sites. These non-nest sites are termed inhabited area controls (Figure 2.2). No *F. picea* nests were found in the immediate vicinity of any inhabited area controls upon establishment of the transects. While establishing Transect 1, I noticed that the area immediately north of it was unoccupied by *F. picea* nests, although it did not appear obviously different in terms of vegetation from the occupied area to its south. I therefore added Transect 4 to provide a set of sites, not occupied by *F. picea* nests and outside the broad area occupied by them, from which measurements could be taken for comparison with those taken from inhabited area controls on Transect 1. These non-nest sites are termed uninhabited area controls (Figure 2.2). Starting at the northern end of Transect 1, I selected points at 5 m intervals while walking in a northerly direction. Intervals of this distance were used because the distance between the northern end of Transect 1 and the railway line marking the northern boundary of the bog was less than 100 m. Therefore using 10 m intervals would have resulted in fewer than 10 uninhabited area control sites, the number that I required for comparison with inhabited area controls on Transect 1. All points on all transects were marked with a tagged bamboo cane.

Ecological parameters

Data regarding ecological parameters, solar volume and phenology were collected in two successive years, with repeated measurements being taken at intervals between 22-05-03 and 26-08-03, and between 04-06-04 and 23-08-04 (Tables 2.1a, b). Ecological parameter data were collected to determine any differences between nest sites and inhabited area controls in the south of the bog, and inhabited area controls on Transect 1 and uninhabited area controls. It is conceivable that the relationship between ecological parameters and patch vary throughout the year. Unfortunately it

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was not possible to take measurements at other times of the year as the solaria are only used in the summer. Even if old solaria were found, it may not be possible to determine if the nest was active or had been abandoned. This means that no comparison involving nest sites could be made. Not all parameters measured in 2003 were measured in 2004; in 2004 I concentrated mainly on measuring parameters that produced significant results in 2003. It is unlikely that any parameters not measured in 2004 would have produced different results to those shown in 2003, as of the 12 parameters measured in both years only one differed between years (see *Results*).

F. picea is a ground-nesting species, and it is conceivable that pH of the soil could influence the suitability of a patch for colonisation. Approximately 25 ml of soil was taken from one position to the east of each patch, directly next to each solarium or marker cane. The pH of a 5 : 1 distilled water : soil mixture (Allen 1989) was measured using a digital pH meter. Measurements of the same mixture were taken three times and the mean calculated.

As solaria are brood incubating chambers, it seemed possible that the temperature and relative humidity of the immediate surroundings would play some part in choice of nest patch. These readings were taken with a digital thermometer / hygrometer. Measurements were taken both at head-level (approximately 180 cm above ground level) and at nest-level, i.e. directly above each solarium. At inhabited and uninhabited controls, where nests were absent, this latter measurement was taken at an equivalent level. This was usually on a dense covering of dead *Molinia* grass, upon which the thermometer / hygrometer would rest. A digital probe thermometer was used to record the temperature 10 cm under the ground directly next to the solarium or marker cane. For all measurements the thermometer / hygrometer and digital thermometer were left in position for 5-10 s until readings became stable.

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The insolation of patches is also likely to affect the incubation properties of the solaria. This was measured using a lux meter, again at both head-level and directly above each solarium (or equivalent level). Readings were taken with the sensor facing directly upwards and one reading was taken directly after the other, minimising the effect of change in cloud cover. The difference between head-level and nest-level insolation was calculated and defined as the degree of vegetational shading (since vegetation height on the bog rarely reaches head height).

The size of each nest's solarium on the transects was measured three times throughout each season, to estimate the rate of nest growth. In the absence of permission to excavate nests, the rate of nest growth was used as a surrogate for overall nest productivity. Height was measured, using a tape measure, from the top of the nest to ground level, or to where the vegetation became especially thick; a dense covering of dead *Molinia* grass sometimes obscured the position of ground level. Nest width was measured along the long and short axes of the solarium as seen from above. When these axes were not obvious, measurements were taken north-south and east-west.

Vegetation structure was measured as percentage cover of each species present, maximum vegetation height and vegetation diversity. To measure percentage cover, a 1 m² quadrat, divided into 100 10 cm² squares, was placed over the nest or control site and presence or absence of each plant taxon within each square was recorded. Plant species present were *Molinia caerulea* (Purple Moor Grass), *Erica tetralix* (Bell Heather), *Trichophorum cespitosum* (Deer Grass), *Calluna vulgaris* (Heather), *Myrica gale* (Bog Myrtle) and *Hypnum* moss. Percentage cover was measured as the number of squares within which a particular genus or species was recorded. To measure maximum vegetation height, a micro-transect was run through each point on the transects. The maximum height of any vegetation was recorded

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multiple times; at the centre of the solarium or control site, then at 10 cm intervals for 40 cm north, east, south and west. Flowering stalks of *Molinia* were not recorded, as they occurred at a low density but were always the tallest vegetation where present. At inhabited and uninhabited area controls, vegetation height was measured from ground level. At nest sites, vegetation height was measured from the same height as the top of each solarium. For comparison between nest sites and inhabited area controls, the height of the solarium (measured at the same time) was added to vegetation height measurements taken at nest sites. Vegetation diversity was calculated as Simpson's Index of Diversity (the inverse of Simpson's Index), the probability that two randomly selected plants would be different species (Peet 1974):

$$\text{Simpson's Index of Diversity (1-D)} = 1 - (\sum n(n-1)/N(N-1))$$

where n is the number of individuals of a given species within the studied area (in this case given as the number of quadrat squares which contained a given species) and N is the total number of individuals within the studied area (given as the sum of n over all species). From the percentage cover data for each species, I noticed that *E. tetralix* and *T. cespitosum* seemed to be much more abundant than other species, with the exception of *Molinia caerulea* which was almost always present at ~100% cover. To determine if *E. tetralix* and *T. cespitosum* were driving the pattern of vegetation diversity, Simpson's Index of Diversity was calculated with these two species removed from the data set.

Phenology was checked at each nest approximately every two weeks throughout both study periods. A pencil was inserted approximately 5 cm into the solarium, and the dried vegetation slightly prised apart. The presence of eggs, larvae, cocoons and adults was recorded. Worker cocoons were distinguished from sexual cocoons by size – the former being approximately half the size of the latter. Adult sexuals are also much larger than adult

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workers, although only one sexual was observed throughout the study (see *Results*). Eggs and larvae were not categorised into castes, due to their size (approximately 1 mm and 3 mm in length, respectively) and uniform appearance. Given the few sexual cocoons and adults seen, however, it is likely that the majority of eggs and larvae observed were of the worker caste.

Statistical methods

All statistical analyses were performed on SPSS version 12.0.1. I first analysed all ecological parameter data from Transects 1-3 for variance between nest sites and inhabited area controls. Data were checked for a normal distribution using the one-sample Kolomogorov-Smirnov test, and visualised as histograms. Each variable was measured on between one and six separate days, across both years. Firstly, each variable for each day was analysed separately, using the one-way ANOVA test. Where a variable was measured on two or more days, the data for each variable on all days were analysed using the more powerful univariate General Linear Model (GLM) test (restricted mixed model). This model provides analysis of variance for one dependent variable by one or more factors, analogous to a two-way ANOVA. The variance of ecological parameters could therefore be partitioned into that attributable to site (nest or control) and day. Although the variation in ecological parameters due to site type was of most interest in this project, as differences between days were expected, the univariate GLM model also allowed an effective increase in sample size over using one-way ANOVA to test each day separately. Data were collected over two years to increase the effective size of the data sets and increase the statistical power of the test, not to analyse the variation of parameters across years. Therefore although data from both years were pooled for analysis, the year in which data were collected was not factored into the analyses. Only 18 out of 31 data sets (58%) between 2003 and 2004 showed a normal distribution (Tables 2.2 and 3). However, the univariate GLM was used in all cases as analysis of

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variance is robust to deviations from normality if sample sizes are roughly equal (McKillup 2005). As SPSS version 12.0.1 uses an unrestricted model for this test, F values had to be recalculated from the mean squares (MS) using the following equations (Sokal and Rohlf 1981):

$$F (\text{Random Factor}) = MS_{\text{Random}} / MS_{\text{Error}}$$

$$F (\text{Fixed Factor}) = MS_{\text{Fixed}} / MS_{\text{Interaction}}$$

$$F (\text{Interaction}) = MS_{\text{Interaction}} / MS_{\text{Error}}$$

where a random factor is one whose values are a sample from a larger set of values, a fixed factor is one whose values of interest are all contained within the data file and an interaction is the interaction of the fixed and random factors. Day (the day on which a given parameter was measured) was the random factor and site (nest sites, inhabited area controls or uninhabited area controls) was the fixed factor. As site, the fixed factor, was the factor of interest in this study, the effects of the random factor and the interaction are not displayed in the results section.

Variance between uninhabited area controls (Transect 4) and inhabited area controls (non-nests on Transect 1) was also analysed using both a one-way ANOVA for each individual day's data and a univariate GLM for data from multiple days.

For each year, the mean number of days after the first phenology checks at which each stage of the ant was first and last seen was calculated, averaged over all nests for which full data were available. Data were plotted as range bar graphs. In 2003, the initial phenology check was 13 days earlier than the same check in 2004. For comparison of data between years, the first 13 days of data for 2003 were removed from each range bar. Solarium volume was

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calculated from height, width and length, assuming a half-ellipsoid shape. The difference in mean solarium volume between days on which solarium volume data were collected was analysed using paired samples t-tests.

Results

Random factor and interaction effects

As site, the fixed factor, was the factor of interest in this study, the effects of the random factor (day) and the interaction between site and day are not given in detail. Nest- and head-level temperature, relative humidity and insolation displayed significant differences between days in all data sets (2003, 2004 and both years combined) for which data were collected, with the exception that head-level relative humidity displayed no significant difference between days in 2004. These results are unsurprising as weather was highly variable between days. All other parameters measured displayed no significant difference between days with the exception of percentage cover of dead grass. The observed significant difference in percentage cover of dead grass between days in 2003 may have arisen from decomposition of the dead grass throughout the season, or it being obscured by new vegetation growth. The interaction of day and site had no significant effect on any parameter measured with the exception of nest- and head-level temperature in 2004 and nest-level relative humidity when both years data sets were combined.

Nest sites vs. inhabited area controls

In 2003, mean percentage cover of *E. tetralix* and *T. cespitosum*, mean head level relative humidity, mean maximum vegetation height and mean vegetation diversity were all significantly greater at nest sites compared to inhabited area controls (Figure 2.4, Table 2.2). Vegetation diversity calculated without *E. tetralix* and *T. cespitosum* showed no significant difference

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between sites (Table 2.2). Percentage cover of *C. vulgaris* and *M. gale* and mean vegetational shading was significantly greater at the inhabited area controls compared to nest sites (Figure 2.4, Table 2.2). Other parameters did not differ significantly between nest sites and inhabited area controls (Table 2.2). Out of the 12 parameters measured or calculated again in 2004, only the percentage cover of *M. gale* produced a different result, showing no significant difference between nest sites and inhabited area controls (Figure 2.5, Table 2.3). When data from both years for these 12 parameters were pooled and analysed together, the results were consistent with 2004, with the exception of the percentage cover of moss (Figure 2.6, Table 2.4). Results from the pooled data set showed that the percentage cover of moss was significantly greater at nest sites compared to inhabited area controls (Figure 2.6, Table 2.4).

Uninhabited area controls vs. inhabited area controls

In 2003, mean percentage cover of *E. tetralix* and *T. cespitosum* and mean vegetation diversity were significantly greater at uninhabited area controls compared to inhabited area controls (Figure 2.4, Table 2.2). Vegetation diversity calculated without *E. tetralix* and *T. cespitosum* showed no significant difference between sites. Mean ground temperature, mean nest-level relative humidity and mean vegetation height were significantly greater at inhabited area controls compared to uninhabited area controls (Figure 2.4, Table 2.2). Of the 12 parameters measured again in 2004, only the mean percentage cover of *C. vulgaris* produced a different result, being significantly greater at inhabited area controls compared to uninhabited area controls in 2004 (Figure 2.5, Table 2.3). When data from both years for these 12 parameters were pooled, results were consistent with 2004 with the exception of mean percentage cover of *M. gale* and vegetation diversity (calculated without *E. tetralix* and *T. cespitosum*), which both became significantly greater

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at inhabited area controls compared to uninhabited area controls (Figure 2.6, Table 2.4).

Phenology

In both years, all adult ants seen within the nests were workers (Figure 2.7). Sexual cocoons were seen within solarium in 2004 (Figure 2.7), although the only adult sexual seen over both years was an alate queen on 27-08-03 (Figure 2.8), upon a post at the NW corner of the permanent monitoring grid. Phenology showed a predictable pattern, similar in both years, of eggs appearing before larvae, which appeared before cocoons. Adult workers were present throughout the period of study in both years. The mean first and last sightings of larvae and worker cocoons occurred earlier in 2004 than in 2003 (Figure 2.7). It was not possible to compare the mean first sightings of eggs, as these were present in all nests from the first phenology check in 2004 (Figure 2.7). In 2003, eggs were not present in all nests when the initial phenology check was conducted.

Solarium volume

In 2003, there was no significant difference between mean solarium volume on 15-06-03 and 03-07-03 ($t = -0.967$, d.f. = 19, $p = 0.346$; Figure 2.9), although there were significant differences between mean solarium volume on 03-07-03 and 06-08-03 ($t = -3.791$, d.f. = 19, $p = 0.001$; Figure 2.9) and between 15-06-03 and 06-08-03 ($t = 4.020$, d.f. = 19, $p < 0.001$; Figure 2.9). In 2004, there was no significant difference between mean solarium volume measured on any day (16-06-04 and 15-07-04; $t = 0.923$, d.f. = 19, $p = 0.370$, 15-07-04 and 28-07-04; $t = 0.080$, d.f. = 19, $p = 0.937$, 16-06-04 and 28-07-04; $t = 1.148$, d.f. = 19, $p = 0.268$; Figure 2.10). Mean solarium volume within each year (\pm SE, calculated over measurements taken on multiple days) ranged from $442.65 \pm 100.40 \text{ cm}^3$ to $7458.55 \pm 653.76 \text{ cm}^3$ in 2003 and from

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309.80 ± 17.20 cm³ to 8622.53 ± 944.98 cm³ in 2004. Nest productivity, estimated as either increase in solarium volume or percentage increase in solarium volume, did not correlate significantly with any other parameter measured in either year (data not shown).

Discussion

Nest sites vs. inhabited area controls

The nest sites vs. inhabited area controls analysis was performed to elucidate which of the studied parameters were more or less favourable for *F. picea*. Higher percentage cover of *E. tetralix*, *T. cespitosum* and *Hypnum* moss, along with greater vegetation height, vegetation diversity and head-level relative humidity, were all found to be parameters possibly favourable to *F. picea*. Percentage cover of moss may be the least important of these factors to *F. picea*, as it was only shown to be significantly greater at nest sites compared to inhabited area controls when data from both years was combined, increasing the sample size. Contrastingly, a higher percentage cover of *C. vulgaris* and greater shading by vegetation were found to be unfavourable to *F. picea*. The percentage cover of *M. gale* was also shown to be significantly greater at inhabited area controls in 2003, but, in 2004 and when data were combined, this relationship was not apparent. This suggests that either the percentage cover of *M. gale* changed between years, or that its relationship with nest occurrence was relatively weak. Insolation and, therefore, vegetation shading, was only measured in 2003. This means it was not possible to determine whether its relationship with nest occurrence would remain significant between years. However, out of the 12 parameters re-measured in 2004, only the percentage cover of *M. gale* produced results that differed between years. It therefore seems likely that the relationship of vegetation shading with nest occurrence would stay consistent across years.

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Explanations can be put forward for most of the relationships between these parameters and either nest sites or inhabited area controls. Workers of *F. picea* were often observed on the flowers of *E. tetralix* (personal observation), presumably feeding on nectar, supporting the result that increased percentage cover of *E. tetralix* contributed to a favourable habitat. *Formica picea* frequently construct their solaria from moss. It is therefore easy to see how the presence of moss may make a site more favourable. However, the majority of *F. picea* nests in Cors Goch appeared to be constructed from fragments of grasses rather than moss (personal observation), possibly explaining the relatively weak correlation between *Hypnum* moss and nest occurrence. While it is not obvious why a higher percentage cover of *T. cespitosum* was present at nest sites compared to inhabited area controls, due to its lack of nectar producing flowers, it may be that it is also a favoured solarium-building material or that it requires the same environment for growth as *E. tetralix*. The results show that the greater vegetation diversity found at nest sites was due largely to the increased presence of *E. tetralix* and *T. cespitosum*, as no significant difference in vegetation diversity omitting these two species was revealed between nest sites and inhabited area controls. Solaria are incubating chambers for brood and it is therefore unsurprising that significantly greater vegetational shading and percentage cover of dense, spreading *C. vulgaris*, two parameters that seem likely to be linked, was found at inhabited area controls compared to nest sites. Orledge and Lucas (2002) have previously shown a negative association between *C. vulgaris* percentage cover and *F. picea* nest density, supporting this latter finding. However, the observed relationship between site type and vegetational shading seems to contradict the finding that mean vegetation height was significantly greater at nest sites compared to inhabited area controls, as it seems logical to assume that higher vegetation would produce more shade. An explanation for the observed pattern could be that the relationship between vegetational shading and site type was caused mainly by large, although relatively less common, *C. vulgaris* plants (see mean percentage

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cover estimates, Figures 2.4, 2.5 and 2.6), but the relationship between vegetation height and site type was caused by the more frequently occurring *M. caerulea*; percentage cover of *M. caerulea* approached 100% at all sites (data not shown). It is possible that a significant positive relationship between percentage cover of *M. caerulea* and site type does exist, but was not discovered in this study. This could result in the significant difference in vegetation height between nest sites and inhabited area controls, as *M. caerulea* is a particularly tall species and where conditions favour increased percentage cover they probably also favour increased vegetation height. However, due to the mostly vertical leaves of *M. caerulea* grass, a small increase in height may not have led to a detectable increase of vegetation shading. An explanation for how a relationship between percentage cover of *M. caerulea* and site type could have been missed is that the quadrat used to measure vegetation percentage cover rested on top of the vegetation and may have pushed down the flexible *M. caerulea* leaves, causing them to pass across a large number of quadrat squares. Therefore this method may not have been sensitive enough to discriminate between levels of percentage *M. caerulea* cover above a certain threshold. Studies have noted that *F. picea* often constructs solaría around tussocks of *M. caerulea* and that there is a significant positive relationship between nest density and *M. caerulea* cover in Cors Goch Llanllwch (Orledge and Lucas 2002). In the New Forest Cooke (2001) showed that percentage cover of *M. caerulea* was greater on hummocks, where *F. picea* exclusively occurred, compared to hollows, where it was absent. These studies support the idea that a relationship between *F. picea* nest distribution and percentage cover of *M. caerulea* may have been missed in this investigation.

As well as being responsive to the levels of various micro-environmental parameters, it is possible that the presence of a *F. picea* nest itself could have affected certain ecological parameters (i.e. soil nutrient composition and therefore local vegetation composition) in the immediate

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vicinity of the nest, as this has been well documented in other ant species, including *Formica* (Dean et al. 1997; Folgarait et al. 2002; Dostal et al. 2005; Lane and BassiriRad 2005; Ohashi et al. 2005). However, the species investigated usually build large conspicuous mounds, turning over a great amount of soil in the process. *Formica picea* is a ground nesting species with relatively small nests approximately 30 cm below ground (Fowles and Hurford 1996) and only the solaria show above ground during the summer months. It has also been suggested that *F. picea* colonies are relatively mobile (Orledge 2002), so individual nests may be short-lived. It therefore seems unlikely that nests of *F. picea* could significantly alter local ecological parameters.

Uninhabited area controls vs. inhabited area controls

This comparison was performed in an attempt to discover which parameters make the north section of the bog, uninhabited by *F. picea*, an apparently unfavourable habitat. The greater percentage cover of *E. tetralix* and *T. cespitosum* at uninhabited area controls compared to the inhabited area controls suggests that, in terms of these parameters, the northern section of the bog should be favourable to *F. picea*, yet nests were absent from this section. Vegetation diversity showed a similar and significant pattern. However, as with the analysis of nest sites versus inhabited area controls, when *E. tetralix* and *T. cespitosum* were removed from the data set there was no difference in vegetation diversity between areas in either year. When data sets from both years were pooled, a significant difference was observed in vegetation diversity between areas. However, this time vegetation diversity was significantly greater at inhabited area controls compared to uninhabited area controls, suggesting that, again, percentage cover of *E. tetralix* and *T. cespitosum* was driving the initial pattern of vegetation diversity. Despite the nest site versus inhabited area controls results suggesting that *C. vulgaris* contributes to an unfavourable habitat, its mean percentage cover was higher at inhabited area controls compared to uninhabited area controls. These

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results therefore apparently contradicted those from the nest site versus inhabited area controls comparisons. Hence, they suggest that there is some parameter preventing colonisation of the northern section of the bog, despite it being favourable in terms of other parameters. In 2003, mean ground temperature, nest-level humidity and maximum vegetation height were significantly greater at inhabited area controls than at uninhabited area controls. The former two parameters did not differ significantly between nest sites and inhabited area controls, whereas the latter parameter was significantly greater at nest sites than at inhabited area controls. This suggested that these parameters could be factors promoting the lack of *F. picea* nests in the northern section of the bog. However, although the mean ground temperatures of uninhabited area controls and inhabited area controls deviated significantly from each other, the difference between means was only 0.68°C. This small difference seems unlikely to affect the colonisation of a site by *F. picea*, although it may reflect a tendency that could be enhanced at other times of the year or at night. From the 2003 data it seemed that nest-level relative humidity was more likely to affect colonisation of a patch, given the obvious importance of keeping brood within the solaria from desiccation. However, when nest level relative humidity was measured in 2004, no significant difference between uninhabited area controls and inhabited area controls was discovered.

In addition, the presence of *C. vulgaris* may have both costs and benefits to *F. picea*. During both years, workers of *F. picea* were observed on flowers of *C. vulgaris*, presumably feeding upon nectar. North (1998a) considers *C. vulgaris* flowers to be an important food source for *F. picea*. It may therefore be that nesting too close to *C. vulgaris* is detrimental to brood incubation due to shading, but nesting where *C. vulgaris* is still accessible is favourable due to its use as a food source.

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It seems possible that the difference between the northern, uninhabited, section of the bog and the southern, inhabited, section of the bog could be linked to water level. From personal observations, the north of the bog seems much dryer and after heavy rainfall there was often standing water in the south, but very rarely in the north. These observations are supported by the maximum vegetation height and relative humidity results from 2003, but not by relative humidity results from 2004. A possible explanation for this apparent inconsistency, however, could be the disparity in rainfall between the two years. In summer 2003 (June – August), total rainfall in south Wales was 201 mm, whereas in summer 2004 (June – August) total rainfall in the same region was 273 mm (www.met-office.gov.uk). Extremely heavy rainfall during the 2004 field season often led to large amounts of standing water, occasionally even in more northern sections of the bog (personal observation). It is possible that the excessive rainfall in 2004 saturated the bog to the extent that relative humidity was homogenised across the entire bog. Further studies investigating the ecological requirements of *F. picea* on Cors Goch should incorporate an examination of the water table, specifically any differences between the northern and southern sections. An analysis of the water table at Rhossili Down (Orledge et al. 1998) using screw augers to examine the soil profile revealed that *F. picea* appeared to build nests in areas with relatively higher water tables, although a year round high water table did not appear necessary for colonisation.

Phenology

That only a single sexual adult was seen throughout both years could have multiple explanations. It is possible that the emergence of sexuals took place either outside the sampling period or on days when the bog was not visited. However, genetic analyses revealing high levels of polygyny and colony reproduction by budding (Chapters 3 and 4) suggest that *F. picea* mating flights are either small-scale or absent, queens possibly mating within, and

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being recruited back into, the natal nest. A recent study conducted on *F. picea* within the Netherlands (Mabelis and Chardon 2005) noted that queens were only occasionally caught in traps, and flying queens were never observed, concurring with results from this study. Another possibility, explaining the scarcity of males and queens in both studies, is that very few sexuals were produced in both years. The finding of sexual brood in 2004 in only three solarium supports this possibility. Previous data on *F. picea* phenology is scarce, although some information is available concerning the timing of sexual occurrence. Seifert (1996) notes that *F. picea* sexuals are present in July and August in Germany and both Donisthorpe (1927) and Orledge et al. (1998) report male pupae in nests towards the end of July. Assuming that there is no difference in the timing of occurrence between males and queens (as the gender of sexual cocoons reported in this study is unknown), it seems that sexual occurrence at Cors Goch in 2004 was slightly earlier than previously reported as, on average, the last sightings of sexual cocoons were in early July, presumably due to sexuals eclosing. In this study, the sighting of an alate queen in late August suggests that either the nest from which this queen emerged continued to produce sexuals later in the season than the nests studied, or that the observed queen had not recently eclosed.

The presence of sexual brood in 2004 suggests that this may have been a more productive year than 2003. In addition the earlier presence of both larvae and worker cocoons in 2004 implies that optimum conditions for reproduction occurred earlier in this year. However, if we assume that a more productive nest builds a larger solarium to enable the incubation of more brood, then the results from the measurements of mean solarium volume, which was found to increase throughout the latter part of the study period in 2003 but was unchanged throughout the study period in 2004, seem to contradict the finding that 2004 was the more productive year. Both greater productivity and unchanging mean solarium volume, however, may be

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explained by the greater rainfall in 2004. This may have made the habitat more favourable for brood production and incubation if *F. picea* requires a relatively high water table, but extremely heavy rainfall may have damaged the delicate solaria, preventing a noticeable increase their volume.

In conclusion, the results suggest that small-scale micro-geographical ecological parameters can affect the distribution of *F. picea* nests within a population. Greater percentage cover of *E. tetralix*, an observed source of nectar, and *T. cespitosum*, possibly a building material for solaria, as well as increased vegetation height appear to be favourable parameters for nest site choice. In contrast, the presence of *C. vulgaris* and increased vegetation shading in the immediate vicinity of the nest appear to be detrimental, probably inhibiting insolation of the solaria. However, observations of *F. picea* on the flowers of *C. vulgaris* suggest that this species is also an important food source and that access to *C. vulgaris* can enhance habitat quality for *F. picea*. The northern, uninhabited area of the bog appeared to be favourable in terms of most ecological parameters measured, although in 2003 nest-level relative humidity was significantly greater in the southern, inhabited area of the bog. Combined with vegetation height being significantly greater in the southern, inhabited area of the bog and the observation that this section seemed to flood more readily following heavy rainfall, it seems likely that the factor contributing to the lack of *F. picea* nests in the northern section of the bog is linked to the water table. If this is the case, then this study highlights the specificity of *F. picea* nest site choice, as without a favourable water table, even the abundance of a food source such as *E. tetralix* cannot promote site colonisation. A recent study by Mabelis and Chardon (2005) on the fragmentation of *F. picea* habitat, conducted in The Netherlands, revealed that this species was present at only 33% of patches deemed to be suitable for habitation. This suggests that in The Netherlands *F. picea* may also have highly specific ecological requirements that are currently poorly understood.

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Table 2.1

Dates on which data were successfully collected for (a) analyses of ecological parameters and (b) phenology and change in solaria volume. Adverse weather conditions sometimes meant that data could not be collected or were not useable. Only one data set was collected for the North-South analyses in 2003 as the northern section of the transect (Transect 4) was only established late in the 2003 season. For explanation of Nest-Control and North-South comparisons see Table 2.2.

(a)

	2003		2004	
	Nest-Control	North-South	Nest-Control	North-South
% Cover <i>Molinia caerulea</i>				
% cover <i>Erica tetralix</i>				
% cover <i>Trichophorum cespitosum</i>	15/06/03		15/07/04	15/07/04
% cover dead grass	03/07/03	06/08/03	28/07/04	28/07/04
% cover <i>Hypnum</i> moss	06/08/03		11/08/04	11/08/04
% cover <i>Calluna vulgaris</i>				
% cover <i>Myrica gale</i>				
Soil pH	07/08/03	07/08/03	n/a	n/a
Ground temperature				
Nest-level temperature				
Head level temperature	12/06/03		28/07/04	28/07/04
Nest level RH	02/07/03	27/07/03	11/08/04	11/08/04
Head level RH	27/07/03			
Nest level insolation				
Head level insolation				
Mean maximum vegetation height	03/07/03 06/08/03	06/08/03	n/a	n/a

(b)

	2003	2004
	Phenology	22/05/03
30/05/03		
10/06/03		04/06/04
20/06/03		16/06/04
02/06/03		30/06/04
14/07/03		15/07/04
27/07/03		28/07/04
07/08/03		13/08/04
21/08/03		23/08/04
26/08/03		
Solaria volume	15/06/03	16/06/04
	03/07/03	15/07/04
	06/08/03	28/07/04

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Table 2.2

Results of all ecological parameters measured in 2003. Nest = nest site, Control = inhabited area control, North = uninhabited area control, South = inhabited area control on Transect 1. For Nest vs. Control analyses, all parameters were measured on three separate days, with the exception of * = parameters measured on one day, and ** = parameters measured on two separate days. For North vs. South analyses, all parameters were measured on a single day. *p* values in bold are statistically significant. N/A is displayed for 'Variable significantly greater at:' when there is no significant difference between analysed sites. Vegetation diversity 2 = vegetation diversity calculated by omitting *E. tetralix* and *T. cespitosum*.

Data set	Normal distribution	Nest vs. Control				North vs. South			
		Test	F	<i>p</i>	Variable significantly greater at:	Test	F	<i>p</i>	Variable significantly greater at:
% Cover <i>Molinia caerulea</i>	no	Univariate GLM	F _{1,2} = 0.558	0.533	n/a	One-way ANOVA	F _{1,18} = 0.39	0.845	n/a
% cover <i>Erica tetralix</i>	yes	Univariate GLM	F _{1,2} = 434.027	0.020	Nest	One-way ANOVA	F _{1,18} = 14.199	0.001	North
% cover <i>Trichophorum cespitosum</i>	yes	Univariate GLM	F _{1,2} = 19.129	0.049	Nest	One-way ANOVA	F _{1,18} = 9.274	0.007	North
% cover dead grass	no	Univariate GLM	F _{1,2} = 7.230	0.115	n/a	One-way ANOVA	F _{1,18} = 0.892	0.358	n/a
% cover <i>Hypnum</i> moss	no	Univariate GLM	F _{1,2} = 14.038	0.064	n/a	One-way ANOVA	F _{1,18} = 0.358	0.557	n/a
% cover <i>Calluna vulgaris</i>	no	Univariate GLM	F _{1,2} = 26.168	0.036	Control	One-way ANOVA	F _{1,18} = 0.526	0.478	n/a
% cover <i>Myrica gale</i>	no	Univariate GLM	F _{1,2} = 386.286	0.003	Control	One-way ANOVA	F _{1,18} = 1.747	0.203	n/a
Soil pH*	yes	One-way ANOVA	F _{1,38} = 0.223	0.639	n/a	One-way ANOVA	F _{1,18} = 0.225	0.641	n/a
Ground temperature	yes	Univariate GLM	F _{1,2} = 0.208	0.693	n/a	One-way ANOVA	F _{1,18} = 5.878	0.026	South
Nest-level temperature	yes	Univariate GLM	F _{1,2} = 0.852	0.453	n/a	One-way ANOVA	F _{1,18} = 0.570	0.460	n/a
Head level temperature	yes	Univariate GLM	F _{1,2} = 1.943	0.298	n/a	One-way ANOVA	F _{1,18} = 2.276	0.149	n/a
Nest level RH	yes	Univariate GLM	F _{1,2} = 0.052	0.841	n/a	One-way ANOVA	F _{1,18} = 4.799	0.042	South
Head level RH	yes	Univariate GLM	F _{1,2} = 122.510	0.008	Nest	One-way ANOVA	F _{1,18} = 3.089	0.096	n/a
Nest level insolation	yes	Univariate GLM	F _{1,2} = 4.080	0.181	n/a	One-way ANOVA	F _{1,18} = 0.175	0.681	n/a
Head level insolation	yes	Univariate GLM	F _{1,2} = 0.323	0.627	n/a	One-way ANOVA	F _{1,18} = 0.702	0.413	n/a
Shading	yes	Univariate GLM	F _{1,2} = 61.696	0.016	Control	One-way ANOVA	F _{1,18} = 3.464	0.079	n/a
Mean maximum vegetation height**	yes	Univariate GLM	F _{1,1} = 11354.613	0.006	Nest	One-way ANOVA	F _{1,18} = 11.129	0.004	South
Vegetation diversity	yes	Univariate GLM	F _{1,2} = 208.526	0.005	Nest	One-way ANOVA	F _{1,18} = 6.499	0.020	North
Vegetation diversity 2	no	Univariate GLM	F _{1,2} = 1.409	0.357	n/a	One-way ANOVA	F _{1,18} = 0.538	0.473	n/a

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Table 2.3

Results of univariate General Linear Model analyses for all ecological parameters measured in 2004. Nest = nest site, Control = inhabited area control, North = uninhabited area control, South = inhabited area control on Transect 1. N = number of days on which data were collected. Significance (p) values in bold are statistically significant. N/A is displayed for 'Variable significantly greater at:' when there is no significant difference between analysed sites. Vegetation diversity 2 = vegetation diversity calculated by omitting *Erica tetralix* and *Trichophorum cespitosum*.

Data set	Normal distribution	Nest vs. Control				North vs. South			
		N	F	p	Variable significantly greater at:	N	F	p	Variable significantly greater at:
% Cover <i>Molinia caerulea</i>	No	3	$F_{1,2} = 0.726$	0.484	n/a	3	$F_{1,2} = 4.206$	0.177	n/a
% Cover <i>Erica tetralix</i>	No	3	$F_{1,2} = 495.27$	0.002	Nest	3	$F_{1,2} = 57.409$	0.017	North
% Cover <i>Trichophorum cespitosum</i>	No	3	$F_{1,2} = 105.296$	0.009	Nest	3	$F_{1,2} = 75.162$	0.013	North
% Cover <i>Hypnum</i> moss	No	3	$F_{1,2} = 2.748$	0.239	n/a	3	$F_{1,2} = 0.112$	0.770	n/a
% Cover <i>Calluna vulgaris</i>	No	3	$F_{1,2} = 38.33$	0.025	Control	3	$F_{1,2} = 67.819$	0.014	South
% Cover <i>Myrica gale</i>	No	3	$F_{1,2} = 0.333$	0.622	n/a	3	$F_{1,2} = 10.64$	0.083	n/a
Nest level temperature	Yes	2	$F_{1,1} = 0.299$	0.586	n/a	2	$F_{1,1} = 0.111$	0.795	n/a
Head level temperature	Yes	2	$F_{1,1} = 2.316$	0.370	n/a	2	$F_{1,1} = 0.469$	0.618	n/a
Nest level RH	Yes	2	$F_{1,1} = 0.0004$	0.988	n/a	2	$F_{1,1} = 4.909$	0.270	n/a
Head level RH	Yes	2	$F_{1,1} = 42849$	0.003	Nest	2	$F_{1,1} = 159.061$	0.051	North
Vegetation diversity	Yes	3	$F_{1,2} = 112.252$	0.009	Nest	3	$F_{1,2} = 230.414$	0.004	North
Vegetation diversity 2	no	3	$F_{1,2} = 3.589$	0.199	n/a	3	$F_{1,2} = 10.688$	0.082	n/a

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Table 2.4

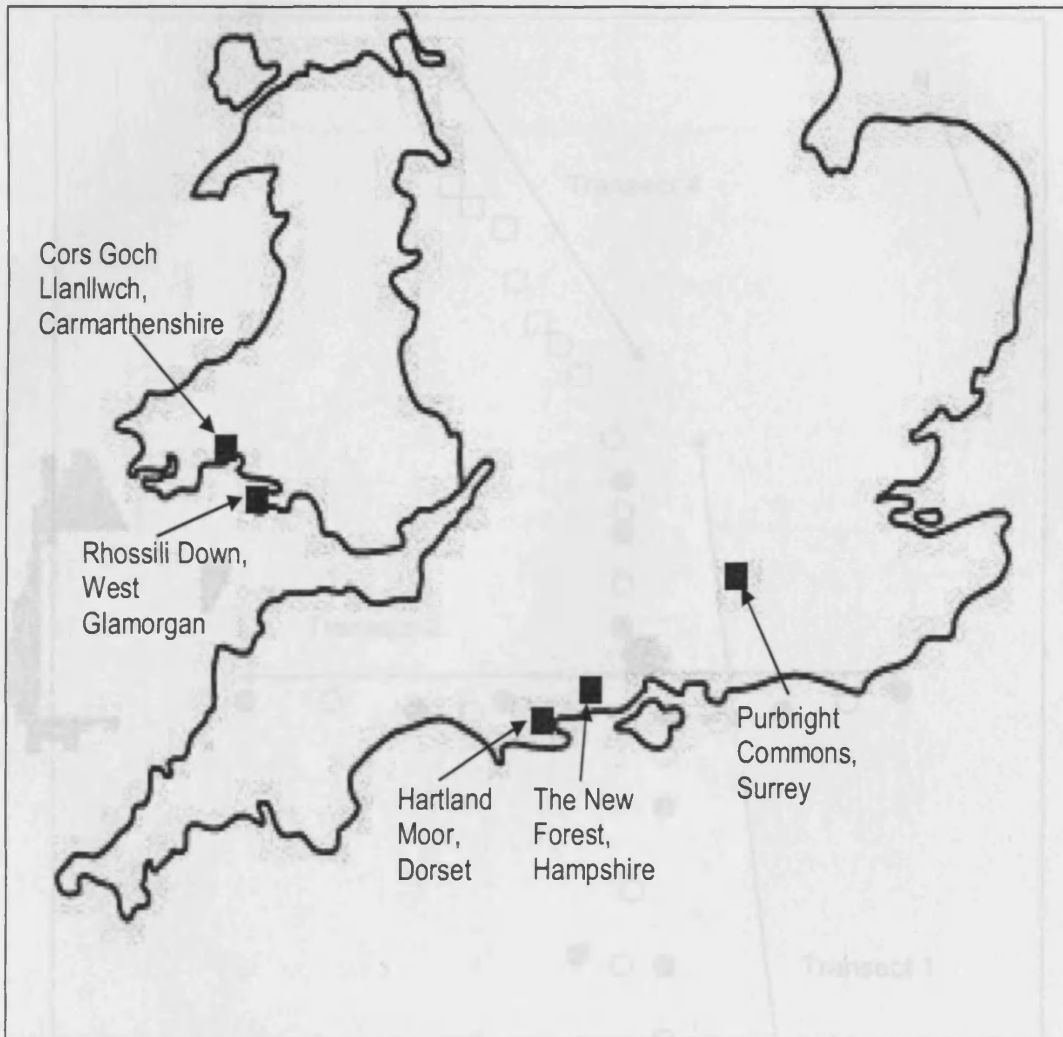
Results of univariate General Linear Model analyses for all ecological parameters measured in both 2003 and 2004. Nest = nest site, Control = inhabited area control, North = uninhabited area control, South = inhabited area control on Transect 1. N = number of days on which data were collected. Significance (p) values in bold are statistically significant. N/A is displayed for 'Variable significantly greater at:' when there is no significant difference between analysed sites. Vegetation diversity 2 = vegetation diversity calculated by omitting *Erica tetralix* and *Trichophorum cespitosum*.

Data set	Normal distribution	Nest vs. Control				North vs. South			
		N	F	p	Variable significantly greater at:	N	F	p	Variable significantly greater at:
% Cover <i>Molinia caerulea</i>	No	6	$F_{1,5} = 1.024$	0.358	n/a	4	3.278	$F_{1,3} = 0.168$	n/a
% Cover <i>Erica tetralix</i>	No	6	$F_{1,5} = 562.424$	<0.001	Nest	4	50.491	$F_{1,3} = \mathbf{0.006}$	North
% Cover <i>Trichophorum cespitosum</i>	No	6	$F_{1,5} = 63.881$	<0.001	Nest	4	60.118	$F_{1,3} = \mathbf{0.004}$	North
% Cover <i>Hypnum</i> moss	No	6	$F_{1,5} = 9.632$	0.027	Nest	4	0.941	$F_{1,3} = 0.403$	n/a
% Cover <i>Calluna vulgaris</i>	No	6	$F_{1,5} = 150.849$	<0.001	Control	4	32.063	$F_{1,3} = \mathbf{0.011}$	South
% Cover <i>Myrica gale</i>	No	6	$F_{1,5} = 0.0008$	0.993	n/a	4	14.411	$F_{1,3} = \mathbf{0.032}$	South
Nest level temperature	Yes	5	$F_{1,4} = 1.743$	0.257	n/a	3	0.484	$F_{1,2} = 0.559$	n/a
Head level temperature	Yes	5	$F_{1,4} = 1.485$	0.290	n/a	3	1.456	$F_{1,2} = 0.351$	n/a
Nest level RH	Yes	5	$F_{1,4} = 0.040$	0.851	n/a	3	0.178	$F_{1,2} = 0.714$	n/a
Head level RH	Yes	5	$F_{1,4} = 21.925$	0.009	Nest	3	0.534	$F_{1,2} = 0.541$	n/a
Vegetation diversity	Yes	6	$F_{1,5} = 292.667$	<0.001	Nest	4	209.601	$F_{1,3} = \mathbf{0.001}$	North
Vegetation diversity 2	No	6	$F_{1,5} = 5.160$	0.072	n/a	4	22.324	$F_{1,3} = \mathbf{0.018}$	South

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Figure 2.1

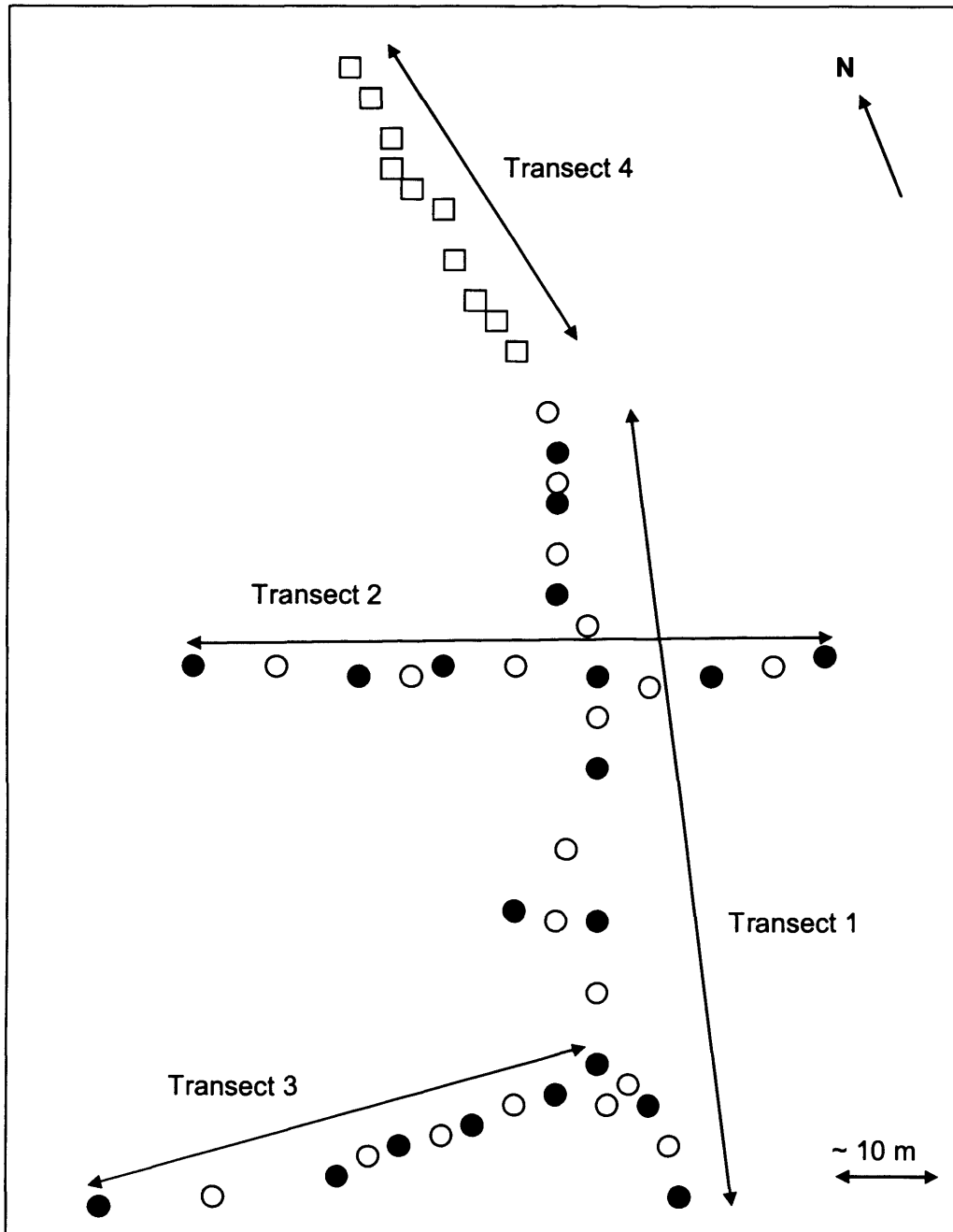
Five currently known UK sites of *F. picea*, two in Wales and three in England. The site at Purbright Commons, Surrey, was discovered too late for inclusion in this study.



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Figure 2.2

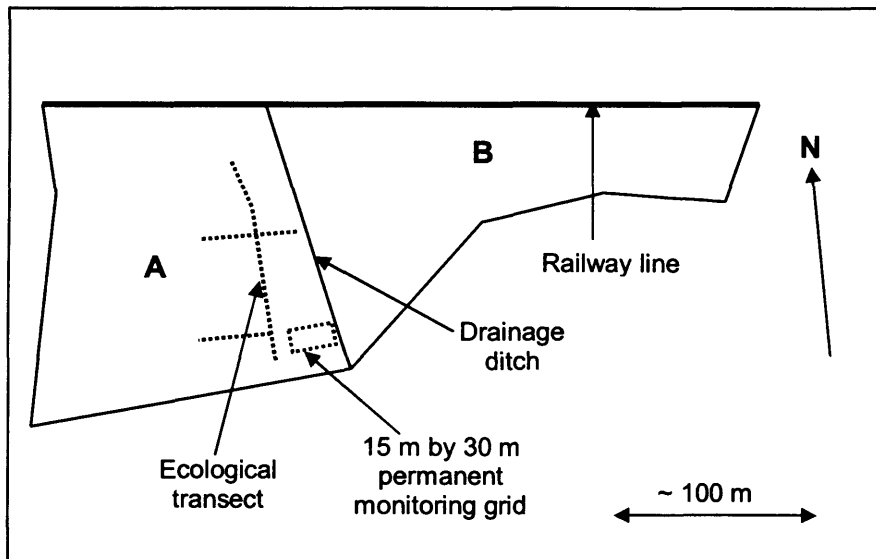
Transects placed at Cors Goch Llanllwch for the measurement of ecological parameters. Filled circles are nest sites, open circles are inhabited area controls and open squares are uninhabited area controls (no *F. picea* nests were found in the northern section of the bog).



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Figure 2.3

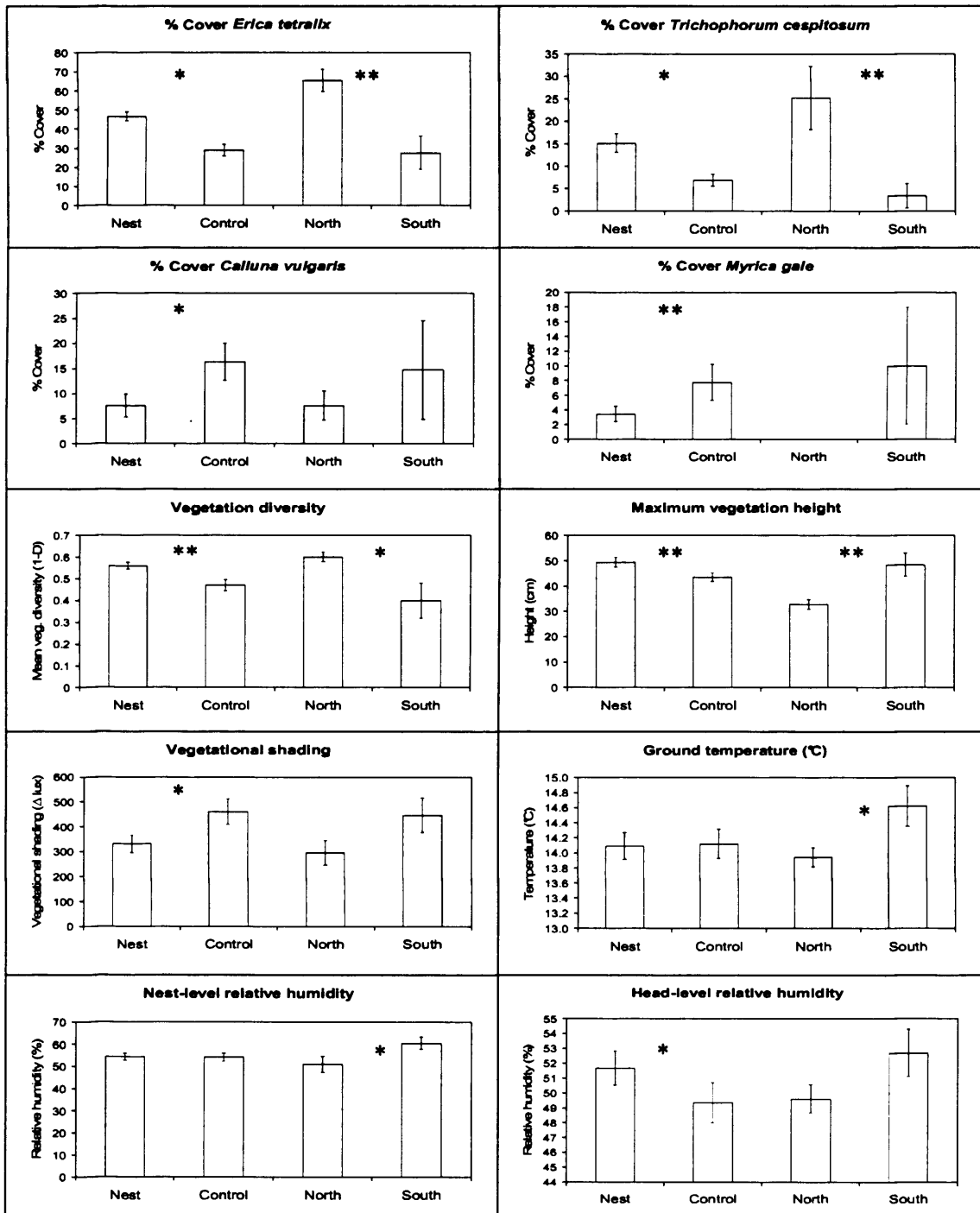
Approximate map of Cors Goch Llanllwch, showing the western, inhabited area of the bog (A) and the eastern, uninhabited area of the bog (B), separated by a drainage ditch. The ecological transect, the permanent monitoring grid and the railway line are also displayed.



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Figure 2.4

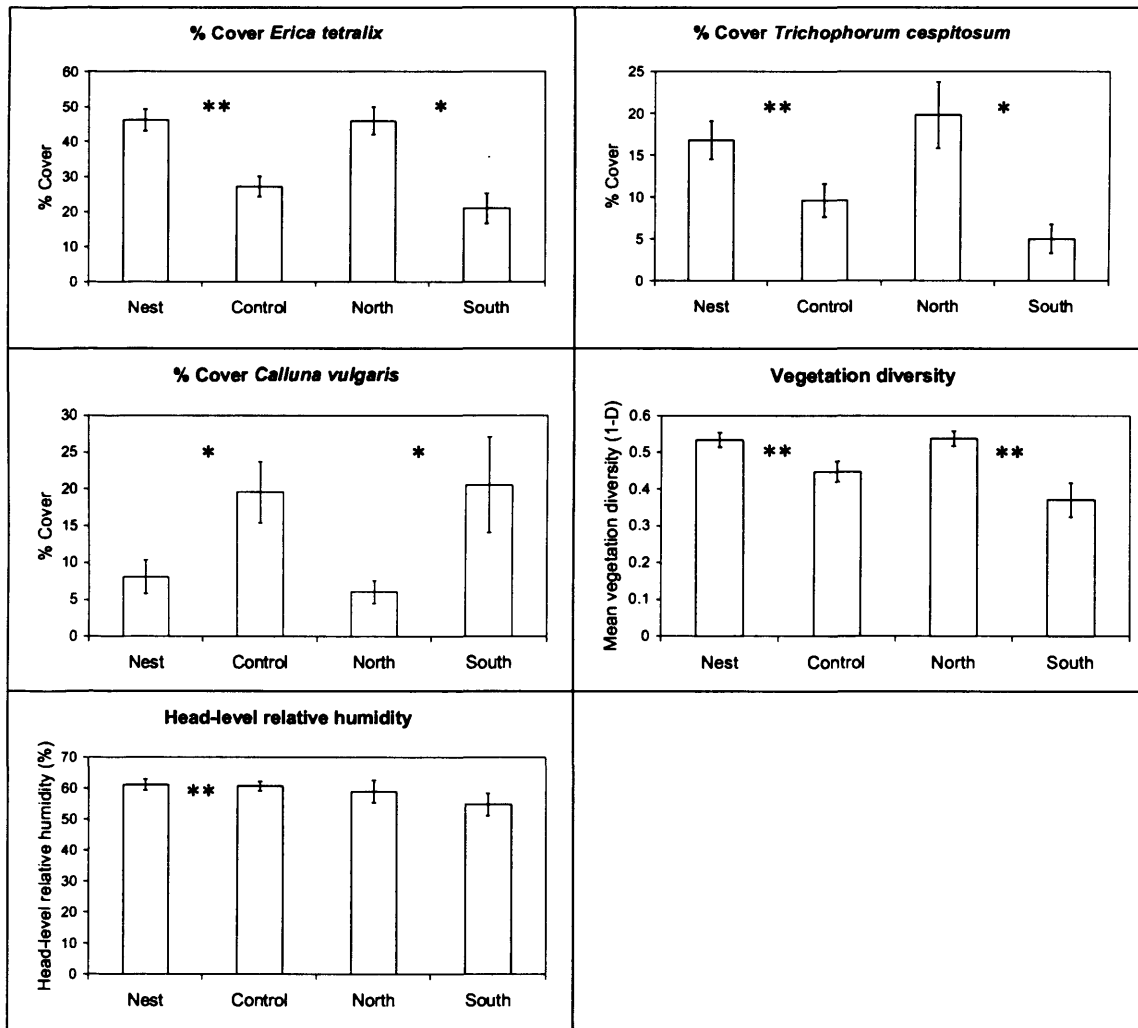
Significant ecological parameter results for 2003. Nest = nest sites. Control = inhabited area controls. North = uninhabited area controls. South = inhabited area controls on Transect 1. Error bars = \pm one SE. Comparisons made between Nest and Control, and North and South. Asterisks represent significant differences between these data sets. * = $p < 0.05$, ** = $p < 0.01$. For test statistics see Table 2.2.



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Figure 2.5

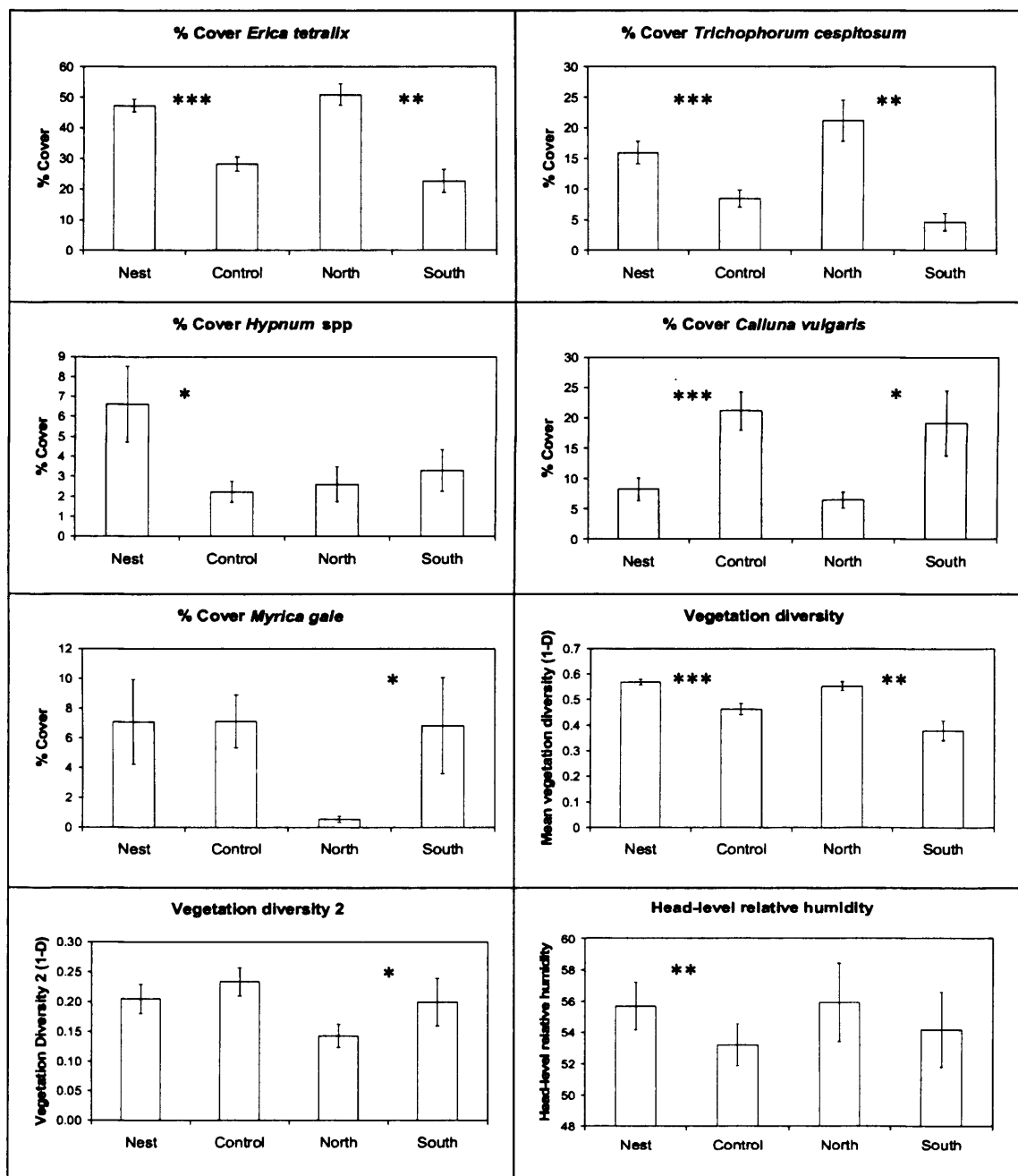
Significant ecological parameter results for 2004. Nest = nest sites. Control = inhabited area controls. North = uninhabited area controls. South = inhabited area controls on Transect 1. Error bars = \pm one SE. Comparisons made between Nest and Control, and North and South. Asterisks represent significant differences between these data sets. * = $p < 0.05$, ** = $p < 0.01$. For test statistics see Table 2.3. It may be noted that head-level relative humidity is significantly different between Nest and Control sites, despite very similar means. This is due to both the low standard errors and the way the test statistical test works. The graph shows the mean value of each parameter and its standard error calculated over all days on which that parameter is measured. The univariate GLM analysis, however, takes account of the relationship between factors within days. Although head-level relative humidity is only slightly higher in Nest compared to Control sites, this relationship is present in both days on which data were collected, increasing its significance.



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Figure 2.6

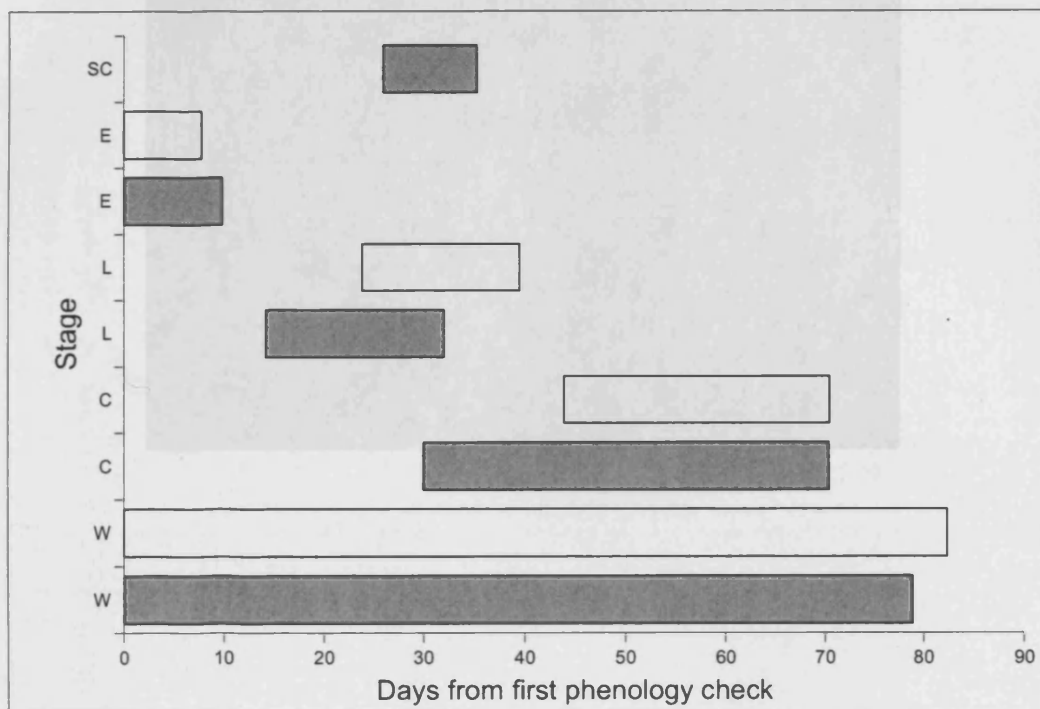
Significant ecological parameter results for 2003 and 2004 combined. Nest = nest sites. Control = inhabited area controls. North = uninhabited area controls. South = inhabited area controls on Transect 1. Error bars = \pm one SE. Comparisons made between Nest and Control, and North and South. Asterisks represent significant differences between these data sets. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. For test statistics see Table 2.4.



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Figure 2.7

Phenology results for 2003 and 2004. Bars represent ranges between mean first and last sightings of each stage, averaged over 17 nests for which full data were available. Open bars = 2003, filled bars = 2004. W = adult workers, E = eggs, L = larvae, C = worker cocoons, SC = sexual (queen or male) cocoons. As the first phenology check in 2003 was 13 days earlier than the same check in 2004, to allow a correct comparison the first 13 days of data from 2003 have been removed. Therefore day zero corresponds to the 4th June in both years (in 2003 the first phenology check was actually conducted on the 22nd May).



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Figure 2.8

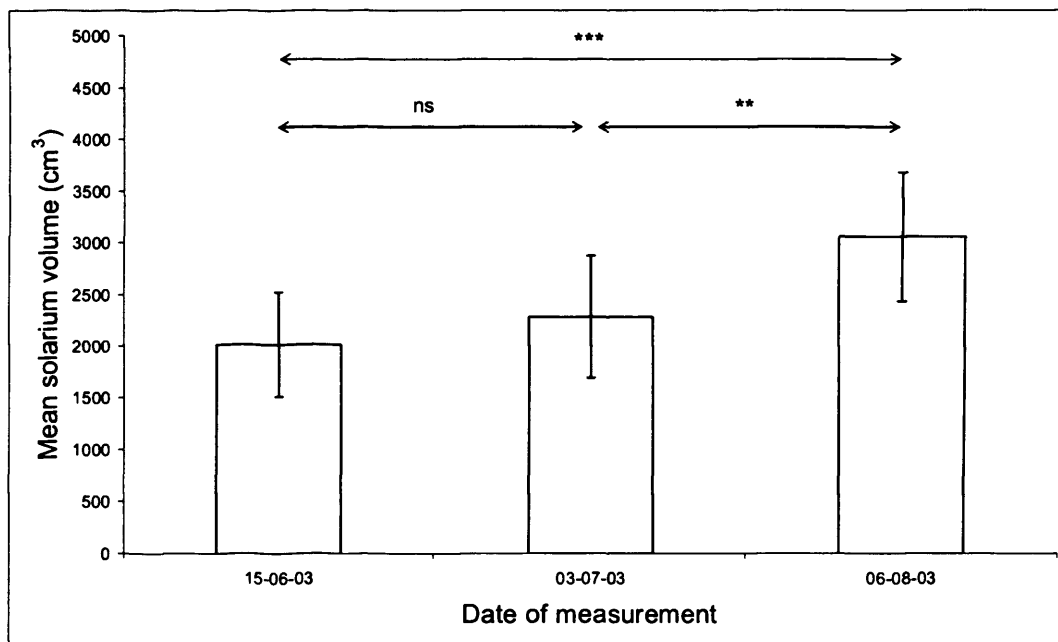
Alate *F. picea* queen seen on the north west corner-post of the permanent monitoring grid at Cors Goch Llanllwch, Carmarthenshire, on 27-08-2003. This was the only adult sexual observed in either 2003 or 2004.



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Figure 2.9

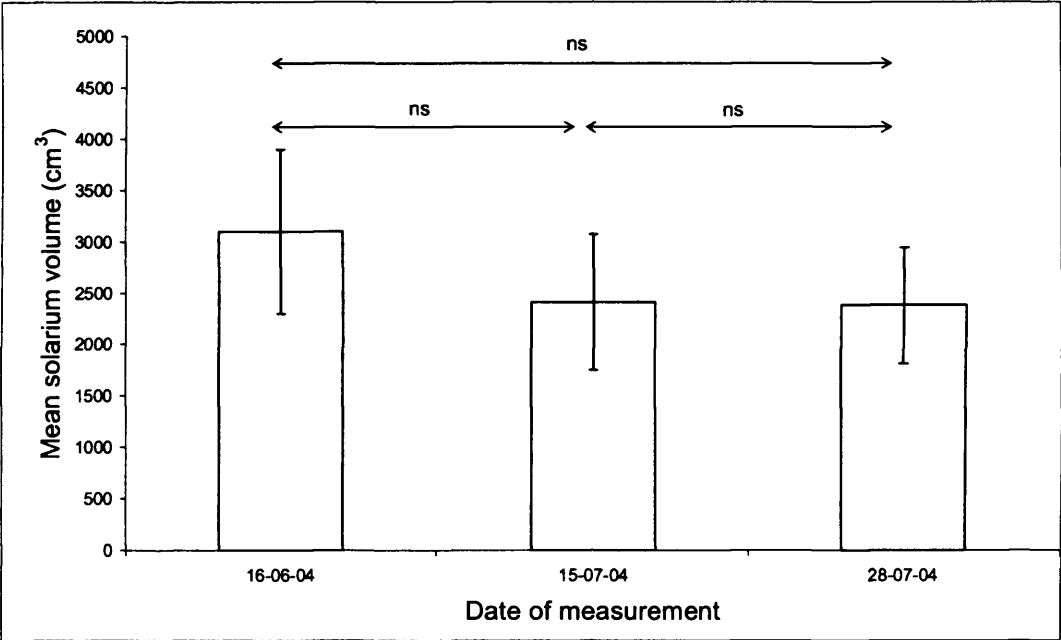
Mean solarium volume increase throughout 2003 study period. Error bars represent \pm one standard error. Significance of any difference in mean solarium volume between dates on which data were collected, tested using a paired samples t-test, is shown directly above arrows delineating the two data sets were tested. *** = $p < 0.001$, ** = $p < 0.01$, ns = non significant.



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Figure 2.10

Mean solarium volume decrease throughout the 2004 study period. Error bars represent \pm one standard error. Significance of any difference in mean solarium volume between dates on which data were collected, tested using a paired samples t-test, is shown directly above arrows delineating the two data sets were tested. ns = non significant.



CHAPTER 3

Relatedness, queen number and mating system of UK populations of *Formica picea*

Abstract

In this study I used microsatellite markers to investigate the genetic structure of colonies and populations of the endangered Black Bog Ant, *Formica picea*, within the UK. The four study populations (Cors Goch Llanllwch, Carmarthenshire; Rhossili Down, West Glamorgan; Hartland Moor, Dorset; the New Forest, Hampshire) were the only known UK populations at the start of the study. Approximately 25 workers from 34 nests in Cors Goch Llanllwch and 12 workers from 10 nests in each of the other populations were collected for genetic analysis in August 2003. To compare social and genetic structure temporally, approximately 25 workers from 26 nests were collected from Cors Goch Llanllwch in August 2004. There was significant inbreeding in two of the four study populations and inbreeding-corrected nestmate worker relatedness estimates were never significantly greater than zero, suggesting a large number of reproducing queens per nest. The main study site, Cors Goch Llanllwch, displayed a level of intra-population social polymorphism. In this population, worker nestmate relatedness estimates (whether corrected for inbreeding or not) for colonies in an area with high nest density (grid sample) were not significantly greater than zero, whereas worker nestmate relatedness estimates for colonies in an area with lower nest density (transect sample) were significantly greater than zero. There was no significant difference in nestmate worker relatedness estimates between the 2003 and 2004 grid samples. These samples did show a marginally significant

difference in inbreeding estimates, although neither estimate was significantly greater than zero.

Introduction

Social insect populations exhibit great diversity in their social and genetic organisation. In ants, a primary factor contributing to this diversity is the number of effectively reproducing females (queens) in a colony. Ant populations and colonies are defined as being either monogynous (each colony containing one reproducing queen) or polygynous (each colony containing multiple reproducing queens). Whether populations and colonies are monogynous or polygynous has a large effect on social organisation and population structure (Hölldobler and Wilson 1990). There is a great deal of social polymorphism within the genus *Formica*, both within and between species (Pamilo et al. 1997), with many species showing both monogyny and polygyny (Sundström 1993; Goropashnaya et al. 2001; Gyllenstrand and Seppä 2003; Seppä et al. 2004). Monogynous populations are characterised by a nuptial flight followed by newly-mated queens independently establishing a colony, resulting in very little genetic structuring. No *Formica* species is obligately monogynous (Sundström et al. 2005), but many have separate monogynous and polygynous populations, including *F. exsecta* (Seppä et al. 2004), *F. truncorum* (Sundström 1993) and *F. cinerea* (Lindstrom et al. 1996). Owing to their nuptial flights, monogynous populations are typically thought to contain individuals with good dispersal ability. Conversely, polygynous populations are characterised by the absence of a nuptial flight and possible mating within the natal nest. Newly-mated queens can either be recruited back into the natal nest, or can take a number of workers and establish a new nest within the vicinity of the natal nest, a mode of colony reproduction termed budding (Rosengren and Pamilo 1983). Budding leads to genetic structuring of populations, where nests are genetically more similar to neighbouring nests than to more distant nests, potentially creating the

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phenomenon of isolation by distance. This genetic structuring is present in many *Formica* species including *F. paralugubris* (Chapuisat et al. 1997) and *F. lugubris* (Gyllenstrand and Seppa 2003). As a result of their mating systems and mode of colony reproduction, polygynous populations are typically thought to have poor dispersal ability, limiting gene flow between populations (Pamilo et al. 1997). Despite producing differing social and genetic structures, monogyny and polygyny are not unlinked. Monogyny can give rise to polygyny through the recruitment of daughter queens into the natal nest in certain situations where the costs of independent colony founding are high (Bourke and Heinze 1994; Sundström 1995; Heinze and Keller 2000).

Social organisation can have consequences for the viability and persistence of ant populations (Gyllenstrand and Seppa 2003). The sterile worker caste present in many species, with only queens and males being able to reproduce, means that effective population size is dramatically lower than the number of individuals in the population. Haplo-diploidy itself reduces effective population size as males carry only half the genetic material of the females (Pamilo et al. 1997). It also reduces genetic variation within populations, leading more readily to inbreeding depression (Chapman and Bourke 2001) and reducing the capacity of a population to respond to environmental change (Darvill et al. 2006). In Hymenoptera with complementary sex determination, such as is thought to be the case in ants, sex is determined by a single locus (Cook 1993). When genetically-related individuals mate, there is an increased chance that this locus will be homozygous, which leads to the production of diploid males instead of diploid females. Production of these males contributes nothing to the colony, as they are sterile, and reduces the number of females produced. This may cause a reduction in population growth rate and size, leading to genetic drift increasing homozygosity at the sex determination locus which, in turn, results in the production of more diploid males. Such a 'diploid male vortex' could drastically increase the

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probability of population extinction (Zayed and Packer 2005; Hedrick et al. 2006). Effective population size in monogynous species is closely tied to the number of colonies, but can be dramatically increased by polygyny. A large polygynous population may therefore prove relatively robust to fluctuating population size, as only a severe bottleneck would cause a decrease in genetic variation (Maki-Petays et al. 2005). However, decreased dispersal stemming from polygyny may also result in reduced gene flow between populations and a reduced ability to colonise new habitat.

The Black Bog Ant *F. picea* (formerly *F. candida*, see Chapter 2) is a scarce habitat specialist found only in bog and wet heathland habitat and confined within the United Kingdom to only five populations. Throughout Europe *F. picea* is a rare relict species (Czechowski et al. 2002). Within the UK it is classed as Endangered on the G.B. Red List and is a Biodiversity Action Plan (BAP) Priority species. Habitat loss due to drainage for afforestation and agriculture is thought to be the leading factor contributing to this species's rarity. Very little is known about its social and genetic structure. The only previous genetic studies of this species were conducted on Finnish populations using allozyme markers. Populations were thought to include both monogynous and polygynous colonies (Pamilo 1982a) and their queens were found to mate only once (Pamilo 1982b). Relatively high differentiation of allele frequencies between populations suggested limited dispersal (Pamilo 1983). However, due to the intra-specific social polymorphism shown to be present in many *Formica* species and the physical isolation of UK populations from one another, it is possible that UK populations of *F. picea* differ considerably from both each other and from populations elsewhere in Europe in their social and genetic organisation. As a scarce-habitat specialist, this species permits investigators to address questions about the genetic structure of colonies and populations of social insects (and of the ant genus *Formica* in particular) with restricted ranges. In addition, the use in the present study of highly polymorphic microsatellite markers allowed a detailed

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analysis of social and genetic organisation not before conducted in this species. Using microsatellite markers, I aimed to investigate the genetic structure of UK *F. picea* colonies and populations, including within-nest relatedness and inbreeding.

Methods

Genetic Sampling

Formica picea was sampled at four UK populations in the summer of 2003, two in Wales and two in England (Table 3.1). The population from which the majority of individuals were sampled, Cors Goch Llanllwch, was re-sampled in the summer of 2004 (Table 3.2). These sites represented all known UK localities for *F. picea* at the time of sampling. A fifth UK population of *F. picea* was discovered in Surrey in 2003 (Baldock 2003), too late for inclusion in the present study. At Cors Goch Llanllwch up to 25 workers were collected from each of 34 nests (Table 3.2) in 2003, for use in the detailed intra-population analysis of mating system and social structure in *F. picea*. Of these nests, 19 were situated along a transect (Cors Goch Transect) of approximately 100 m previously established for ecological analysis (see Chapter 2). The spatial scale of this sampling allows for the possible detection of isolation by distance, as if nests reproduce by budding this may be more detectable when the physical distance between nests is greater. Fifteen of the nests sampled at Cors Goch Llanllwch in 2003 were situated within an area of 7 m by 8 m, itself located within a 15 m by 30 m permanent monitoring grid (Cors Goch Grid 03) established in 1995 (Fowles and Hurford 1996), using a location previously described as containing a high density of nests (Franks 1993). Nest counts within this grid between 1993 and 2002 vary from 60 to 83. In 2002, this amounted to a nest density of 0.18 nest m⁻¹ (Orledge 2002). Although nest density was not measured outside the grid, difficulty in finding nests there suggested that it was considerably lower than within the grid

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(personal observations). Sampling this cluster of nests within the grid allowed a detailed analysis of micro-geographic spatial and genetic structure (Chapter 4). To study this further and to analyse the fidelity of social and genetic structure between years, up to 25 workers per nest from 26 nests in a 9 m by 9 m area of the permanent monitoring grid (incorporating the 7 m by 8 m area initially sampled in 2003) were sampled in August 2004 (Table 3.2). Only 10 of the Cors Goch Grid 03 nests originally sampled were active in 2004. These were all included in the 26 nests re-sampled in 2004, along with 7 nests outside the original 7 m by 8 m sampling area and 9 nests discovered in 2004. All sampled nests were identified by inserting a tagged and numbered bamboo cane into the ground directly next to the nest. This allowed for identification of nests between years. In both years, the location of all sampled nests in Cors Goch was mapped. Nests on Cors Goch Transect were mapped with a Magellan Meridian™ handheld GPS receiver. Nests within Cors Goch Grid were mapped by measuring and recording the distance and bearing from north of each nest from one of the four posts marking the corners of the permanent monitoring grid. This was thought to be more accurate than using the GPS, which is accurate to within approximately 3 m (www.magellangps.com), due to the small distances between nests. To differentiate between samples from Cors Goch Grid taken in 2003 and 2004, the suffixes 03 and 04 have been added to the name Cors Goch Grid throughout this report. In the three other populations, Rhossili Down, Hartland Moor and the New Forest, up to 12 workers were collected from each of 10 nests (Table 3.2), to be used mainly for the analysis of population differentiation (Chapter 4). *Formica picea* nests have been previously found at a number of small sites within the New Forest (North 1998b); for this study nests on Ridley Plain were sampled. Due to the scarcity of nests at Hartland Moor and the New Forest (personal observations), the first ten nests discovered while walking a linear transect arbitrarily placed within suitable looking habitat were sampled. At Rhossili Down, the first ten nests discovered walking a transect (Transect no. 1893) previously used for monitoring *F. picea*

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(Orledge et al. 1998) were sampled. For all populations, nests were sampled by making a small hole in the solarium, an above-ground incubating chamber constructed from fragments of vegetation. An aspirator was then used to collect the first workers to emerge. For transportation from the field, workers were temporarily transferred to a Falken tube containing vegetation, before being stored in 70% Ethanol at -20°C.

Genetic Methods

DNA was extracted from whole workers using a Wizard™ SV96 Genomic DNA Purification System (Promega, catalogue number A2371). Manufacturer's instructions were followed with the exception that all reagent volumes were halved, due to the small amount of tissue (a single ant) being used, and RNase was omitted. This latter alteration was made as RNA quickly denatures unless kept near 0°C, negating the need for RNase in this procedure. These alterations made no discernible difference to subsequent polymerase chain reactions (PCR), as amplification intensity appeared unchanged.

All workers were genotyped at six microsatellite loci, FL12 and FL21 (Chapuisat 1996) and FE19, FE38, FE49 and FE51 (Gyllenstrand et al. 2002). Multiplex PCR amplification was carried out in a 10µl reaction volume containing approximately 12ng DNA, 5µl Qiagen® Multiplex PCR Master Mix (Catalogue number 206145) and varying concentrations of each primer (Table 3.3). Amplification conditions consisted of initial denaturation at 95°C for 15 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 50°C for 90 s and extension at 72°C for 60 s followed by a final extension step at 60°C for 30 min. Amplified products were visualised on an ABI PRISM® 3100 Genetic Analyser, and allele sizes scored against the size standard Liz-250® using Genemapper® software. Microsatellite genotyping errors can potentially confound genetic analysis (Hoffman and Amos 2005). Error rates were analysed by repeating the genotyping of 384 of 1701 (23%)

individuals sampled for genetic analysis. Repeats consisted of a new PCR from the original extraction, using all six loci.

Statistical Methods

Relatedness between nestmate workers results in non-independence of genotypes, as workers within a nest are more likely to share the same allele at any given locus. To circumvent this problem, two re-sampling techniques were initially used. For the first (here termed 'nest-based re-sampling method'), the multi-locus genotype of one individual worker was sampled randomly from each nest per population, with this procedure being repeated 20 times. This resulted in 20 data sets per population, with each data set consisting only of non-nestmates. For the second re-sampling technique (here termed 'full-sib re-sampling method') a maximum likelihood method, implemented in the programme COLONY Version 1.2 (Wang 2004), was used to partition individuals from each population into putative full-sib families. The multi-locus genotype of one individual worker was sampled randomly from each full-sib family per population, with this procedure being repeated 10 times. This resulted in 10 data sets per population, data sets containing no putative full-sibs. The procedure was only repeated 10 times as each population contained many more full-sib families than nests. For each population the inbreeding coefficient (F_{IS} , Weir and Cockerham 1984) was calculated with the programme FSTAT 2.9.3.2 (Goudet 2002) using all individuals (no re-sampling method), the nest-based re-sampling method and the full-sib re-sampling method. F_{IS} is a measure of the proportion of variance within a sub-population contained in an individual (subscripts i and s denote individual and sub-population). In this study, however, each site of occurrence is termed a population, without sub-populations, so F_{IS} is a measure of the proportion of the variance within a population contained in an individual. This corresponds to inbreeding relative to panmixia within populations. Standard errors (SE) of the mean were calculated over loci when no re-sampling

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method was used and over re-sampled data sets when re-sampling techniques were used. The three sets of F_{IS} results were compared to determine if either of the re-sampling methods produced different results from using all individuals per population. The nest-based re-sampling method was deemed to be the most useful (see *Results*). Therefore this re-sampling method was used for all analyses except estimates of worker nestmate relatedness, where no re-sampling method was used. For the analyses of F_{IS} and genetic diversity, where estimates were compared either with zero or/and between populations using a t-test, 20 re-sampled data sets were generated. A greater level of re-sampling was not conducted for these analyses as it was felt that this would artificially increase the degrees of freedom and therefore the significance of any differences observed. For the analysis of linkage disequilibrium, heterozygote deficiency and heterozygote excess, where the output of the program was a significance value and the final significance value given corresponds to the proportion of the re-sampled data sets in which a significant value was observed, 100 re-sampled data sets were generated, as it was felt that this would increase the accuracy of the analyses.

Linkage disequilibrium is the non-random association of alleles at two or more loci. If two loci are in linkage disequilibrium then they are not independent, leading to pseudo-replication. Linkage disequilibrium can have a number of causes. Epistasis may lead to linkage disequilibrium if a combination of alleles at multiple loci contributes to fitness. These alleles may be selected for and appear linked. Decreased genetic diversity, possibly caused by genetic drift, a population bottleneck or population structure, can also increase the probability that, by chance, two or more alleles are in linkage disequilibrium.

For the analysis of linkage disequilibrium, heterozygote deficiency and heterozygote excess, the final null hypotheses being tested were those of effect, rather than the more common hypotheses of no effect. This is due to

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the way in which the results given by FSTAT 2.9.3.2 (Goudet 2002) were analysed, rather than a function of the program itself. Using the analysis of linkage disequilibrium as an example, within each population, for each of the re-sampled data sets analysed, the null hypothesis was actually one of no effect (i.e. $p < 0.05$ corresponds to significant linkage disequilibrium). However, for each population the final significance value given in this study corresponds to the proportion of the re-sampled data sets that show significant linkage disequilibrium. If 100 re-sampled data sets were analysed, 5% of these would be expected to show significant linkage disequilibrium by chance. Therefore if five out of these 100 re-sampled data sets showed significant linkage disequilibrium, the final p -value would be 0.05, which would equate to no significant overall linkage disequilibrium in the population. Correspondingly, a p -value of greater than 0.05 would indicate that more of the re-sampled data sets showed linkage disequilibrium than expected by chance and that there was significant overall linkage disequilibrium in the population. The null hypothesis being rejected here is that a given pair of loci will show significant linkage disequilibrium. This treatment of significance also applies to the analyses of heterozygote deficiency and heterozygote excess.

Linkage disequilibrium was tested using the program FSTAT 2.9.3.2 (Goudet 2002) and employing a sequential Bonferroni correction (Rice 1989) for tests with multiple pairs of loci. As described above, significance values for each locus correspond to the proportion of the 100 re-sampled data-sets (generated using the nest-based re-sampling method) in which linkage disequilibrium was observed.

Deviation from Hardy-Weinberg equilibrium (heterozygote deficiency or excess), per locus within each of 100 re-sampled data sets (generated using the nest-based re-sampling method), was tested using the randomisation technique implemented in FSTAT 2.9.3.2 (Goudet 2002) with 1000 randomisations. As for the analyses of linkage disequilibrium and Hardy-

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Weinberg equilibrium, this resulted in 100 p -values per locus, for both heterozygote deficiency and heterozygote excess. A sequential Bonferroni correction for multiple loci (Rice 1989) was applied to the results from both tests and the final significance values for both heterozygote excess and heterozygote deficiency per locus corresponded to the percentage of significant values at that locus.

Relatedness between nestmate workers within each population was calculated using the software RELATEDNESS 5.0 (Goodnight 1994) using the algorithm of Queller and Goodnight (1989). Nests were weighted equally and standard errors were obtained by jackknifing over loci, as it was thought likely that loci were more independent than nests. Although I analysed Cors Goch Grid 03 and Cors Goch Transect together (as Cors Goch) and separately, the background allele frequencies used in these calculations were always those of the entire Cors Goch population, as the two sectors were deemed unlikely to represent separate sub-populations.

Inbreeding can inflate relatedness estimates and confound analysis of social organisation. To remove this effect, when the inbreeding estimate was significantly greater than zero, the method of Pamilo (1985) was implemented

$$r^* = \frac{(r-2F)/(1+F)}{((1-2F)/(1+F))}$$

where r^* = inbreeding-adjusted relatedness, r = relatedness and F = inbreeding coefficient.

If we assume that queens reproducing within a colony are as related to each other as are nestmate workers and that males breeding within a colony are unrelated, then the effective number of queens per colony (N_Q) is given by the equation (Pamilo 1993; Seppä 1994)

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$$N_Q = \frac{4(0.25 + (0.5/M_e)) - r}{(3r)}$$

where M_e is the effective queen mating frequency and r is the mean nestmate worker relatedness. In a study using allozyme markers to analyse *F. picea* populations in southern Finland, it was shown that M_e was 1.03 (Pamilo 1982b). Due to the endangered status of *F. picea* in the UK, it was not possible to sample queens for the determination of mating frequency in the present study, so the above value of M_e was used when calculating effective queen number. A high number of mates per queen in *Formica* species is rare (Boomsma and Ratnieks 1996; Chapuisat 1998), so this value is likely to be representative.

Single and multi-locus genetic diversity within populations ($H_e \pm SE$ calculated over re-sampled data-sets generated using the nest-based re-sampling method), observed heterozygosity ($H_o \pm SE$ calculated over loci), allele number (N_a) and inbreeding coefficient ($F_{IS} \pm SE$ calculated over re-sampled data-sets generated using the nest-based re-sampling method) were calculated using the program FSTAT 2.9.3.2 (Goudet 2002). Population-wide values are presented as means of the 20 re-sampled data-sets, or as whole values in the case of N_a . Significant deviation of F_{IS} from zero was estimated with a two-tailed t-test. The number of nests sampled can affect the number of alleles discovered, so allelic richness (A_r) was calculated using a slightly altered re-sampling technique. This technique was based around the minimum sample size of ten nests (Table 3.2). For populations and sectors where sampling exceeded the minimum sample size, one individual was randomly selected from each of ten randomly selected nests and the number of alleles recorded. This was repeated 20 times. Estimates of A_r (mean \pm SE) for each population and sector were calculated over these re-sampled data sets.

Results

Re-sampling methods

Very little difference was observed between F_{IS} results obtained from using all individuals per population (no re-sampling method) and using the nest-based re-sampling method (Table 3.4). However, due to a large variation in F_{IS} estimates between loci, standard errors calculated over loci (no re-sampling method) are large; this resulted in estimates not significantly greater than zero in any population when all individuals were used. Although quantitative differences were observed between the full-sib based re-sampling method and the nest-based re-sampling method (Table 3.4), only three results differed qualitatively. Using the full-sib based re-sampling method all populations and sectors showed F_{IS} estimates significantly greater than zero, whereas using the nest-based re-sampling method the New Forest, Cors Goch Transect and Cors Goch Grid 04 produced estimates not significantly greater than zero (Table 3.4). However, the New Forest, Cors Goch Transect and Cors Goch Grid 04 produced the lowest estimates regardless of the re-sampling method used (Table 3.4). Given the qualitative similarity between F_{IS} estimates obtained using both re-sampling methods and the fact that individuals in separate full-sib families may still be related (if multiple reproducing queens are related), these results provide no clear evidence that the full-sib re-sampling method overcomes the problem of non-independence of genotypes more successfully than either the nest-based re-sampling method or using all individuals per population. As the smaller standard errors produced by the nest-based re-sampling method allow a more accurate analysis of the significance of results compared to using no re-sampling method, the nest-based sampling method was deemed the most useful. This sampling method was used for all analyses with the exception of nestmate worker relatedness.

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Analyses of error rates, linkage disequilibrium and Hardy-Weinberg equilibrium

Out of 2304 genotypes repeated for the analysis of genotyping errors (384 individuals genotyped at six microsatellite loci each), 80 genotypes could not be compared due to scoring difficulties of either the initial or repeated PCR product. Of the remaining 2224 genotypes, repeats differed from the initial scoring only 36 times (1.6 %). These mismatches were of four types: Homo-Heterozygote (allele scored as homozygote initially, then as a heterozygote when genotyped for a second time), Homo-Homozygote (allele scored as homozygote initially, then as a different homozygote when genotyped for a second time), Hetero-Homozygote (allele scored as heterozygote initially, then as a homozygote when genotyped for a second time) and Hetero-Heterozygote (allele scored as heterozygote initially, then as a different heterozygote when genotyped for a second time). The frequencies of each of these types were very similar (ratio of 9:7:10:10, respectively) and not significantly different from a ratio of 9:9:9:9 ($\chi^2 = 0.667$, d.f. = 3, $p = 0.881$). This suggested that the low level of error detected in the genotyping and scoring would not have systematically biased any of the results.

The null hypothesis that all loci are in linkage disequilibrium was not rejected in Cors Goch between loci FE51 and FE49, FE49 and FL12, and FL12 and FE51 (Table 3.5; $p = 0.360$, 0.110 and 0.150 , respectively). However, when Cors Goch was divided into Cors Goch Grid 03 and Cors Goch Transect, the null hypothesis was only accepted in Cors Goch Grid 03 between loci FE51 and FE49 ($p = 0.160$). The null hypothesis was rejected in Cors Goch Grid 04 ($p < 0.05$ for all loci pairs; Table 3.5). Cors Goch was divided into sectors (Cors Goch Grid 03 and Cors Goch Transect) following inspection of worker nestmate relatedness estimates (see subsection, *Inbreeding, worker relatedness and effective queen number*). All other analyses were then performed on both Cors Goch and its component sectors. As it is highly

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unlikely that linkage disequilibrium exists between these loci in 1 of 4 populations, and differs within sectors of a single population, all loci were included in further analyses. No significant linkage has been reported in any other study of *Formica* species between any of the loci used here (e.g. Beye et al. 1998; Goropashnaya et al. 2001; Gyllenstrand and Seppa 2003; Chapuisat et al. 2004; Gyllenstrand et al. 2004; Elias et al. 2005; Pamilo et al. 2005). Therefore, it is likely that the linkage disequilibrium detected between three pairs of loci in Cors Goch occurred by chance.

The null hypothesis that all loci display heterozygote excess was rejected in all populations (Table 3.6a). The null hypothesis that all loci display heterozygote deficiency was not rejected in Cors Goch at loci FE49 and FE51 (Table 3.6b; $p = 0.410$ and $p = 0.510$, respectively) and in Rhossili Down at locus FE51 (Table 3.6b; $p = 0.100$). However, as with the tests for linkage disequilibrium, when Cors Goch was divided into Cors Goch Grid 03 and Cors Goch Transect only the former showed significant results, at loci FE49 and FE51 (Table 3.6b; $p = 0.320$ and $p = 0.670$, respectively). No significant heterozygote excess was observed in Cors Goch Grid 04 (Table 3.6b).

Genetic diversity within populations

Allele number (N_a) per locus over all four populations (Table 3.7) ranged from 2 (FE19 and FL21) to 13.3 (FE38). Single locus estimates of genetic diversity ($H_e \pm SE$) varied greatly, ranging from 0.197 ± 0.100 (FE19) to 0.720 ± 0.109 (FE38) (Table 3.7). The number of alleles (N_a) per population (Table 3.8) averaged over the re-sampled data-sets ranged from 38 (Cors Goch) to 31 (Rhossili Down). Allelic richness (avoiding bias of sample size) showed a different pattern, with Rhossili Down and the New Forest producing the highest and lowest estimates, respectively ($A_r \pm SE = 4.13 \pm 0.06$ and 3.283 ± 0.065 , Table 3.8). Genetic diversity (Table 3.8; $H_e \pm SE$) within populations varied between 0.562 ± 0.008 (Rhossili Down) and 0.469 ± 0.008 (Hartland

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Moor). When Cors Goch was divided into sectors Cors Goch Grid 03 and Cors Goch Transect, the estimates for A_r (3.817 ± 0.098 and 3.433 ± 0.084 , respectively) and H_e (0.502 ± 0.010 and 0.467 ± 0.005) differed significantly (Two-sample t-test: A_r : $t = 3.061$, d.f. = 38, $p = 0.002$, H_e : $t = 3.284$, d.f. = 38, $p = 0.002$). When Cors Goch Grid 03 and Cors Goch Grid 04 were compared, estimates for H_e and A_r were both significantly different from each other (Two-sample t-test: $t = 3.530$, d.f. = 38, $p = 0.004$; $t = 7.283$, d.f. = 38, $p < 0.001$, respectively).

Inbreeding, worker relatedness and effective queen number

Inbreeding (F_{IS}) estimates (Table 3.9) within populations ranged from 0.119 ± 0.014 (Cors Goch) to 0.001 ± 0.026 (New Forest). All populations produced inbreeding estimates significantly greater than zero, with the exception of the New Forest (Cors Goch: $t = 8.823$, d.f. = 19, $p < 0.001$, Rhossili Down: $t = 6.569$, d.f. = 19, $p < 0.001$, New Forest: $t = 0.050$, d.f. = 19, $p = 0.961$, Hartland Moor: $t = 2.234$, d.f. = 19, $p < 0.004$). When Cors Goch Transect, Cors Goch Grid 03 and Cors Goch Grid 04 were analysed separately, F_{IS} estimates were 0.037 ± 0.021 , 0.196 ± 0.022 and -0.019 ± 0.017 , respectively, although only the Cors Goch Grid 03 estimate was significantly greater than zero (Cors Goch Transect; $t = 1.741$, d.f. = 19, $p = 0.098$, Cors Goch Grid 03; $t = 8.825$, d.f. = 19, $p < 0.001$, Cors Goch Grid 04; $t = 1.094$, d.f. = 19, $p = 0.288$ respectively). Inbreeding estimates for Cors Goch Transect, Cors Goch Grid 03 and Cors Goch Grid 04 all differed significantly from each other (Cors Goch Transect – Cors Goch Grid 03: $t = 5.285$, d.f. = 38, $p < 0.001$, Cors Goch Transect – Cors Goch Grid 04: $t = 2.096$, d.f. = 38, $p = 0.043$, Cors Goch Grid 03 – Cors Goch Grid 04: $t = 7.870$, d.f. = 38, $p < 0.001$).

Average nestmate worker relatedness (r) varied between populations from 0.218 ± 0.023 in Rhossili Down to 0.084 ± 0.055 in the New Forest (mean \pm

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SE: Table 3.9). All estimates were significantly greater than zero, with the exception of worker relatedness in the New Forest (Cors Goch: $t = 6.424$, d.f. = 5, $p = 0.001$, Rhossili Down: $t = 9.316$, d.f. = 5, $p < 0.001$, New Forest: $t = 1.637$, d.f. = 5, $p = 0.163$, Hartland Moor: $t = 4.369$, d.f. = 5, $p = 0.007$). Inspection of Cors Goch relatedness estimates suggested a difference between nests from the grid sample and nests from the transect sample. When Cors Goch was divided into Cors Goch Grid 03 and Cors Goch Transect, estimates changed to 0.036 ± 0.030 and 0.177 ± 0.025 respectively, with only the Cors Goch Transect estimate being significantly greater than zero (Cors Goch Grid 03: $t = 1.197$, d.f. = 5, $p = 0.285$, Cors Goch Transect: $t = 7.203$, d.f. = 5, $p < 0.001$). The worker nestmate relatedness for Cors Goch Grid 04 was also not significantly different from zero (mean \pm SE = 0.058 ± 0.035 , $t = 1.652$, d.f. = 5, $p = 0.159$). In only two out of the four populations were estimates of nestmate worker relatedness significantly lower than 0.25 (Cors Goch: $t = 8.282$, d.f. = 5, $p < 0.001$, Rhossili Down: $t = 1.368$, d.f. = 5, $p = 0.230$, New Forest: $t = 3.217$, d.f. = 5, $p = 0.024$, Hartland Moor: $t = 1.432$, d.f. = 5, $p = 0.212$), the lowest possible relatedness value resulting from monogyny with multiple mating (Bourke and Franks 1995). The mean estimate of relatedness for Cors Goch Transect was significantly different from the estimates for Cors Goch Grid 03 and Cors Goch Grid 04 (Two sample t-test: $t = 23.861$, d.f. = 10, $p < 0.001$ and $t = 16.673$, d.f. = 10, $p < 0.001$ respectively). Estimates of relatedness differed only marginally between Cors Goch Grid 03 and Cors Goch Grid 04 (Two sample t-test: $t = 2.607$, d.f. = 10, $p = 0.026$).

Correcting for the effects of inbreeding (Pamilo 1985) produced nestmate worker relatedness estimates (r^* , Table 3.9) varying from 0.068 (Hartland Moor) to -0.132 (Cors Goch). Negative relatedness values suggest that nestmates are less related to each other than they are to the population as a whole. None of the three populations at which inbreeding was significantly greater than zero produced estimates of r^* significantly greater than zero

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(Rhossili Down: $t = 1.224$, d.f. = 5, $p = 0.276$, Hartland Moor: $t = 1.582$, d.f. = 5, $p = 0.175$); the r^* estimate produced by Cors Goch was significantly lower than zero (Cors Goch: $t = 7.766$, d.f. = 5, $p < 0.001$). As, of the sectors within Cors Goch, only Cors Goch Grid 03 showed significant inbreeding, r^* was only estimated for this sector and was significantly lower than zero (Cors Goch Grid 03: $t = 14.520$, d.f. = 5, $p < 0.001$). All estimates of r^* were significantly less than 0.25 (Cors Goch: $t = 22.472$, d.f. = 5, $p < 0.001$, Rhossili Down: $t = 9.460$, d.f. = 5, $p < 0.001$, Hartland Moor: $t = 4.219$, d.f. = 5, $p = 0.008$, Cors Goch Grid 03: $t = 22.882$, d.f. = 5, $p < 0.001$).

Applying the equation for effective queen number from the Methods resulted in estimates of N_Q (Table 3.9) ranging from 11.3 (New Forest) to 4.2 (Rhossili Down). Dividing Cors Goch ($N_Q = 8.6$) into Cors Goch Transect, Cors Goch Grid 03 and Cors Goch Grid 04 produced values of 5.2, 27.1 and 16.7 respectively. Inbreeding-corrected estimates of effective queen number (N_Q^*), using r^* in place of r , were only calculated when inbreeding was significantly greater than zero and when r^* was positive. Where r^* estimates were negative, N_Q^* was likely to be high, although the equation could not be applied. The estimates of N_Q^* produced were always greater than the corresponding N_Q values (Table 3.9).

The greatly differing inbreeding estimates of Cors Goch Grid 03 and Cors Goch Grid 04 seemed to represent an anomaly. As two loci (FE49 and FE51) displayed evidence of heterozygote excess in Cors Goch Grid 03, these loci were removed from the data set and the F_{IS} estimated again. Using only four loci, the F_{IS} estimate for Cors Goch Grid 03 was greatly lowered (0.047 ± 0.022) and not significantly greater than zero ($t = 2.096$, d.f. = 19, $p = 0.050$). The new inbreeding estimate for Cors Goch Grid 03 and the inbreeding estimate for Cors Goch Grid 04 differed only slightly, but significantly, from each other ($t = 2.391$, d.f. = 38, $p = 0.022$). The two estimates of F_{IS} for Cors Goch Grid 03, using 6 and 4 loci respectively, were significantly different from

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each other ($t = 4.838$, $d.f. = 38$, $p < 0.001$). To achieve an F_{IS} estimate for the Cors Goch population, loci FE49 and FE51 were also removed from the Cors Goch Transect data set before F_{IS} was recalculated (0.016 ± 0.022). This made little difference to the estimate of F_{IS} in Cors Goch Transect, as the two estimates, using six and four loci respectively, did not differ significantly from each other ($t = 0.720$, $d.f. = 38$, $p = 0.476$). The two estimates of F_{IS} for Cors Goch, using six and four loci respectively (F_{IS} in Cors Goch using four loci = 0.021 ± 0.013), differed significantly from each other ($t = 5.396$, $d.f. = 38$, $p < 0.001$). Estimates of F_{IS} for Cors Goch Grid 03 and Cors Goch Transect, both using four loci, did not differ significantly from each other ($t = 1.022$, $d.f. = 38$, $p = 0.313$).

Relatedness estimates for Cors Goch Grid 03 and Cors Goch Transect using four loci did not differ qualitatively from estimates calculated using all six loci ($r = 0.010$ and 0.147 , respectively). These estimates differed significantly from each other ($t = 101.149$, $d.f. = 10$, $p = < 0.001$), suggesting that it was not the two loci displaying homozygote excess causing the difference in relatedness estimates between the two sectors. As with relatedness estimates calculated from all loci, using four loci the estimate for Cors Goch Grid was not significantly greater than zero ($t = 0.605$, $d.f. = 5$, $p = 0.571$), in contrast to the estimate for Cors Goch Transect ($t = 13.821$, $d.f. = 5$, $p = < 0.001$). Given that none of the newly calculated F_{IS} values differed significantly from zero, nestmate relatedness corrected for inbreeding (r^*) was not calculated with just four loci.

Discussion

This study aimed to address questions concerning the social and genetic organisation of endangered UK populations of the Black Bog Ant (*F. picea*), with a view to its conservation. No inbreeding was detected in the New Forest population, although all three other populations were significantly inbred.

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However, within Cors Goch this estimate was driven by two loci showing heterozygote deficiency in the Cors Goch Grid 03 samples. When these loci were removed from the data set no significant inbreeding was detected within Cors Goch or its component sectors. Nestmate worker relatedness was low in all populations and, when corrected for inbreeding, was not significantly greater than zero in any population, suggesting a large number of queens per nest. Intra-population polymorphism in social and genetic organisation was observed in Cors Goch. When Cors Goch Grid 03 and Cors Goch Transect (sectors of Cors Goch, occupying areas with high and low nest densities, respectively) were analysed separately, they differed significantly in nestmate worker relatedness and genetic diversity (H_e and A_r). F_{IS} estimates for the two sectors differed significantly from each other when all loci were included in the analysis, but not when two loci displaying heterozygote deficiency in Cors Goch Grid 03 were omitted from the analysis. When the grid was sampled the following year (Cors Goch Grid 04), it differed significantly from Cors Goch Grid 03 in genetic diversity (H_e and A_r) but not nestmate worker relatedness. As with the comparison between Cors Goch Grid 03 and Cors Goch Transect, F_{IS} estimates for Cors Goch Grid 03 and Cors Goch Grid 04 differed significantly from each other when all loci were included in the analysis. When the two loci displaying heterozygote deficiency in Cors Goch Grid 03 were omitted from the analysis, F_{IS} estimates within the two sectors differed only slightly, but significantly, from each other, although neither estimate was significantly greater than zero.

Genetic relatedness of nestmate workers and inbreeding

The finding that relatedness was low in all populations and inbreeding was present in two out of four populations concurs with studies showing that low nestmate relatedness is linked to inbreeding across most *Formica* species (Sundström et al. 2005). This seems to be a paradoxical association, as it is logical to assume that lower relatedness between nestmates would lead to

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reduced levels of inbreeding. The biological interpretation, however, is that high levels of polygyny are associated with poor dispersal, queens sometimes mating within the natal nest. This increases the chance that mating will occur between relatives, as although the mean estimate of nestmate relatedness may be close to zero, due to the distribution of nestmate relatedness there will still be a small number of highly related individuals within the nest.

Inbreeding inflated the estimates of relatedness, as relatedness corrected for inbreeding did not differ significantly from zero in any population. Relatedness estimates corrected for inbreeding were also significantly less than 0.25 (the lowest possible relatedness estimate under monogyny with multiple mating) in all populations and sectors, confirming that low relatedness is indeed due to polygyny. The lack of inbreeding in Cors Goch Grid 04 compared to the significant inbreeding exhibited by Cors Goch Grid 03 raises the question of how this difference could arise. Relatedness estimates were low and not significantly different across years, evidence for a similar social structure. Two loci within Cors Goch Grid 03 (FE49 and FE51) displayed evidence for heterozygote deficiency, which may have driven the high inbreeding estimate. Significant linkage disequilibrium was also observed between these two loci within Cors Goch Grid 03, another possible artefact of increased homozygosity, as decreased variation at two loci can increase the probability that they will appear linked. It is unlikely that these estimates represent true linkage disequilibrium as the pattern is not seen in Cors Goch Grid 04, and recombination cannot cause high levels of linkage disequilibrium to be lost in one generation. Null alleles were discounted as a reason for the heterozygote deficiency, as no such deficiency was present in the Cors Goch Transect sector. Another possible cause was hidden structure of the Cors Goch Grid 03 sector. However, this should affect all neutral loci equally, so does not seem a comprehensive enough explanation. When the two heterozygote deficient loci in question were dropped from the Cors Goch Grid 03 data set, the inbreeding estimate dropped dramatically and was no longer significantly

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different from zero. The new F_{IS} estimate was still different from the estimate produced by Cors Goch Grid 04, but with only marginal statistical significance. To investigate in more detail the apparently anomalous results within Cors Goch Grid 03, heterozygote deficiency and linkage disequilibrium were estimated within each nest using FSTAT 2.9.3.2 (Goudet 2002). Three nests (44, 85 and 86) displayed significant levels of linkages disequilibrium between loci FE49 and FE51. This was probably driven by the significant heterozygote excess displayed by these loci at the three nests (data not shown), with the exception of FE49 within nest 85, which narrowly missed statistical significance after a bonferroni correction for multiple tests. These results suggested that it had been the two heterozygote deficient loci, possibly within specific nests, driving the majority of the high F_{IS} estimate and that neither true inbreeding nor linkage disequilibrium between FE49 and FE51 is present within the Cors Goch population.

A lack of relatedness between nestmate workers suggests a social organisation whereby individuals are exchanged freely between nests, sometimes termed unicoloniality. This phenomenon is not unknown within the *Formica* ants, having been shown to occur in *F. paralugubris* (Chapuisat and Keller 1999) and *F. truncorum* (Elias et al. 2005). Populations of the ants *F. exsecta* (Seppä et al. 2004), *F. lugubris* (Gyllenstrand and Seppä 2003) and *F. cinerea* (Zhu et al. 2003) also show the presence of many queens per colony as evidenced by low nestmate worker relatedness. Estimates of effective queen number per colony (N_Q^*) were made using relatedness corrected for inbreeding, but as these relatedness values were not significantly different from zero, the effective queen numbers may be underestimates. However, the finding of the present study that, on average, nests contain many reproductive queens agrees with previous observations at Cors Goch (A. Abbot, personal communication), where 27 dealate (i.e. potentially reproductive) queens were found in one nest. The number of queens as measured by censusing nest populations is expected to be greater

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than the effective number of queens revealed by genetic methods, as it is often the case that multiple queens in a single nest do not reproduce equally (Bourke and Franks 1995; Bourke et al. 1997).

Social polymorphism

Although unlooked for, a significant difference in genetic structure and social organisation was found between sectors (Cors Goch Grid 03 and Cors Goch Transect) within one of the study populations (Cors Goch). This social polymorphism is not uncommon within *Formica* ants and in the most extreme case monogynous and highly polygynous colonies can be found in the same population (Chapuisat et al. 2004). In this study the two sectors differed significantly in nestmate worker relatedness and genetic diversity (H_e and A_r). A possible explanation for the observed pattern is the variation in ecologically favourable habitat (Chapter 2). The southern section of Cors Goch, where the sampled nests are found, appears to be more favourable for *F. picea* in terms of certain micro-geographical ecological variables. Although these variables have not been measured within the permanent monitoring grid containing the Cors Goch Grid 03 nests, the density of these nests (0.18 nests m^{-1} in 2002) suggests that this area is ecologically favourable. It is possible that within the permanent monitoring grid at Cors Goch Llanllwch, high nest density could result in an increased cost to queens of colony foundation due to a lack of favourable unoccupied habitat. This would promote daughter queen recruitment into the natal nest, increasing queen number and decreasing worker nestmate relatedness per nest.

In conclusion, the results suggest that UK populations of *F. picea* are highly polygynous. Despite the putatively high effective population size conferred by many queens per colony, the apparent geographic isolation of these populations may still be of conservation concern. It is possible that the two populations displaying significant levels of inbreeding may be subjected to an

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increased risk of extinction compared to populations exhibiting no inbreeding (Saccheri et al. 1998), especially given the complimentary sex determination mechanism thought to be present in most Hymenoptera (Zayed and Packer 2005). The levels of genetic diversity observed in UK populations of *F. picea*, however, appear relatively high, and are comparable with other *Formica* species (Table 3.10). It seems unlikely that this level of genetic diversity would lead to the production of diploid males and their associated genetic load upon the population. Of more pressing concern is be that if dispersal is limited, as is the case in many highly polygynous ants, then the ability of all populations to colonise new suitable habitat may be compromised. Any damage done to existing populations could therefore have a devastating effect on the persistence of *F. picea* within the UK.

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Table 3.1

Name and location of each studied site. Grid references are for areas where *F. picea* nests were found. For map refer to Figure 2.1.

Site	Location	Grid Ref
Cors Goch Llanllwch (CG)	Wales	SN363185
Rhossili Down (RD)	Wales	SS428893
New Forest (NF)	England	SU210066
Hartland Moor (HM)	England	SY944854

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Table 3.2

Genetic sampling per population expressed as the number of nests sampled (N_{Nests}), total number of individual workers genotyped ($N_{\text{Genotyped}}$), total number of individuals successfully scored (N_{Scored}), mean number of individuals successfully scored per nest (N_{Mean}) and collection date. CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type. Ten of the CGG04 nests are the same nests that were sampled for the CGG03 data set.

	N_{Nests}	$N_{\text{Genotyped}}$	N_{Scored}	N_{Mean}	Date
CG	34	789	758	22.3	N/A
CGG03	15	322	311	20.7	27.08.03
CGT	19	467	447	23.5	07.08.03
CGG04	26	553	524	20.2	27.07.04
RD	10	120	120	12.0	26.07.03
NF	10	120	109	10.9	21.07.03
HM	10	119	116	11.6	22.07.03
Total	64	1701	1627	N/A	N/A

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Table 3.3

Fluorescent label used and concentration of each primer (P_{CONC} , forward and reverse) per Multiplex PCR reaction for microsatellite genotyping.

Locus	Label	P_{CONC} (μM)
FE19	PET	0.15
FE38	6-FAM	0.20
FE49	NED	0.30
FE51	PET	0.20
FL12	VIC	0.15
FL21	6-FAM	0.20

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Table 3.4

Inbreeding estimates ($F_{IS} \pm SE$) for all populations and sectors using all samples per population (SE calculated over loci), using the 'nest-based re-sampling method' (generating 20 data sets, each consisting of one randomly selected individual per nest; SE calculated over re-sampled data sets) and using the 'full-sib based re-sampling technique' (generating 10 data sets, each consisting of one randomly selected individual from each full-sib family; SE calculated over re-sampled data sets). Full-sib families estimated using the programme COLONY (Wang 2004). CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ (deviation of mean from zero, t-test).

Method	All samples		Nest-based		Full-sib based	
	F_{IS}	SE	F_{IS}	SE	F_{IS}	SE
CG	0.114	± 0.067	0.119	± 0.014 ***	0.063	± 0.009 ***
CGG03	0.199	± 0.109	0.196	± 0.022 ***	0.140	± 0.008 ***
CGT	0.036	± 0.033	0.037	± 0.021	0.062	± 0.008 ***
CGG04	-0.004	± 0.058	-0.019	± 0.017	0.025	± 0.006 ***
RD	0.101	± 0.084	0.108	± 0.016 ***	0.122	± 0.010 ***
NF	0.012	± 0.047	0.001	± 0.026	0.041	± 0.013
HM	0.061	± 0.105	0.069	± 0.021 **	0.139	± 0.018 ***

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Table 3.5

Linkage disequilibrium between locus pairs, per population. CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type. Values (significance levels, p) correspond to percentage of re-sampled data sets in which significant disequilibrium occurred. Hence $p \leq 0.050$ corresponds to a lack of significant linkage disequilibrium.

Loci	CG	CGG03	CGT	CGG04	RD	NF	HM
FE19 X FE38	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE19 X FE49	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE19 X FE51	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE19 X FL12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE19 X FL21	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE38 X FE49	0.010	0.000	0.000	0.000	0.000	0.000	0.000
FE38 X FE51	0.020	0.000	0.000	0.000	0.000	0.000	0.000
FE38 X FL12	0.000	0.010	0.010	0.000	0.000	0.000	0.000
FE38 X FL21	0.040	0.000	0.000	0.000	0.000	0.000	0.000
FE49 X FE51	0.360	0.160	0.031	0.000	0.000	0.000	0.000
FE49 X FL12	0.110	0.010	0.000	0.000	0.000	0.000	0.000
FE49 X FL21	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE51 X FL12	0.150	0.030	0.050	0.000	0.000	0.000	0.000
FE51 X FL21	0.000	0.000	0.020	0.000	0.000	0.000	0.000
FL12 X FL21	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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Table 3.6

Heterozygote excess (a) and heterozygote deficiency (b) per locus in each population. CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type. Values (single locus significance levels, p) correspond to percentage of re-sampled data sets in which significant heterozygote excess (a) or deficiency (b) occurred. Hence $p \leq 0.050$ corresponds to a lack of significant heterozygote excess (a) or deficiency (b).

(a)

Locus	CG	CGG03	CGT	CGG04	RD	NF	HM
FE19	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE38	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE49	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE51	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FL12	0.000	0.000	0.000	0.000	0.000	0.020	0.000
FL21	0.000	0.000	0.000	0.000	0.000	0.000	0.000

(b)

Locus	CG	CGG03	CGT	CGG04	RD	NF	HM
FE19	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FE38	0.050	0.000	0.050	0.000	0.000	0.000	0.000
FE49	0.410	0.320	0.030	0.000	0.000	0.040	0.000
FE51	0.510	0.670	0.030	0.010	0.100	0.030	0.040
FL12	0.040	0.010	0.010	0.000	0.020	0.010	0.000
FL21	0.000	0.000	0.000	0.010	0.000	0.000	0.000

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Table 3.7

Mean allele number (N_a) and expected heterozygosity ($H_e \pm SE$) per locus over all populations.

Locus	N_a	H_e
FE19	2	0.197 ± 0.100
FE38	13.3	0.720 ± 0.109
FE49	4.8	0.596 ± 0.112
FE51	5	0.612 ± 0.061
FL12	7	0.628 ± 0.088
FL21	2	0.311 ± 0.096

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Table 3.8

Genetic diversity per population, expressed as expected heterozygosity ($H_e \pm SE$), observed heterozygosity ($H_o \pm SE$), allelic richness ($A_r \pm SE$) and allele number (N_a). N_{Nests} = number of nests sampled per population/sector. SE calculated over re-sampled data sets (H_e and A_r) or loci (H_o). CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type.

	N_{Nests}	H_e	H_o	A_r	N_a
CG	34	0.487 ± 0.004	0.428 ± 0.127	3.808 ± 0.111	38
CGG03	15	0.502 ± 0.010	0.402 ± 0.138	3.817 ± 0.098	36
CGT	19	0.467 ± 0.005	0.449 ± 0.122	3.433 ± 0.084	37
CGG04	26	0.427 ± 0.005	0.435 ± 0.149	3.400 ± 0.072	34
RD	10	0.562 ± 0.008	0.501 ± 0.124	4.133 ± 0.062	31
NF	10	0.509 ± 0.006	0.507 ± 0.115	3.283 ± 0.065	33
HM	10	0.469 ± 0.008	0.435 ± 0.101	3.325 ± 0.064	36

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Table 3.9

Relatedness of nestmate workers ($r \pm SE$), inbreeding coefficient ($F_{IS} \pm SE$), inbreeding corrected relatedness of nestmate workers ($r^* \pm SE$), effective queen number per nest calculated using r (N_Q) and effective queen number per nest calculated using r^* (N_Q^*). *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ (deviation of mean from zero, t-test). ... = $p < 0.001$, .. = $p < 0.01$, . = $p < 0.05$ (deviation of mean from 0.25, t-test). CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type. The abbreviation N/A is displayed when either r^* cannot be calculated due to an F_{IS} estimate not significantly greater than zero, or when N_Q^* cannot be calculated due to a negative r^* . F_{IS} estimates shown are identical to those shown in Table 3.4.

	r		F_{IS}		r^*		N_Q	N_Q^*
CG	0.109 ± 0.017 ***	...	0.119 ± 0.014 ***		-0.132 ± 0.017 ***	...	8.6	N/A
CGG03	0.036 ± 0.030	...	0.196 ± 0.022 ***		-0.434 ± 0.030 ***	...	27.1	N/A
CGT	0.177 ± 0.025 ***	.	0.037 ± 0.021		N/A		5.2	N/A
CGG04	0.058 ± 0.035	..	-0.019 ± 0.017		N/A		16.7	N/A
RD	0.218 ± 0.023 ***		0.108 ± 0.016 ***		0.029 ± 0.023	...	4.2	33.9
NF	0.084 ± 0.055	.	0.001 ± 0.026		N/A		11.3	N/A
HM	0.188 ± 0.043 **		0.069 ± 0.021 **		0.068 ± 0.043	..	4.9	14.1

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Table 3.10

Comparison of mean number of alleles per population or sub-population (N_a) and genetic diversity (H_e) between UK populations of *F. picea* and four other *Formica* species. Levels of genetic variation within UK populations of *F. picea* appear comparable to other species.

	N_a	H_e	Loci	Study populations	Reference
<i>F. picea</i>	5.7	0.511	6	UK	This study
<i>F. lugubris</i>	5.3	0.607	7	UK	Gyllenstrand and Seppa 2003
<i>F. aquilonia</i>	5.8	0.480	12	Russia	Maki-Petays et al. 2005
<i>F. selysi</i>	5.0	0.467	9	Switzerland	Chapuisat et al. 2004
<i>F. cinerea</i>	4.0	0.544	5	Scandinavia, Estonia	Goropashnaya et al. 2001

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Intra- and inter-population genetic spatial structuring and unicoloniality in *Formica picea*

Abstract

I used microsatellite and mitochondrial DNA markers to study the genetic spatial structuring within and between four UK populations of the ant *Formica picea* (Cors Goch Llanllwch, Carmarthenshire; Rhossili Down, West Glamorgan; Hartland Moor, Dorset; the New Forest, Hampshire). Inter-population analyses show that all population pairs were significantly genetically differentiated from each other, with the exception of the geographically closest pair, the New Forest and Hartland Moor, suggesting that gene flow either occurs between these two populations or has done in the recent past. Within populations, results indicated that dispersal of both sexes is extremely limited, as significant isolation by distance (colonies being genetically more similar to physically closer colonies) was detected within both Cors Goch Llanllwch and the New Forest. The Rhossili Down and Hartland Moor populations showed a similar, but non-significant, trend. These results also suggested that colonies reproduce by budding, a process by which daughter queens establish new colonies in close proximity to the natal colony. The mitochondrial DNA sequence studied, the Cytochrome Oxidase subunit 1 gene, displayed low levels of variation, with one haplotype in the English populations and one in the Welsh populations. Given the homogeneity of this mitochondrial region in the present study, female mediated gene flow could not be determined, either within or between populations. Significant evidence for genetic bottlenecks was observed in two populations, Rhossili Down and the New Forest. Aggression was almost

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completely lacking between all *F. picea* nests in the main study population, Cors Goch Llanllwch, regardless of the distance between them. Using genetic methods, evidence for the presence of polydomy (multiple nests per colony) was found in one sector of this population where the highest density of nests occurred. However, using the method employed (an approach designed for this study, based on con-coloniality of nest pairs), not all nests could be allocated to polydomous colonies and those polydomous colonies revealed were not spatially discrete. Overall the results suggested that the main study site contains a population that approximates unicoloniality, with individuals potentially moving freely between nests, by consisting of aggregations of highly polygynous and polydomous colonies in large patches of suitable habitat and polygynous and monodomous colonies where suitable habitat is more sparse.

Introduction

Ant colonies and populations are typically associated with two basic social types, monogyny (a single reproducing queen per colony) and polygyny (multiple reproducing queens per colony). Monogynous and monodomous (single nest) colonies are often seen to be the prototype social structure in ants and are the least complex forms of colony organisation. At the other end of the scale are polygynous and polydomous (multiple nest) colonies and populations. The ant genus *Formica* is a socially polymorphic group, with species ranging from monogynous, through intermediate levels of gyny to highly polygynous. Social polymorphism within *Formica* ants is not confined to the inter-species level, as there is a great deal of variation within species (Sundström 1993; Goropashnaya et al. 2001; Gyllenstrand and Seppä 2003; Seppä et al. 2004). It is even possible for colonies within the same population to vary between monogyny and high levels of polygyny (Chapuisat et al. 2004). Monogyny is generally associated with good dispersal ability, independent nest founding by new queens and a lack of genetic structure

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within populations. Contrastingly, in polygynous colonies and populations new nests are founded by a process termed budding (Rosengren and Pamilo 1983). In this process, queens characteristically do not have a nuptial flight and can even mate within the nest. They are then either recruited back into the natal nest or found a new nest a short distance away, taking workers from the natal nest with them. This form of colony reproduction can therefore produce within-population genetic structuring, involving individuals from physically closer nests being genetically more similar, potentially leading to isolation by distance. Once a bud nest has been established, exchange of workers with the mother nest may cease, creating an independent colony. However, if exchange of individuals continues then polydomy arises, with both nests effectively being part of the same colony (Hölldobler and Wilson 1990). There are obvious advantages to colonies consisting of multiple nests, one being increased foraging efficiency and another being the reduced risk to the colony of predation or stochastic damage of a single nest (Rosengren and Pamilo 1983). Despite these advantages, polydomy is very rarely associated with monogynous colonies and populations (Pedersen and Boomsma 1999). This appears to stem from polydomy generally arising from polygyny and reproduction by colony budding. Rarely, extremely high levels of polygyny and polydomy can lead to a phenomenon termed unicoloniality. In unicolonial populations, the distribution of relatedness between nestmates is such that mean estimates approach zero and individuals can potentially move freely between nests because aggression against non-nestmates is lacking (Hölldobler and Wilson 1990; Bourke and Franks 1995). This should result in a lack of genetic structure within the population, as seen in a population of the ant *F. truncorum* (Elias et al. 2005), but largely unicolonial colonies and populations can retain genetic viscosity due to budding and poor dispersal, as in *F. paralugubris* (Chapuisat et al. 1997; Chapuisat and Keller 1999). Unicolonial populations and species characteristically dominate their habitat due to high densities of workers and nests and the advantages conferred by polydomy (Ross et al. 1996; Tsutsui et al. 2000; Giraud et al. 2002).

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A number of studies have investigated aggressive interactions between workers of ant colonies to determine the basis of nestmate recognition (Stuart and Herbers 2000; Chapuisat et al. 2005) or large scale colony boundaries (Giraud et al. 2002). However, although genetic data have also been used to determine colony boundaries (Pedersen and Boomsma 1999; DeHeer and Herbers 2004), the two have rarely been used together to elucidate specific small scale colony boundaries.

The Black Bog Ant *Formica picea* (formerly *F. candida*) is a little studied habitat specialist, occurring only in areas of wet heathland and bog. Its range covers much of northern Europe where it is a rare relict species (Czechowski et al. 2002). Within the United Kingdom, however, *F. picea* is classed as endangered on the GB Red List and is a Biodiversity Action Plan (BAP) Priority species. Habitat loss due to drainage of bog and wet heathland for afforestation and agriculture is most often cited as the main cause of decline. *F. picea* is now restricted to five sites within the UK, two in Wales and three in England. In the only genetic study on UK populations of *F. picea*, I have previously shown the four populations studied to be highly polygynous, with inbreeding-corrected estimates of nestmate worker relatedness not significantly greater than zero (Chapter 3). Within Cors Goch Llanllwch, where the most extensive sampling was done, two sectors showed some social polymorphism. A densely populated 7 m by 8 m grid (Cors Goch Grid 03) showed levels of nestmate worker relatedness not significantly greater than zero, whereas a less densely populated ~100 m transect (Cors Goch Transect) had significantly higher relatedness estimates, suggesting fewer reproducing queens per nest. Given it is an endangered species (within the UK) with apparently isolated populations, studies of *F. picea* will contribute to the knowledge of social insects with restricted ranges. Detailed investigation into the inter- and intra-population genetic structure of this species will also aid its conservation by revealing gene flow and elucidating factors that may have promoted its current social and genetic organisation. In the present

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study I investigated in detail the social and genetic organisation of *F. picea* in the UK, both within and between populations, with particular emphasis on the relationship between genetic and spatial structure. The use of polymorphic microsatellite markers allows a detailed analysis of social and genetic structure within and between populations, whereas mitochondrial DNA markers allow the partitioning of gene flow by sex, as mitochondrial DNA is inherited maternally. These two types of markers have been successfully used in tandem to study other *Formica* species (Gyllenstrand and Seppä 2003; Seppä et al. 2004). In this study I attempted to discover the answers to the following questions: does gene flow occur between UK *F. picea* populations? Do populations of *F. picea* show evidence of isolation by distance and colony reproduction by budding? Do workers behave aggressively to workers from foreign colonies? Do nests form polydomous colonies? Can aggression of workers be used in conjunction with genetic data to determine putative colony boundaries?

Methods

Genetic sampling

All data on worker genotypes at microsatellite loci collected from the four studied populations (Cors Goch, Rhossili Down, the New Forest and Hartland Moor) for analyses in Chapter 3 were used for analyses in this chapter. For detailed sampling methods, see Chapter 3. In brief, approximately 25 workers from 34 nests were collected from the main study site, Cors Goch Llanllwch, in August 2003. Of these nests, 19 were situated on a transect approximately 100 m in length and 15 were situated within a 7 m by 8 m section of a permanent monitoring grid, containing a high density of nests (Chapter 3). In August 2004, approximately 25 workers from 26 nests were collected from a 9 m by 9 m area of the permanent monitoring grid incorporating the area sampled in 2003. Nest density was higher within the grid than on the transect

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(Chapter 3). Approximately 12 workers from 10 nests were collected from the three other populations (Rhossili Down, the New Forest and Hartland Moor) in the summer of 2003 (Chapter 3).

Genetic methods

a) Microsatellite DNA

All workers were genotyped at six microsatellite loci, FL12 and FL21 (Chapuisat 1996) and FE19, FE38, FE49 and FE51 (Gyllenstrand et al. 2002). Polymerase chain reaction (PCR) conditions, visualisation of PCR products and allele size scoring methods were described in Chapter 3.

b) Mitochondrial DNA

Eight individuals from two nests at Cors Goch Llanllwch and four individuals from two nests at each of the other populations were initially analysed for mitochondrial variation, to determine the number of individuals that should be analysed from each nest overall. As no intra-nest variation was observed, one individual from every nest in the total sample for each population was used for population-wide analysis. A 1kb sequence of the mitochondrial region Cytochrome Oxidase subunit I gene (COI) was initially targeted using the primers CI13 and CI24 (Hasegawa et al. 2002). PCR amplification was carried out in a 25µl reaction volume containing approximately 12ng DNA, 10µl Qiagen® Multiplex PCR Master Mix (catalogue number 206145) and 400µM of each primer. PCR conditions consisted of initial denaturation at 94°C for 5 min followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 45°C for 1 min and extension at 60°C for 3 min followed by a final extension at 72°C for 7 min. PCR products were purified with a QIAquick® PCR Purification Kit (Qiagen, catalogue number 28104) using a microcentrifuge. Purified products were then used as the template for

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subsequent sequencing PCR reactions. Four target fragments were amplified separately using the primers CI13, CI14, CI21 and CI24 (Hasegawa et al. 2002) in 15µl reaction volumes. Each reaction contained 3µl purified PCR product, 1µl ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Mix (catalogue number 4337455), 5µl Better Buffer (Microzone, catalogue number 3BB1.5-5) and 1.6µM sequencing primer. PCR conditions were: initial denaturation at 96°C for 5 min followed by 28 cycle s of denaturation at 96°C for 15 s, annealing at 45°C for 10 s and extension at 60°C for 4 min. Excess BigDye® was removed from PCR products following the ethanol/EDTA protocol suggested by Applied Biosystems, and products were sequenced using an ABI PRISM® 3100 Genetic Analyser. Sequences were determined using DNA Sequencing Analysis Software v4.0 and aligned using Sequencher™.

Aggression tests

Aggression tests were performed on the 6th and 7th of July, 2005, between the times of 11:00 am and 5:00 pm. A nest was termed active and viable for use in aggression tests if workers immediately exited a small hole made in the solarium with a pencil. Out of the 19 nests sampled from Cors Goch Transect for genetic analyses in August 2003, only eight remained active in July 2005. Remains of the other solaria were present, but were empty and in obvious disrepair. All nine active nests were used in aggression tests. An equal number of nests were also chosen from Cors Goch Grid. However, out of the 15 nests sampled from Cors Goch Grid 03, only five remained active in 2005. Therefore the first three nests discovered in Cors Goch Grid 04, within the originally sampled 7 m by 8 m section, were also used for aggression tests. Nests sampled for genetic analyses in 2003 were used where possible so that results from aggression tests could be linked to genetic structure, assuming nest identity and behavioural relationships between given pairs of nests are constant across years.

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Aggression between pairs of nests was tested implementing a slightly altered version of the 'Live 5-5' method of Roulsten et al. (2003). This method was chosen as Roulsten et al. (2003) found that it yielded the most consistent results between repeated aggression assays and was the most likely to detect acts of aggression out of four bioassays compared using the Argentine ant *Linepithema humile*. In the present study, arenas for aggression tests were circular plastic dishes (7 cm diameter, 3 cm high side walls). The inner surfaces of the outer walls were coated with the dry lubricant Fluon (polytetrafluoroethylene) to prevent the ants' escape. To provide a natural substrate for the ants to move over, the floor of each dish (the arena) was covered in fine sand to a depth of approximately 5 mm. The sand was replaced after each test to ensure that any chemical cues deposited by workers did not interfere with subsequent tests using the same arena. An inner ring of plastic 4 cm in diameter and 3 cm high and coated on both sides with Fluon was placed within the main arena. This ring was pushed down through the sand so that ants could not burrow underneath. Using an aspirator, five workers from one nest were deposited within the inner ring and five workers from another nest were deposited outside the inner ring. After acclimatisation for at least 1 min, the inner ring was lifted out and the ants were free to interact. To minimize the time between capture of workers and their use in the tests, all aggression tests were conducted in the field. To achieve this, six arenas were attached to the bottom of a large plastic bucket approximately 30 cm in diameter, using adhesive tape. Once all workers were deposited within the arenas, all inner rings were removed as simultaneously as possible. Each arena was observed for 5-10 s every 1 min for 5 min. The number of aggressive interactions observed during each 5-10 s time period was recorded. Aggression was defined as behaviours corresponding to points 3 ('aggression'; including lunging, brief biting and pulling) and 4 ('fighting'; including prolonged 'aggression', abdomen curling and attempts to spray defensive compounds) on the 0-4 scale of Suarez et al. (1999), as used in Roulsten et al. (2003). Because the workers used in the tests were unmarked,

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it was unknown if any aggression observed was occurring between nestmates or individuals of different nests. Therefore, intra-nest tests were performed for each nest as controls. As additional controls (for distance between nests and for species identity), two nests were randomly selected for tests with the *F. picea* nest on the bog most distant from the sampling sites (a nest approximately 200 m west of the grid, i.e. Control A) and with worker ants of the taxon *Lasius* s. str. collected from the bog (Control B). Each day, pairs of nests to be tested were selected at random. Each aggression test, whether inter-nest, intra-nest or control, was performed once.

Eight nests from both Cors Goch Grid and Cors Goch Transect were selected for aggression tests, giving a maximum of 120 possible inter-nest pair combinations. After 50 inter-nest tests (42%) had been conducted, testing was ended since only very low levels of aggression were recorded in all tests (see Results section). Intra-nest tests for all 16 nests were completed for controls.

Statistical methods

The suitability of the six microsatellite loci (genotyping error rates, null alleles and linkage disequilibrium) has been addressed previously (Chapter 3). As error rates and evidence for null alleles and linkage disequilibrium were low, all loci were used in genetic analyses.

i) Population genetic differentiation

Inter-population genetic structure was assessed using estimates of pairwise F_{ST} , calculated using the programme ARLEQUIN version 2 (Schneider et al. 2000). The nest-based re-sampling method (Chapter 3) was used to generate 20 data sets per population. One of these data sets was randomly chosen from each population or sector to be analysed, to form a final data set that

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could be used for estimating F_{ST} , this process being performed 20 times until all data sets produced with the nest-based re-sampling method were used. Estimates given are means of the 20 F_{ST} values obtained by analysing these final 20 data sets (\pm SE, calculated over 20 final data sets). Significance of F_{ST} estimates were assessed by 1000 permutations of genotypes among populations, implemented in ARLEQUIN version 2 (Schneider et al. 2000). This permutation provides the null distribution of pairwise F_{ST} values under the hypothesis of no genetic differentiation between populations. The significance value for each re-sampled data set corresponds to the proportion of permutations that resulted in F_{ST} estimates equal to or greater than the observed. Final significance values correspond to the proportion of re-sampled data sets which showed no significant genetic differentiation between populations (therefore $p \leq 0.05$ equates to a significant F_{ST} estimate). Analysis of isolation by distance between populations was performed by plotting mean pairwise F_{ST} estimates against log.-transformed pairwise distances. These distances were calculated as the shortest over-land distance between populations. Significance of the correlation was assessed using a matrix correlation test (Mantel 1967) implemented in Microsoft Excel with the POPTOOLS version 2.6 add-in (Hood 2005).

The genetic structure of populations was also assessed using the program STRUCTURE version 2 (Pritchard et al. 2000), which implements a Bayesian based clustering method to infer population structure and assign individuals to populations. Given a putative number of populations (K) for the multi-locus genotype data, the model assigns individuals to one or more (if admixture is allowed within the model) of the putative populations, finding clusters of individuals that are at Hardy-Weinberg and linkage equilibrium. For each value of K , the estimated log probability of the data ($\ln \Pr(X/K)$, where X represents the genotypes of the sampled individuals) is given, indicating the most likely number of populations. However, the authors of STRUCTURE version 2 warn that $\ln \Pr(X/K)$ is an *ad hoc* approximation of the number of

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populations and should only be used as a guide (Pritchard et al. 2000; Pritchard and Wen 2003). In this study, therefore, the most biologically likely estimate for K is determined both graphically (plotting $\ln \text{Pr}(X/K)$ against K) and by examining the assignment of individuals to populations, given that the sampling location for each individual is known. Pritchard and Wen (2003) suggest that when plotting $\ln \text{Pr}(X/K)$ against K , the more biologically likely value of K is often the value at the start of a plateau (even if $\ln \text{Pr}(X/K)$ continues to rise with increasing values of K), i.e. it is the smallest value of K that captures the major structure. It is also suggested that when no population structure is present, assignment of individuals is roughly symmetrical between populations (Pritchard and Wen 2003). In this study the options for admixture and correlated allele frequencies were chosen, as recommended by Falush et al. (2003) for detecting subtle population structure. A burn-in length of 30000 was used, together with a Markov Chain Monte Carlo run length of 100000, as this produced consistent estimates of $\ln \text{Pr}(X/K)$ (as shown by the small standard errors, see Results). Initially, multi-locus genotypes from Cors Goch, Rhossili Down, the New Forest and Hartland Moor were analysed, testing values of K from 1 to 6, each repeated three times to obtain standard errors, calculated over repeated runs. To examine the genetic structure within Cors Goch (both within and between years), multi-locus genotypes from Cors Goch (Cors Goch Transect, Cors Goch Grid 03 and Cors Goch Grid 04) were analysed separately, testing values of K from 1 to 4, each repeated three times to obtain standard errors, calculated over repeated runs. All analyses were repeated with 10 separate re-sampled data sets, generated using the nest-based re-sampling method (Chapter 3).

ii) Within-population isolation by distance

To determine whether isolation by distance was present within populations, F_{ST} estimates between all pairs of nests within each population were calculated using the program ARLEQUIN version 2 (Schneider et al. 2000).

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No re-sampling method was used for this analysis as multiple individuals within each nest are required to produce pairwise F_{ST} estimates. For each population these values were plotted graphically against the log.-transformed physical distance between pairs of nests. Significance of the correlation was calculated using a Mantel test (Mantel 1967) implemented in Microsoft Excel with the PopTools version 2.6 add-in (Hood 2005).

iii) Population genetic bottlenecks

Loss of genetic variation due to demographic bottlenecks, a recent reduction in effective population size, was tested for using the programme BOTTLENECK (Cornuet and Luikart 1996). When a population experiences a genetic bottleneck, both the number of alleles and gene diversity decrease. However, allele number decreases at a higher rate than gene diversity, a phenomenon that can often be detected using multi-allelic systems such as microsatellites, where rare alleles can be lost rapidly. The result of this effect is observed gene diversity higher than the expected equilibrium gene diversity, calculated from the observed number of alleles assuming a constant size population. The model used to test for genetic bottlenecks was the two-phase model of mutation (TPM), allowing 90% one-step mutations and 10% multi-step mutations. Studies have shown that microsatellite markers better fit the TPM than either the infinite allele model (IAM, where all mutations may be multi-step) or the stepwise mutational model (SMM, where all mutations are one-step) (Di Rienzo et al. 1994). To determine whether a significant number of loci display gene diversity excess relative to equilibrium gene diversity, a Wilcoxon sign-rank test implemented in the programme BOTTLENECK (Cornuet and Luikart 1996) was employed. One hundred re-sampled data sets, generated using the nest-based re-sampling method, were used for this analysis, resulting in 100 significance values (one for each re-sampled data set). The final significance value given corresponds to the proportion of re-sampled data sets that display significant gene diversity excess relative to

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equilibrium gene diversity (therefore $p \leq 0.05$ is evidence for a lack of genetic bottleneck). Due to the way the results are analysed, the null hypothesis being rejected here is one of no effect (as for the analyses of linkage disequilibrium and Hardy-Weinberg equilibrium in Chapter 3).

iv) Worker Aggression

Both frequency and level of pairwise aggression were calculated. Frequency of aggression was calculated as the percentage of nest pairs tested that displayed aggressive interactions. The level of aggression was calculated as the mean number of aggressive interactions per 5-10 s scan over all tested nest pairs. Within- and between-sector interactions, within-nest interactions and controls A and B were analysed separately.

v) Polydomy

To estimate polydomous colony boundaries within a population or sector, the first step was to calculate con-colonality (i.e. membership of the same colony) between all possible nest pairs, utilising the technique of Pedersen and Boomsma (1999). Low levels of aggression between non-nestmate workers (see Results) meant that behavioural data were not incorporated into colony boundary estimation. Average worker relatedness within nests has been calculated previously (Chapter 3). Average worker relatedness between all nest pairs was calculated using the programme Relatedness 5.0 (Goodnight 1994), which is based on the algorithm of Queller and Goodnight (1989). Background allele frequencies used to calculate relatedness values were always those of the entire Cors Goch population, as analyses of population differentiation revealed that the component sectors of Cors Goch were not separate populations (see Results; *Population genetic differentiation*). For the analysis of Cors Goch Grid 04, the background allele frequencies used were calculated from Cors Goch Grid 04 and Cors Goch

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Transect, as nests on the transect were not sampled in 2004. This assumes that allele frequencies on the transect did not change significantly between 2003 and 2004. Given the lack of genetic differentiation between Cors Goch Grid 03 and Cors Goch Grid 04 (see Results; *Population genetic differentiation*), this seems to be a valid assumption. Con-coloniality between pairs of nests was estimated using the following equation (Pedersen and Boomsma 1999):

$$\Delta r = r_{1 \leftrightarrow 2} - \frac{n_1 r_1 + n_2 r_2}{n_1 + n_2}$$

where $r_{1 \leftrightarrow 2}$ is inter-nest relatedness, i.e. the mean relatedness between workers from nests 1 and 2, r is the mean worker nestmate relatedness within either nest (with the subscript denoting the nest) and n is the number of individuals sampled from either nest (again with the subscript denoting the nest), weighting the intra-nest relatedness estimate by sample size. The hypothesis that two nests are con-colonial is accepted if inter-nest relatedness ($r_{1 \leftrightarrow 2}$) \geq mean intra-nest relatedness ($(n_1 r_1 + n_2 r_2) / (n_1 + n_2)$); that is, if Δr is positive. The rationale of this method is the fact that a positive inter-nest relatedness alone cannot confirm con-coloniality, as it could be due to allele sharing arising from one nest budding from the other, or both nests budding from the same nest (Pedersen and Boomsma 1999). In 2003 almost all con-colonial nest pairs were within Cors Goch Grid 03. Two con-colonial nest pairs were between Cors Goch Grid 03 and Cors Goch Transect but there were no con-colonial nest pairs within Cors Goch Transect. As con-coloniality between nests within Cors Goch Transect was absent, all subsequent analyses were performed on Cors Goch Grid 03 and Cors Goch Grid 04 only.

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vi) Polydomous colony boundaries

Colony boundaries were analysed using the presence of con-colonial nest pairs. In a theoretical model where polydomous colonies within a population are discrete and isolated from other polydomous colonies, each nest within a colony should be con-colonial with all nests within the colony but not con-colonial with any other nests. This situation is unlikely to occur in natural populations, especially if habitat saturation is high, worker nestmate relatedness is low and colonies are capable of moving to new nest-sites. I designed the following technique to detect clusters of nests that are con-colonial with as many other nests in the cluster as possible.

A matrix of all nest pairs was created, and con-colonial pairs highlighted (e.g. Figure 4.1). This created a "barcode" system of identification, where two nests which were con-colonial with the same other nests would have the same barcode. As in this matrix each nest pair combination appears twice, the lower left section of the matrix (below diagonal) was identical to the upper right section (above diagonal). To make individual barcodes more comparable, intra-nest pairs were also highlighted as con-colonial. Omitting this step reduces the power of the method; if nest Y is con-colonial with nest Z, the barcode method will only fully capture this similarity if nest Z is 'con-colonial' with itself. The nest that was con-colonial with the most other nests was thought most likely to belong to a relatively large polydomous colony. This nest (nest X) was selected and moved to the far left column of the matrix. All other nests were then sorted by column in descending order according to the number of con-colonial nests they shared with nest X (similarity of "barcode"). Starting with the nest, or nests, possessing the least similar barcode, nests were removed from both row and column of the matrix if they did not share $\geq 50\%$ con-colonial pairings ('barcode' similarity) with nest X. If the removed nest, or nests, were con-colonial with nest X, then the number of con-colonial pairings shared with nest X in order to be included in

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the matrix was recalculated, as the total number of nests that nest X was con-colonial with decreased. When all remaining nests shared $\geq 50\%$ con-colonial pairings with nest X , these nests formed the first polydomous colony. A new matrix was formed with the remaining nests and the process repeated until remaining nests could not be assigned to a polydomous colony.

If, when selecting nest X , several nests were found to share the highest number of con-colonial pairings, the first polydomous colony matrix was constructed for each of the possibilities separately. If nests within matrices did not overlap (each nest belonging to only one matrix) then these matrices were all used as normal. If nests from multiple matrices did overlap, then the likelihood of the cluster of nests being a polydomous colony was estimated and compared between matrices. This likelihood was the percentage of nest pairs within each putatively polydomous colony that were con-colonial. Because, in the extreme case, all nests within a polydomous colony should be con-colonial with all other nests within the colony, the matrix that produced the highest percentage of con-colonial pairings was the most likely to be a true polydomous colony. This matrix was used and the procedure continued.

vii) Validation of method of detecting polydomous colony boundaries

To test whether the pattern of polydomous colonies produced by my method of colony boundary detection described above was different from a pattern produced by a random matrix of con-colonial pairings, I designed a validation technique based on the Cors Goch Grid 04 data and results. A half matrix containing 113 con-colonial pairings (the number of con-colonial pairings within Cors Goch Grid 04) was randomised. This was then converted into a full matrix so that each con-colonial pairing was repeated and intra-nest con-colonial pairings were added, as was done with the real data. This last step was added so that any differences observed between patterns of putative polydomous colonies produced with actual and randomised data could not be

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attributed to the methods I had used to turn the data into a useful matrix. The randomised matrix was then used to produce putative polydomous colonies using the model described above. The whole procedure was repeated ten times. Each time, the number of putative polydomous colonies produced, the average number of nests per colony and the 'likelihood' of each putative colony actually being a colony, calculated as previously explained, were recorded.

If my method of colony boundary detection is valid, then at least some of observed pattern of putative polydomous colonies produced using actual data will be due to relatedness patterns between nests. Conversely, the pattern produced by the randomised data will be due only to random association within the matrix as well as any association that may have arisen from creating a full, useable matrix. Therefore it is reasonable to assume that if my model is valid the statistics recorded (stated above) for each data set should differ significantly between putative colonies produced with actual and randomised data in the following ways. Randomised data should produce a higher number of putative polydomous colonies with a lower average number of nests per colony, as well as having a lower 'likelihood'. The null hypotheses being tested are that the data do not conform to the above expectations. Significance of the validity of the model corresponded to the proportion of times, of the 10 randomisations, that the randomised data violated the above three assumptions, and was calculated for each assumption. As data were randomised ten times, one violation of an assumption would provide a p value of 0.10. This would make the patterns of putative polydomous colonies produced by the method not significantly different from those produced by randomised data, invalidating the method.



Results

Population genetic differentiation

Pairwise F_{ST} estimates revealed significant genetic differentiation between all population pairs with the exception of the New Forest - Hartland Moor pair (Table 4.1; New Forest - Hartland Moor pair, $F_{ST} = 0.045 \pm 0.005$, $p = 0.400$). In Cors Goch, analysing sectors Cors Goch Grid 03 and Cors Goch Transect separately revealed no significant genetic differentiation (Table 4.1; $F_{ST} = 0.008 \pm 0.004$, $p = 0.900$). Comparisons of Cors Goch Grid 04 with Cors Goch Grid 03 also revealed no significant genetic differentiation (Table 4.1; $F_{ST} = 0.011 \pm 0.003$, $p = 0.850$). Significant isolation by distance (Figure 4.2) was found between the four populations (correlation = 0.637, $r^2 = 0.406$, $p < 0.001$).

For each re-sampled data set analysed with the programme STRUCTURE version 2 (Pritchard et al. 2000) for the elucidation of genetic structure, mean values (\pm SE, calculated over three repeated analyses for each value of K) of $\ln \Pr(X/K)$ were plotted against values of K tested and visually analysed. Examining plots from the first analysis (using individuals from Cors Goch, Rhossili Down, the New Forest and Hartland Moor) for either obvious peaks (signifying a particularly high estimate of $\ln \Pr(X/K)$) or plateaus (to find the lowest value of K that captures the major structure) revealed that five re-sampled data sets (data sets 2, 3, 4, 5 and 9; Figure 4.3) showed evidence for $K = 3$ being the most likely number of populations and three re-sampled data sets (data sets 1, 7 and 10; Figure 4.3) showed evidence for $K = 4$ being the most likely number of populations. Two re-sampled data sets (data sets 6 and 8; Figure 4.3) were more difficult to interpret graphically, as they sloped gradually to a small peak at $K = 5$. As the majority of re-sampled data sets suggested that either $K = 3$ or $K = 4$ represented the most likely number of populations, the assignment of individuals when $K = 3$ and $K = 4$ respectively

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was displayed for each re-sampled data set (Table 4a, b). Although the $\text{Ln Pr}(X/K)$ shown is a mean, calculated over three repeated analyses, the assignment of individuals shown is that of the analyses that produced the lowest $\text{Ln Pr}(X/K)$. Examination of assignment of individuals reveals three main clusters, one containing a large proportion of Cors Goch individuals, one containing a large proportion of Rhossili Down individuals and one containing a large proportion of both the New Forest and Hartland Moor individuals (Table 4a, b). This supports the finding of the majority of the graphical plots, that the most likely number of populations is $K = 3$. When $K = 4$, assignment of individuals makes less biological sense, especially given the known geographical location of the sampling sites. The three main clusters usually remain, with the fourth cluster frequently consisting of a small proportion of each population's individuals (Table 4a, b). Examining plots from the second analyses, investigating genetic structure within Cors Goch (within and between years), revealed a lack of obvious peaks (Figure 4.4). Most plots also displayed a lack of slope, what slope there is being negative (decreasing $\text{Ln Pr}(X/K)$ with increasing K ; Figure 4.4). Given that values of K above the 'true' value can produce estimates of $\text{Ln Pr}(X/K)$ similar to or slightly greater than that produced when K is correct (Pritchard and Wen 2003), this suggests that $K = 1$ is the most likely number of populations for the individuals tested. Examining the assignment of individuals to populations when $K = 2$ (Table 4.3a, b) reveals that individuals are assigned relatively symmetrically (each population containing $\sim 1/K$) suggesting a lack of true population structure (Pritchard and Wen 2003). It was noted that this trend continued when $K > 2$ (data not shown). The results from analyses using STRUCTURE version 2 suggest that Cors Goch and Rhossili Down are genetically distinct populations and that the New Forest and Hartland Moor, although being distinct from the two Welsh populations, are not genetically distinct from each other. Analyses within Cors Goch (within and between years) suggest that neither Cors Goch Transect, Cors Goch Grid 03 nor Cors Goch Grid 04 are genetically distinct enough to be classed as separate populations. The results

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of the Bayesian clustering analysis performed implemented in STRUCTURE version 2 closely match the between-population F_{ST} results.

Within-population isolation by distance

All four populations studied showed the same trend of increasing pairwise F_{ST} with increasing physical distance between nests (Figure 4.5), although a large amount of variation within this trend was apparent. A significant positive correlation between pairwise F_{ST} and physical distance between nests was only apparent within Cors Goch (Table 4.4; correlation = 0.265, $R^2 = 0.070$, $p = 0.020$) and the New Forest (Table 4.4; correlation = 0.303, $R^2 = 0.092$, $p = 0.031$) at the 5% level. Within the sectors of Cors Goch only Cors Goch Transect showed evidence for isolation by distance (Table 4.4; correlation = 0.173, $R^2 = 0.030$, $p = 0.033$) whilst Cors Goch Grid 03 displayed a non-significant negative relationship between pairwise F_{ST} and physical distance (Table 4.4; correlation = -0.120, $R^2 = 0.013$, $p = 0.840$). Cors Goch Grid 04 revealed no significant evidence for isolation by distance (Table 4.4; correlation = 0.110, $R^2 = 0.008$, $p = 0.081$). In all populations and sectors that display significant isolation by distance, the amount of variation in pairwise F_{ST} explained by the physical distance between nests was small, as evidenced by low R^2 estimates (Table 4.4).

Population genetic bottlenecks

Two of the four populations studied, Rhossili Down and the New Forest, displayed significant evidence for recent genetic bottlenecks (Table 4.5; $p = 0.230$ and 0.170 respectively). All other populations and sectors, across both years, displayed no significant evidence for recent genetic bottlenecks (Table 4.5).

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Mitochondrial DNA

I sequenced 972 bp of the Cytochrome Oxidase subunit 1 gene in 81 individuals of the 96 analysed. The DNA of 15 individuals was not of sufficient quality to obtain scorable sequences. The successfully sequenced individuals represented 30 nests from Cors Goch Llanllwch, 9 nests from Rhossili Down, 10 nests from the New Forest and 9 nests from Hartland Moor. Comparison with the same mtDNA region sequenced in a closely related species, *F. cunicularia* (Hasegawa et al. 2002), revealed no frame-shift mutations, the start codon being ATT (coding for the amino acid Isoleucine, Figure 4.6). Within the four UK populations of *F. picea* analysed, only two haplotypes were discovered, one in the English populations and one in the Welsh populations. The difference between the two haplotypes was one synonymous nucleotide substitution at the third nucleotide position of a codon. The substitution was GCC (Welsh populations) to GCT (English populations), both codons coding for the amino acid Alanine (Figure 4.6). The variability of the sequenced region was not great enough to perform any formal statistical analyses.

Worker aggression

The total frequency and level of lunging of intra-nest controls (31% and 0.088 respectively; Table 4.6a) was greater than that of inter-nest tests (22% and 0.048 respectively; Table 4.6b), indicating that lunging could not be used to assay between-nest aggression. By contrast, biting only occurred in inter-nest aggression tests, but it did so at an extremely low level (Table 4.6b). Therefore, an analysis of the relationship between aggression and spatial structure of nests could not be performed. Biting was absent from control A (Cors Goch Grid and Cors Goch Transect workers vs. workers of most distant *F. picea* nest in the bog), despite the distance between nests. Frequency and level of aggression within control B (*F. picea* workers vs. worker ants of the

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taxon *Lasius* s. str.) were the highest seen (100% and 2.700, respectively; Table 4.6c). During control B tests both species appeared to be actively aggressive towards the other. Although initiation of aggression was quick and difficult to determine, both species were observed biting the other (personal observations), suggesting that *F. picea* does have the potential to be highly aggressive.

Polydomous colony boundary model validation

Of the ten randomisations, none violated the assumptions of any statistic; the number of putative colonies was always greater and the number of nests per putative colony and 'likelihood' of each putative colony were always lower for results obtained from randomised data sets compared to results obtained from actual data ($p = 0.000$ for all three statistics; Table 4.7). These results validate the method used as a viable technique to detect micro-geographic population structure caused by inter-nest relatedness and polydomy.

Polydomy

Within Cors Goch in 2003, 32 nest pairs out of a possible 561 were con-colonial. Only two of these nest pairs contained nests from Cors Goch Transect and in no pair were both nests from Cors Goch Transect. Within Cors Goch Grid 03, 29% of possible nest pairs were con-colonial, while the corresponding proportion within Cors Goch Grid 04 was 34% (Table 4.8).

Extremely low levels of aggression meant that only genetic data were used to estimate polydomous colony boundaries. Three putative polydomous colonies were discovered within both Cors Goch Grid 03 and Cors Goch Grid 04 (Figure 4.7a,b) using the con-colonial matrix technique. The mean number of nests within colonies was higher in Cors Goch Grid 04 than in Cors Goch Grid 03 (Table 4.8). Out of the five nests within colony A in 2003 that were present

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in 2004, four were part of colony A and one was part of colony B in 2004. Of the three nests within colony B in 2003 that were present in 2004, two were part of colony A and one was part of colony B in 2004. Neither nest within colony C in 2003 was present in 2004. Clusters of putatively polydomous nests were not discrete, as all colony boundaries were found to cross at least one other colony boundary in both Cors Goch Grid 03 and Cors Goch Grid 04. In Cors Goch Grid 04, colonies A and B appeared to be almost spatially separate from each other, with the exception of nests 41 and 51 (Figure 4.7b). However, the boundaries of colony C overlapped those of both A and B.

Discussion

This study aimed to investigate in detail the social and genetic structure within and between UK populations of the endangered Black Bog Ant *Formica picea*, with particular emphasis on the link with spatial structure. Significant genetic differentiation was revealed between all population pairs, with the exception of the New Forest and Hartland Moor, the geographically closest pair. This suggests that either gene flow occurs between these two populations, or has done in the recent past. Despite nestmate worker relatedness estimates not significantly greater than zero, significant isolation by distance was revealed in two out of the four populations, indicating that, in at least these two populations, workers are more genetically similar to workers from physically closer nests and that, therefore, it is highly likely that colony reproduction occurs by budding. Evidence for recent genetic bottlenecks was displayed in two populations, Rhossili Down and the New Forest. Within Cors Goch, a lack of aggression was found between almost all nest pairs tested and so behavioural data were not used to estimate colony boundaries. Three putative polydomous colonies were estimated within both Cors Goch Grid 03 and Cors Goch Grid 04, although analysis of the temporal fidelity of boundaries proved difficult due to the relatively few nests sampled

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in 2003 that remained active in 2004. In both years, colony boundaries overlapped each other, although they were somewhat more defined in Cors Goch Grid 04, where the area and number of nests sampled were greater. As expected, given that nests were approximately 10 m apart, no polydomous colonies were detected in Cors Goch Transect as relatedness within nests was always greater than relatedness between nests.

Population genetic differentiation

Results from the Bayesian clustering analyses closely matched the between-population pairwise F_{ST} results, as individuals from the New Forest and Hartland Moor were clustered together, whereas individuals from Cors Goch and Rhossili Down were clustered separately. High levels of gene flow between local polygynous populations has been noted in other *Formica* species, such as *F. lugubris* (Gyllenstrand and Seppa 2003), but the distances between these populations was much lower than between-population distances in the present study. The lack of genetic differentiation between New Forest and Hartland Moor raises the question of how gene flow could be occurring between two populations over 30km distant from one another. As both populations are highly polygynous (see Chapter 3), dispersal ability of queens is probably poor and it is unlikely that even males could fly the distance between the populations. More feasible is that there are, or were in the very recent past, a number of other populations that might have bridged the gap between the two populations, so permitting gene flow. Within the New Forest there are known to be other small populations of *F. picea* (North 1998b), although the number of these populations is unresolved (Else 1997). However, the distance between the edge of the New Forest and Hartland Moor (~30 km) is still likely to be too great for individuals from a highly polygynous population to fly. Even if there were other populations between the New Forest and Hartland Moor they would have to be both close together and numerous to lead to gene flow over this distance. A possible

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explanation for the observed pattern of inter-population structure is that it is an historic artefact, remaining from a period when not only were there intervening populations but when dispersal ability was greater. *Formica picea* probably colonised the UK at some time after the last glaciation, by crossing the land bridge between the UK and what is now continental Europe, circa 10000 years ago. This colonisation itself suggests a level of dispersal ability and a corresponding decrease in the level of polygyny. Dispersal, and therefore gene flow, between populations may have been more feasible under these circumstances. Polygyny, and a corresponding loss of some dispersal ability, may have since evolved as suitable habitat became scarcer and costs associated with independent colony foundation increased. If no gene flow has occurred between the New Forest and Hartland Moor populations for a considerable period of time, genetic drift would be expected to increase the genetic distance between them unless effective population size is extremely high. That this has not happened suggests that gene flow (possibly due to intervening populations) either is present between the two populations or, as seems more likely, has been in the recent past.

Analysis of isolation by distance revealed a significant negative relationship between geographical distance and genetic differentiation of population pairs. Isolation by distance usually suggests that individuals within a system of local populations can only disperse far enough to reach the closest neighbouring population. However, as has been discussed, the distances involved and the level of polygyny make dispersal between even the closest populations in the present study unlikely. Also, the observed correlation between physical distance and pairwise F_{ST} consists of only four data points and seems to rely heavily on a single point (distance and F_{ST} between New Forest and Hartland Moor) in the bottom left corner (Figure 4.2), decreasing the confidence that it is a biologically relevant relationship.

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Within-population isolation by distance

Two out of the four populations studied (Cors Goch and the New Forest) showed significant isolation by distance. The other two populations (Rhossili Down and Hartland Moor) revealed the same trend of workers being more genetically similar to workers from physically closer nests, although this trend was not significant. As all populations had previously been shown to be highly polygynous, colony reproduction by budding was expected and is supported in at least Cors Goch and the New Forest. In many species of ant there is a dispersal polymorphism between the sexes, with males dispersing over greater distances (Sundström 1995). As microsatellites are nuclear markers, a small amount of male gene flow over this small distance could have homogenised allele frequencies and so prevented the detection of isolation by distance within populations, but this seems not to have occurred. These results suggest that both males and queens have restricted dispersal, with queens possibly mating within the natal colony. It is likely that budding also occurs at Rhossili Down and Hartland Moor but these populations may have been slightly genetically homogenised if dispersal of either males or queens is greater than at Cors Goch and the New Forest. Increased mobility of nests may also hamper the detection of isolation by distance, even if dispersal is poor and nest reproduction by budding occurs.

Population genetic bottlenecks

The recent decrease in effective population size (genetic bottlenecks) displayed by the New Forest and Rhossili Down populations could be due to either the decrease in effective size of already established populations, or to founder effects (a small number of individuals founding the population). This latter cause seems most likely, as a factor causing a dramatic decrease in population size (e.g. loss of suitable habitat) would be unlikely to allow subsequent population expansion. This then raises the question of where

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these founding individuals came from. For Rhossili Down and the New Forest, the only possible donor populations seem to be Cors Goch and Hartland Moor respectively. However, as has been discussed, due to the distance between populations and the apparent limited dispersal ability of *F. picea*, it is unlikely that dispersal, even that aided by wind, could occur between them. For both populations that show evidence of a genetic bottleneck, it is possible that founding queens came from populations that no longer exist or have not been discovered. It is also possible that the founding queens of the New Forest population came from another population within the New Forest.

Mitochondrial DNA

The mitochondrial DNA (mtDNA) region Cytochrome Oxidase I (COI) used in this study proved invariable within populations and only differed by one nucleotide between English and Welsh populations. This raises the question of whether the observed lack of variability is specific to the mitochondrial region used, and therefore not a true representation of *F. picea* genetic structure. The only other study using mtDNA to analyse *F. picea* (Goropashnaya 2003) used a section including Cytochrome b (Cyt b), a region with a high level of variation in UK populations of *F. lugubris* (Gyllenstrand and Seppa 2003). However, Goropashnaya's (2003) study investigated the phylogeographic structure of *F. picea* (among other species) across Eurasia and variation was across large distances. An examination of *F. picea* mtDNA sequences from Goropashnaya's (2003) study, deposited on GenBank, approximately 1.5kb in length, showed that sequences from the UK differed from sequences from Finland and Sweden by only four nucleotide substitutions and one nucleotide substitution respectively (Table 4.9; S. Rees, unpublished observations). As the large distances between these populations should exclude the possibility of gene flow, variation within populations should be lower than variation between populations, suggesting that the Cyt b region would also be relatively invariable within UK populations of *F. picea*. It is

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therefore likely that COI provides a true representation of genetic structure characterised by female-biased dispersal in *F. picea*, rather than being an uncommonly invariable region. Conclusions to be drawn from the observed mitochondrial haplotype distribution are limited. Female gene flow between Welsh populations and between English populations cannot be determined, as they share the same haplotype at a frequency of 1. However, although larger samples might have revealed different results, it seems likely that no female-biased gene flow occurs between the Welsh and English populations. One explanation for the current lack of mitochondrial variation within and between populations is that only very few queens colonised the UK. Another is that the glacial refugia used by *F. picea* (Goropashnaya 2003) underwent some kind of population bottleneck, resulting in the low levels of variation observed in this study. Whatever the reason, it is obvious from this study that the COI region, and possibly mtDNA in general, is not a particularly useful tool for detailed population genetic studies in UK populations of *F. picea*.

Worker Aggression

The almost complete lack of aggression between *F. picea* workers of different nests, even those up to ~200 m distant (control A), supports the theory of a population with an almost uniclonal social organisation, as suggested previously by the very low levels of nestmate worker relatedness (Chapter 3). However, because aggression was uniformly low, these results could not be used to complement the genetic data to estimate colony boundaries. It is somewhat surprising that workers from one sector behave no differently towards workers from another sector than they do towards workers from the same sector, especially given the difference in mean nestmate worker relatedness previously shown (Chapter 3). It may be that workers are not able to recognise other workers from foreign nests, even though nestmate worker relatedness within nests on Cors Goch transect, although low, is significantly greater than zero. It may also be that workers can recognise non-nestmates,

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but are not aggressive towards them (Chapuisat et al. 2005). There are two main theories to explain the evolution of unicoloniality and the lack of aggression between individuals of different nests that accompanies it. One is a loss of genetic variation at recognition cues (Chapman and Bourke 2001), often associated with invasive species undergoing a population bottleneck (Tsutsui et al. 2000). The other is the difficulty in establishing independent colonies when habitat becomes saturated (Chapman and Bourke 2001). In this situation queens become accepted into natal colonies, increasing the level of polygyny and leading to selection for colonies to reproduce by budding. This process can be self-reinforcing and the end result is a population of highly polygynous and polydomous colonies (Keller 1995; Chapuisat and Keller 1999). Due to the high level of polygyny, intra-nest relatedness would also be low. Lack of aggression between nestmates, who may be only slightly related to each other, would be selected for, as aggression towards nestmates would lead to an unproductive colony. As has been shown recently in a population of the ant *F. paralugubris* (Chapuisat et al. 2005), it is possible for unicolonial populations to evolve despite nestmate recognition. Given that *F. picea* is not an invasive species and within-nest relatedness is significantly greater than zero on Cors Goch Transect (Chapter 3), as well as the apparent patchy distribution of suitable habitat even within populations (Chapter 2), it appears likely that habitat saturation drives low nestmate relatedness and unicoloniality in this species.

Polydomous colony boundary model

The fact that putative polydomous colonies detected with actual and randomised data differed significantly in terms of the number of colonies, the number of nests per colony and the 'likelihood' (the percentage of nest pairs within each putatively polydomous colony that were con-colonial; see Methods) of colonies, suggests that the model is a valid method for detecting polydomous colony boundaries. This implies that some portion of the pattern

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of polydomous colonies produced with actual data is due to nests being part of true polydomous colonies, rather than a random association of points within a matrix. However, results produced by actual and randomised data did not differ greatly. This may suggest that the model lacks precision and is only able to reveal a small amount of the relationship between genetic and spatial structure of nests. Another possibility, discussed later in this section, is that this relationship does not differ greatly from a random association and that the model is detecting the small amount of genetic and spatial structure present between nests.

Colony boundaries and polydomy

The lack of con-colonial nest pairs in Cors Goch Transect initially suggests that these colonies are not polydomous, whereas colonies within the grid (Cors Goch Grid 03 and Cors Goch Grid 04) are. Another possibility is that Cors Goch Transect nests were too far apart to be connected, as the distance between closest nests was approximately 10 m and that the sampled nests may have been con-colonial with physically closer nests not sampled. However, this would still represent a much lower level of polydomy as, while initially searching for nests on the transect in 2003, I found nest density to be extremely low when compared to the grid. Considering the difference in effective queen number between the two sectors (Chapter 3), it seems that there is a significant difference in social and genetic organisation. Also, putative polydomous colonies in Cors Goch Grid 04 extended over most of the sampled area, nests from the same putative polydomous colony being approximately 9 m apart. If there were no difference in the level of polydomy between the two sectors, I would expect some of the nests within Cors Goch Transect to be con-colonial with each other. As suggested previously (Chapter 3), this level of apparent social polymorphism may be linked to the availability of suitable habitat. If suitable habitat on the transect is more patchy than on the grid, it may not be possible for bud nests to be founded in

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the immediate vicinity of the mother nest, so aggregates of large polydomous colonies would not be formed. The low worker nestmate relatedness estimate on Cors Goch Transect therefore probably reflects the actual number of reproducing queens within each nest, rather than individuals being exchanged between nests of the same or even different colonies.

Employing the technique utilised in this study, discrete polydomous colony boundaries were difficult to detect, even within the grid. Although three putative polydomous colonies were detected in both years (Cors Goch Grid 03 and Cors Goch Grid 04), boundaries frequently overlapped. Boundaries seem slightly more defined in Cors Goch Grid 04, where the area and number of nests sampled was greater (81 m², 26 nests sampled). It may be that the area and number of nests sampled in Cors Goch Grid 03 (56 m², 15 nests sampled) was too small to detect the multi-nest colonies effectively. A number of nests appear to change colonies between years. Given the lack of aggression observed between *F. picea* workers it is unlikely that this is due to one colony actively taking over another colony's nest, but it may reflect queens being recruited into nests from different polygynous colonies. There is also the possibility, however, that the technique used is not accurate enough to detect such fine-scale spatial structuring. Using a greater number of more polymorphic loci and increasing the sample size would certainly increase the precision of this technique, but it currently seems adequate to detect the presence of polydomy when an area is extensively sampled.

Whether there are discrete polydomous colonies to detect in this population is another question to be addressed. If the colonies were typically unicolonial, with free exchange of individuals between all nests, then there would be no detectable boundaries, although some nests pairs would appear con-colonial by chance. However, it is also possible polydomous boundaries could be difficult to determine even if individuals were only exchanged between a limited number of nests. The assumption that polydomous colony boundaries

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do not overlap may be invalid. If nest density and habitat saturation is high and aggression is low, there seems to be no reason why a bud nest from one polydomous colony may not be founded within the boundaries of another polydomous colony. The frequency of this occurring should be low, as a suitable patch within the boundaries of a theoretical polydomous colony X would be more likely utilised by colony X than another, simply due to the proximity of more of colony X 's nests to that patch. However, even if a nest was founded within the boundaries of another colony infrequently, estimations of colony boundaries could become confounded. Finally, con-coloniality between a pair of nests may signify that one nest has recently budded from another but exchange of workers may have ceased. Con-coloniality between the two nests will persist until a new cohort of workers has been produced (Pedersen and Boomsma 1999). Whether con-coloniality detectable with genetic methods persists after this time will depend on the relatedness of the new queen and her mate to individuals from the original colony. As workers were sampled during the end of August in each year, I would expect some of them to be progeny of the new queen if the nest had been recently founded by budding. Also, due to the high density of nests within the grid and the lack of aggression between non-nestmate workers, it seems likely that a nest recently founded by budding would exchange workers with the 'mother' nest.

Conservation implications of polydomy

Polydomy within Cors Goch has implications for the conservation of this population, relating to effective population size. Although a high effective queen number suggested by low worker nestmate relatedness increases effective population size, this is an estimate per colony. If a polydomous colony has five nests between which workers are freely exchanged, sampling these five nests is essentially sampling the same colony five times. The effective number of queens estimated for the whole colony should theoretically be the same as any one of the associated nests. Therefore the

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number of colonies used to establish a grid-wide queen number and effective population size should be the number of nests divided by the mean number of nests per polydomous colony.

Queen number is often used as a surrogate for effective population size (N_E) in studies of ants where no information about the number of potentially reproductive males per colony is available (Gyllenstrand and Seppa 2003), such as this one. As males are haploid, even if there were an equal number of reproductive males to females, including just the females would still capture two thirds of the effective population size. There are approximately 100 known nests within the grid at Cors Goch in any year. From personal observation, I suggest that there are a minimum of 50 and a maximum of 100 nests outside the grid. Using these figures and the estimates for N_Q calculated in Chapter 1 (Table 3.9, using estimates for CGG03 and CGT), this gives minimum and maximum estimates for total queen number of 2970 and 3230, respectively. If polydomy within the grid is taken into account (again using CGG03, for the estimate of number of nests per colony) these estimates are reduced to 890 and 1150, respectively. Polydomy can therefore drastically reduce effective population size compared to monodomy. Even taking into account polydomy, however, these estimates of effective population size, although small compared to many insects, are not small for an isolated ant population (Gyllenstrand and Seppa 2003). This large effective population size, conferred by polygyny, is probably the source of the relatively high genetic diversity and lack of inbreeding observed within Cors Goch (Chapter 3).

In conclusion, the results from this study show that all populations are genetically differentiated from each other, with the exception of the New Forest and Hartland Moor. It is likely that dispersal is poor and colony reproduction occurs by budding in all populations, so the apparent pattern of gene flow between these two populations may be due to intervening

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populations not yet discovered or recently become extinct. Limited dispersal also suggests that the ability of *F. picea* to colonise new habitat is low. Results from genetic and behavioural analysis of Cors Goch suggest that it is a largely unicolonial-like population, with high numbers of queens per colony, low nestmate worker relatedness and a lack of aggression between non-nestmates. Genetic structuring is maintained within the population by poor dispersal and budding and aggregations of polydomous colonies probably utilise large patches of suitable, stable habitat. To judge from the nest densities casually observed while collecting samples, the colonies sampled within Rhossili Down, the New Forest and Hartland Moor are probably not highly polydomous. Despite this, and considering the lack of aggression between workers from Cors Goch Transect and the low nestmate worker relatedness in all populations, it is possible that all populations could be largely unicolonial.

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Table 4.1

Genetic differentiation between all pairs of populations, expressed as F_{ST} (below diagonal; mean \pm SE, calculated over re-sampled data sets). Significance (p values; above diagonal) given correspond to the proportion of re-sampled data sets that displayed no significant genetic differentiation (therefore $p \leq 0.05$ equates to a significant F_{ST} estimate). CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type.

	CG	CGG03	CGT	CGG04	RD	NF	HM
CG03	-	N/A	N/A	N/A	0.000	0.000	0.000
CGG03	N/A	-	0.900	0.850	N/A	N/A	N/A
CGT03	N/A	0.008 \pm 0.004	-	N/A	N/A	N/A	N/A
CGG04	N/A	0.011 \pm 0.003	N/A	-	N/A	N/A	N/A
RD	0.239 \pm 0.011	N/A	N/A	N/A	-	0.000	0.000
NF	0.209 \pm 0.007	N/A	N/A	N/A	0.172 \pm 0.008	-	0.400
HM	0.271 \pm 0.008	N/A	N/A	N/A	0.182 \pm 0.009	0.045 \pm 0.005	-

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Table 4.2a

Probabilistic clustering of individuals' multi-locus genotypes sampled from four populations (Pop; CG = Cors Goch Llanllwch, RD = Rhossili Down, NF = the New Forest and HM = Hartland Moor), performed with the programme STRUCTURE version 2 (Pritchard et al. 2000). Numbers within clusters are, for each population, the proportion of individuals assigned to that cluster (numbers in bold indicate the highest proportion of each populations assigned individuals). K = putative number of populations, $\text{Ln } P(X/K)$ = likelihood of K given genotype data X (\pm SE, calculated over 3 repeated runs). N/A shown in cluster 4 when $K = 3$. Results shown for re-sampled data sets 1-5.

Data Set	K	Pop	Cluster				Mean $\text{Ln } P(X/K) \pm \text{SE}$
			1	2	3	4	
1	3	CG	0.810	0.166	0.024	N/A	-833 \pm 1.92
1	3	RD	0.116	0.768	0.116	N/A	
1	3	NF	0.019	0.021	0.960	N/A	
1	3	HM	0.035	0.081	0.884	N/A	
1	4	CG	0.807	0.035	0.023	0.136	-783 \pm 0.30
1	4	RD	0.107	0.796	0.087	0.010	
1	4	NF	0.015	0.056	0.921	0.008	
1	4	HM	0.030	0.194	0.768	0.007	
2	3	CG	0.864	0.118	0.018	N/A	-775 \pm 4.00
2	3	RD	0.041	0.914	0.045	N/A	
2	3	NF	0.020	0.156	0.824	N/A	
2	3	HM	0.020	0.023	0.957	N/A	
2	4	CG	0.690	0.151	0.016	0.143	-815 \pm 8.00
2	4	RD	0.033	0.456	0.036	0.475	
2	4	NF	0.019	0.118	0.752	0.110	
2	4	HM	0.017	0.024	0.935	0.024	
3	3	CG	0.845	0.129	0.026	N/A	-841 \pm 1.01
3	3	RD	0.129	0.829	0.042	N/A	
3	3	NF	0.035	0.089	0.876	N/A	
3	3	HM	0.015	0.129	0.856	N/A	
3	4	CG	0.474	0.076	0.025	0.424	-845 \pm 2.30
3	4	RD	0.132	0.675	0.030	0.163	
3	4	NF	0.060	0.133	0.735	0.072	
3	4	HM	0.036	0.175	0.748	0.041	
4	3	CG	0.898	0.081	0.021	N/A	-773 \pm 2.61
4	3	RD	0.017	0.938	0.045	N/A	
4	3	NF	0.022	0.062	0.915	N/A	
4	3	HM	0.016	0.019	0.965	N/A	
4	4	CG	0.792	0.015	0.024	0.169	-779 \pm 2.63
4	4	RD	0.020	0.732	0.024	0.224	
4	4	NF	0.023	0.222	0.695	0.060	
4	4	HM	0.016	0.025	0.933	0.025	
5	3	CG	0.864	0.048	0.089	N/A	-814 \pm 2.11
5	3	RD	0.052	0.883	0.065	N/A	
5	3	NF	0.026	0.197	0.777	N/A	
5	3	HM	0.032	0.286	0.682	N/A	
5	4	CG	0.802	0.031	0.042	0.125	-809 \pm 2.21
5	4	RD	0.026	0.790	0.027	0.157	
5	4	NF	0.022	0.157	0.754	0.067	
5	4	HM	0.026	0.243	0.547	0.184	

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Table 4.2b

As Table 4.2a; results shown for re-sampled data sets 6-10.

Data Set	K	Pop	Cluster				Mean Ln P(X/K) ±
			1	2	3	4	
6	3	CG	0.882	0.094	0.024	N/A	-773 ± 1.42
6	3	RD	0.168	0.812	0.020	N/A	
6	3	NF	0.016	0.013	0.971	N/A	
6	3	HM	0.015	0.075	0.909	N/A	
6	4	CG	0.871	0.007	0.027	0.096	-745 ± 3.66
6	4	RD	0.167	0.809	0.011	0.013	
6	4	NF	0.014	0.014	0.963	0.009	
6	4	HM	0.014	0.247	0.731	0.008	
7	3	CG	0.863	0.108	0.030	N/A	-801 ± 3.27
7	3	RD	0.132	0.850	0.019	N/A	
7	3	NF	0.024	0.094	0.882	N/A	
7	3	HM	0.023	0.069	0.908	N/A	
7	4	CG	0.690	0.030	0.027	0.254	-771 ± 1.33
7	4	RD	0.102	0.730	0.017	0.151	
7	4	NF	0.026	0.074	0.855	0.045	
7	4	HM	0.034	0.040	0.818	0.108	
8	3	CG	0.890	0.076	0.034	N/A	-824 ± 1.48
8	3	RD	0.064	0.784	0.152	N/A	
8	3	NF	0.030	0.227	0.743	N/A	
8	3	HM	0.019	0.055	0.925	N/A	
8	4	CG	0.880	0.021	0.032	0.067	-779 ± 1.98
8	4	RD	0.038	0.882	0.065	0.016	
8	4	NF	0.023	0.034	0.736	0.207	
8	4	HM	0.015	0.062	0.914	0.009	
9	3	CG	0.845	0.140	0.015	N/A	-828 ± 0.61
9	3	RD	0.127	0.800	0.073	N/A	
9	3	NF	0.019	0.092	0.889	N/A	
9	3	HM	0.026	0.115	0.859	N/A	
9	4	CG	0.575	0.101	0.014	0.311	-837 ± 4.21
9	4	RD	0.166	0.456	0.064	0.315	
9	4	NF	0.021	0.125	0.807	0.047	
9	4	HM	0.026	0.116	0.810	0.047	
10	3	CG	0.863	0.108	0.029	N/A	-854 ± 1.67
10	3	RD	0.328	0.608	0.064	N/A	
10	3	NF	0.021	0.228	0.751	N/A	
10	3	HM	0.019	0.120	0.861	N/A	
10	4	CG	0.780	0.133	0.024	0.063	-822 ± 2.89
10	4	RD	0.131	0.729	0.033	0.107	
10	4	NF	0.018	0.032	0.739	0.211	
10	4	HM	0.016	0.034	0.844	0.106	

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Table 4.3a

Probabilistic clustering of individual's multi-locus genotypes sampled from sectors of Cors Goch (Pop; CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04), performed with the programme STRUCTURE version 2 (Pritchard et al. 2000). K = putative number of populations, $\text{Ln } P(X/K)$ = likelihood of K given genotype data X (\pm SE, calculated over 3 repeated runs). N/A shown in cluster 2 when $K = 1$. Results shown for re-sampled data sets 1-5.

Data Set	K	Pop	Cluster		Mean $\text{Ln } P(X/K) \pm \text{SE}$
			1	2	
1	1	CGG03	1.00	N/A	-700 \pm 0.57
1	1	CGT	1.00	N/A	
1	1	CGG04	1.00	N/A	
1	2	CGG03	0.440	0.560	-713 \pm 5.68
1	2	CGT	0.614	0.386	
1	2	CGG04	0.729	0.271	
2	1	CGG03	1.00	N/A	-679 \pm 1.44
2	1	CGT	1.00	N/A	
2	1	CGG04	1.00	N/A	
2	2	CGG03	0.360	0.640	-689 \pm 18.18
2	2	CGT	0.516	0.484	
2	2	CGG04	0.390	0.610	
3	1	CGG03	1.00	N/A	-695 \pm 2.13
3	1	CGT	1.00	N/A	
3	1	CGG04	1.00	N/A	
3	2	CGG03	0.500	0.500	-714 \pm 10.37
3	2	CGT	0.499	0.501	
3	2	CGG04	0.505	0.495	
4	1	CGG03	1.00	N/A	-646 \pm 0.73
4	1	CGT	1.00	N/A	
4	1	CGG04	1.00	N/A	
4	2	CGG03	0.501	0.499	-652 \pm 2.62
4	2	CGT	0.498	0.502	
4	2	CGG04	0.500	0.500	
5	1	CGG03	1.00	N/A	-670 \pm 1.03
5	1	CGT	1.00	N/A	
5	1	CGG04	1.00	N/A	
5	2	CGG03	0.495	0.505	-699 \pm 1.81
5	2	CGT	0.502	0.498	
5	2	CGG04	0.500	0.500	

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Table 4.3b

As Table 4.3a; results shown for data sets 6-10.

Data Set	K	Pop	Cluster		Mean Ln P(X/K) ± SE
			1	2	
6	1	CGG03	1.00	N/A	-669 ± 1.96
6	1	CGT	1.00	N/A	
6	1	CGG04	1.00	N/A	
6	2	CGG03	0.416	0.584	-636 ± 3.01
6	2	CGT	0.462	0.538	
6	2	CGG04	0.331	0.669	
7	1	CGG03	1.00	N/A	-687 ± 0.21
7	1	CGT	1.00	N/A	
7	1	CGG04	1.00	N/A	
7	2	CGG03	0.496	0.504	-704 ± 5.43
7	2	CGT	0.496	0.504	
7	2	CGG04	0.508	0.492	
8	1	CGG03	1.00	N/A	-647 ± 1.35
8	1	CGT	1.00	N/A	
8	1	CGG04	1.00	N/A	
8	2	CGG03	0.479	0.521	-715 ± 30.24
8	2	CGT	0.568	0.432	
8	2	CGG04	0.453	0.547	
9	1	CGG03	1.00	N/A	-723 ± 0.69
9	1	CGT	1.00	N/A	
9	1	CGG04	1.00	N/A	
9	2	CGG03	0.457	0.543	-730 ± 3.64
9	2	CGT	0.610	0.390	
9	2	CGG04	0.609	0.391	
10	1	CGG03	1.00	N/A	-696 ± 0.06
10	1	CGT	1.00	N/A	
10	1	CGG04	1.00	N/A	
10	2	CGG03	0.499	0.501	-714 ± 23.11
10	2	CGT	0.498	0.502	
10	2	CGG04	0.501	0.499	

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Table 4.4

Analysis of isolation by distance within each population and sector, expressed as the correlation coefficient (Correlation), R^2 value and significance (p -value, assessed using a Mantel test (Mantel 1967)) of the relationship between pairwise genetic and physical distances of nests. CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type.

	CG	CGG03	CGT	CGG04	RD	NF	HM
Correlation	0.265	-0.120	0.173	0.110	0.210	0.303	0.347
R^2	0.070	0.013	0.030	0.008	0.049	0.092	0.121
p	0.020	0.840	0.033	0.081	0.073	0.031	0.091

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Table 4.5

Loss of genetic variation due to genetic bottlenecks, within each population and sector. CG = Cors Goch (comprising CGG03 and CGT), CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04, RD = Rhossili Down, NF = New Forest, HM = Hartland Moor. Populations shown in bold, sectors of Cors Goch in normal type. Significance of genetic bottleneck (p value) corresponds to the proportion of re-sampled data sets that displayed significant evidence for genetic bottlenecks (i.e. $p \leq 0.05$ indicates no significant evidence for genetic bottlenecks).

Pop	CG	CGG03	CGT	CGG04	RD	NF	HM
p value	0.000	0.030	0.010	0.020	0.230	0.170	0.000

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Table 4.6

Frequency and level of total aggression (A_{TOTAL}) and aggression split into lunging and biting, calculated for intra-nest aggression tests (a; within nests within sectors), inter-nest aggression tests (b; within and between sectors and over all inter-nest pairings) and controls (c; Control A = CGG and CGT nests vs. the most distant *F. picea* nest on the bog, Control B = CGG and CGT nests vs. nest of *L. s. str.* During Control B tests, personal observations suggest that both species were actively aggressive towards the other). Aggression tests performed in July 2005 using nests from Cors Goch Grid (CGG) and Cors Goch Transect (CGT). N_{TESTS} = number of nest pairs tested. Frequency of aggression calculated as the percentage of nest pairs tested that displayed aggressive interactions. The level of aggression was calculated as the mean number of aggressive interactions per 5-10 s scan over all tested nest pairs.

(a)

	N_{TESTS}	Aggression Frequency			Aggression Level		
		% A_{TOTAL}	% Lunge	% Bite	A_{TOTAL}	Lunge	Bite
CGG	8	40	40	0	0.125	0.125	0.000
CGT	8	25	25	0	0.050	0.050	0.000
Total	16	31	31	0	0.088	0.088	0.000

(b)

	N_{TESTS}	Aggression Frequency			Aggression Level		
		% A_{TOTAL}	% Lunge	% Bite	A_{TOTAL}	Lunge	Bite
CGG-CGG	12	25	25	0	0.066	0.066	0.000
CGT-CGT	10	30	30	0	0.060	0.060	0.000
CGG-CGT	28	25	18	7	0.050	0.036	0.014
Total	50	26	22	4	0.056	0.048	0.008

(c)

	N_{TESTS}	Aggression Frequency			Aggression Level		
		% A_{TOTAL}	% Lunge	% Bite	A_{TOTAL}	Lunge	Bite
Control A	2	50	50	0	0.100	0.100	0.000
Control B	2	100	0	100	2.700	0.000	2.700

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Table 4.7

Results of polydomy model validation. Number of putative colonies, mean number of nests per putative colony and mean 'likelihood' of each putative colony (mean percentage of con-colonial pairings each nest within the putative polydomous colony shares with nest *X*, see Methods) shown, produced by Cors Goch Grid 04 (CGG04) data and randomised (Rand1, 2, 3 etc) data. Significance (*p* value) represents the proportion of randomised data sets that produced results which violated assumptions (see Methods), for each statistic produced (therefore $p \leq 0.05$ indicates that the model used produces results that are significantly different from those produced with randomised data sets, therefore is valid).

Data	Number of colonies	Nests per colony	Likelihood
CGG04	3	7.0	85.6
Rand1	5	5.2	74.0
Rand2	5	4.8	81.4
Rand3	5	4.2	78.4
Rand4	4	6.0	74.5
Rand5	4	6.3	72.0
Rand6	4	5.8	75.5
Rand7	5	4.6	85.0
Rand8	4	6.0	80.3
Rand9	5	5.2	78.0
Rand10	5	4.6	85.0
<i>p</i>	0.000	0.000	0.000

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Table 4.8

Comparison of polydomy analysis between sectors Cors Goch Grid 03 (CGG03) and Cors Goch Grid 04 (CGG04), expressed as area sampled, number of nests sampled (N_{TOTAL}), the frequency (percentage) of all possible nest pairings that are con-colonial (F_{CON}), the number of putative colonies revealed (N_{COLONY}) and the mean number of nests per colony (N_{NESTS}).

	Sector	
	CGG03	CGG04
Area	56 m ²	81 m ²
N_{TOTAL}	15	26
F_{CON}	29%	34%
N_{COLONY}	3	3
N_{NESTS}	4.3	7.0

Table 4.9

Nucleotide substitutions, in the mitochondrial Cytochrome b gene region, between *F. picea* individuals sampled from the UK, Sweden and Finland. Sequences deposited on GenBank by Goropashnaya (2003). Details of substitution given as the number of nucleotides from the start of the sequence deposited on GenBank (Position), the codon in which the substitution takes place, the amino acid (AA) coded for by the codon, and the region of mitochondrial DNA in which the substitution takes place. NC = non-coding, and is given for both AA and Region when the substitution is within a non-coding region of mitochondrial DNA.

Population	Nucleotide differences				GenBank accession number
	1	2	3	4	
	Position/Codon/ AA/Region	Position/Codon/ AA/Region	Position/Codon/ AA/Region	Position/Codon/ AA/Region	
UK	10/AAG/Lys/ND6	312/ATA/NC/NC	755/AAT/Asn/Cyt b	1466/ATC/NC/NC	AY786145
Sweden	10/AAG/Lys/ND6	312/ACA/NC/NC	755/AAT/Asn/Cyt b	1466/ATC/NC/NC	AY786146
Finland	10/AAA/Lys/ND6	312/ACA/NC/NC	755/AAC/Asn/Cyt b	1466/ATA/NC/NC	AY786144

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Figure 4.1

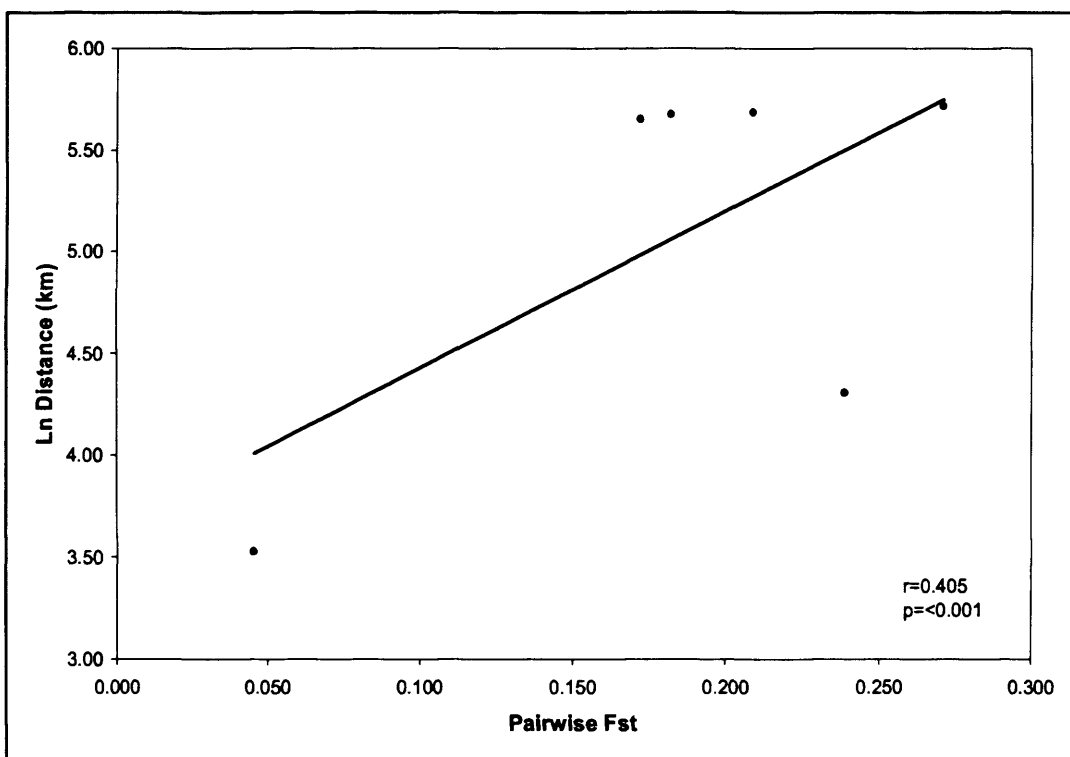
Example of a matrix of con-coloniality, displaying 'barcode'-like identification. Data shown is from Cors Goch Grid 03 nests, prior to sorting according to the number of con-colonial nest pairs shared with nest X (see Statistical Methods). Filled cells denote con-colonial nest pairs.

	CGG 41	CGG 42	CGG 43	CGG 44	CGG 45	CGG 46	CGG 48	CGG 49	CGG 50	CGG 51	CGG 52	CGG 85	CGG 86	CGG 87	CGG 101
CGG 41	■										■				■
CGG 42		■			■		■	■		■					■
CGG 43			■												■
CGG 44				■					■			■	■		
CGG 45		■			■	■	■	■		■	■				■
CGG 46					■										■
CGG 48		■			■		■			■					■
CGG 49		■			■			■		■					■
CGG 50				■					■			■	■		
CGG 51		■			■		■	■		■	■				■
CGG 52	■				■			■		■					■
CGG 65				■					■			■	■		
CGG 86				■					■			■	■		
CGG 87			■											■	■
CGG 101	■				■	■	■	■		■	■				■

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Figure 4.2

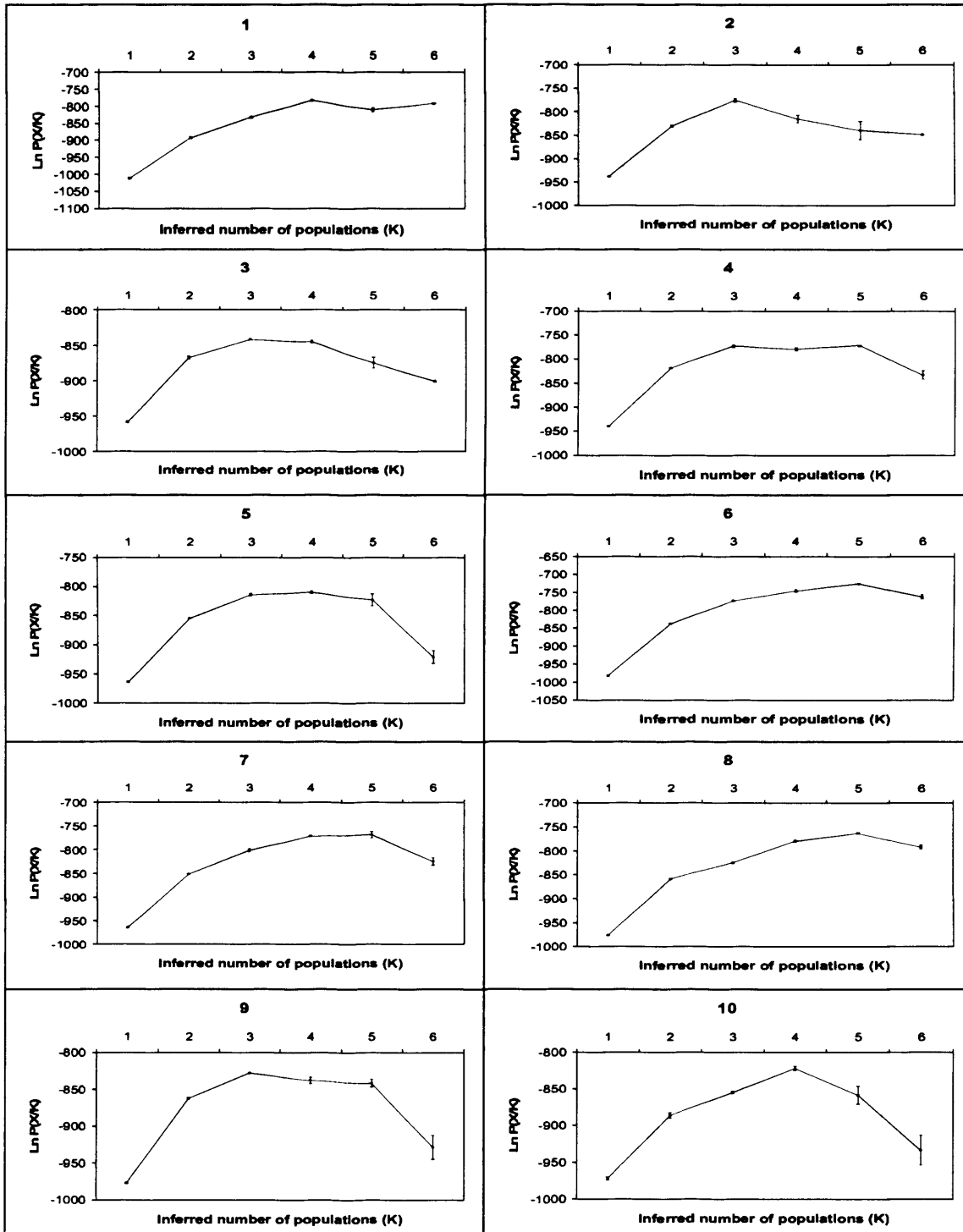
Analysis of isolation by distance between all populations (Cors Goch, Rhossili Down, the New Forest and Hartland Moor). Mean F_{ST} estimates between all population pairs are plotted against the log.-transformed distance between them. Significance of the correlation deduced using a Mantel test (Mantel 1967) implemented in Microsoft Excel with the POPTOOLS version 2.6 add-in (Hood 2005).



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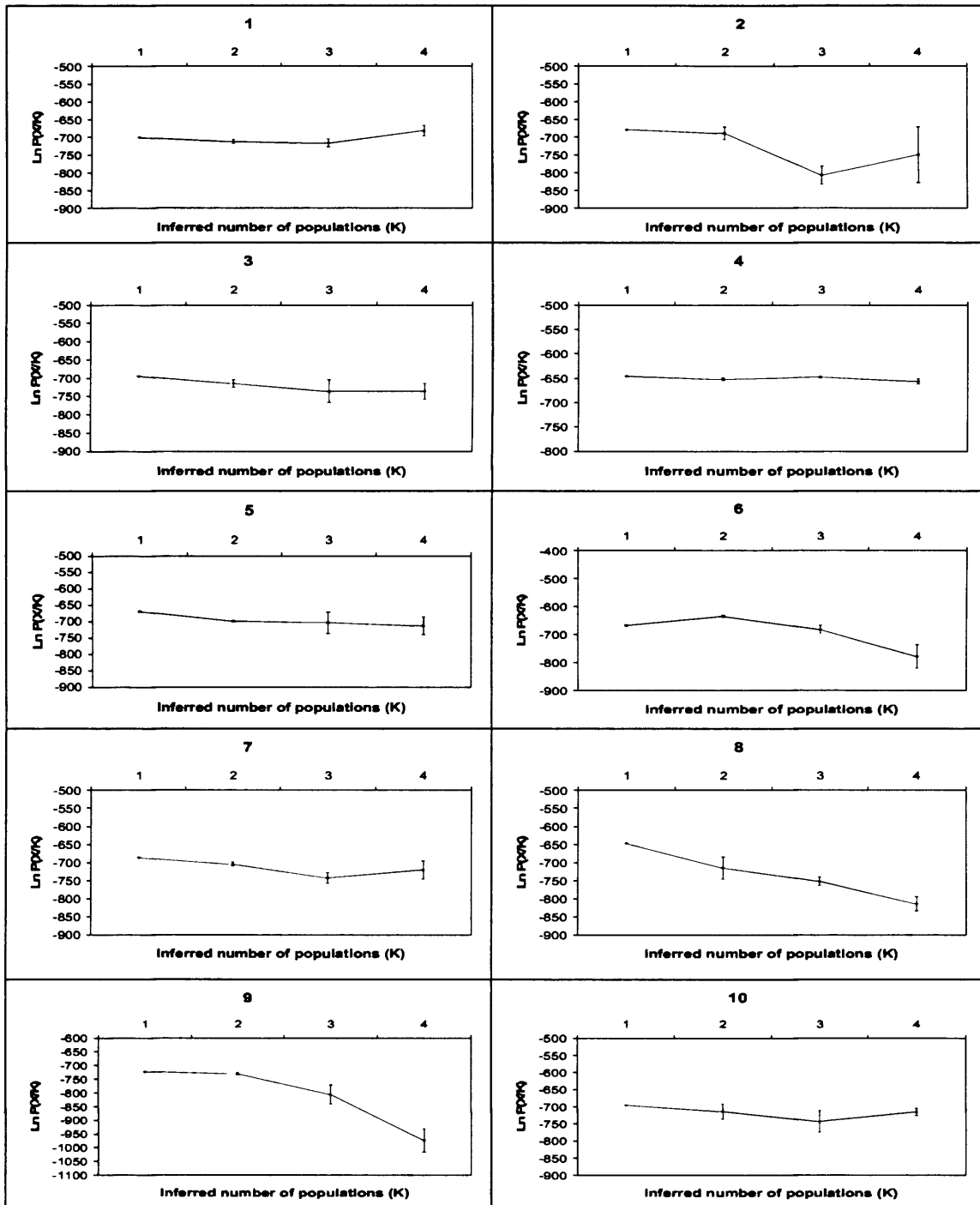
Figure 4.3

Genetic structure of populations (Cors Goch, Rhossili Down, the New Forest and Hartland Moor). Putative number of populations (K) plotted against the likelihood of that value for K , given the observed multi-locus genotypes X ($\ln \Pr(X/K)$); results obtained from the program STRUCTURE version 2 (Pritchard et al. 2000). Error bars represent ± 1 SE, calculated over 3 repeated runs for each putative value of K . Number at the top centre of each graph represents the re-sampled data set used.



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Figure 4.4

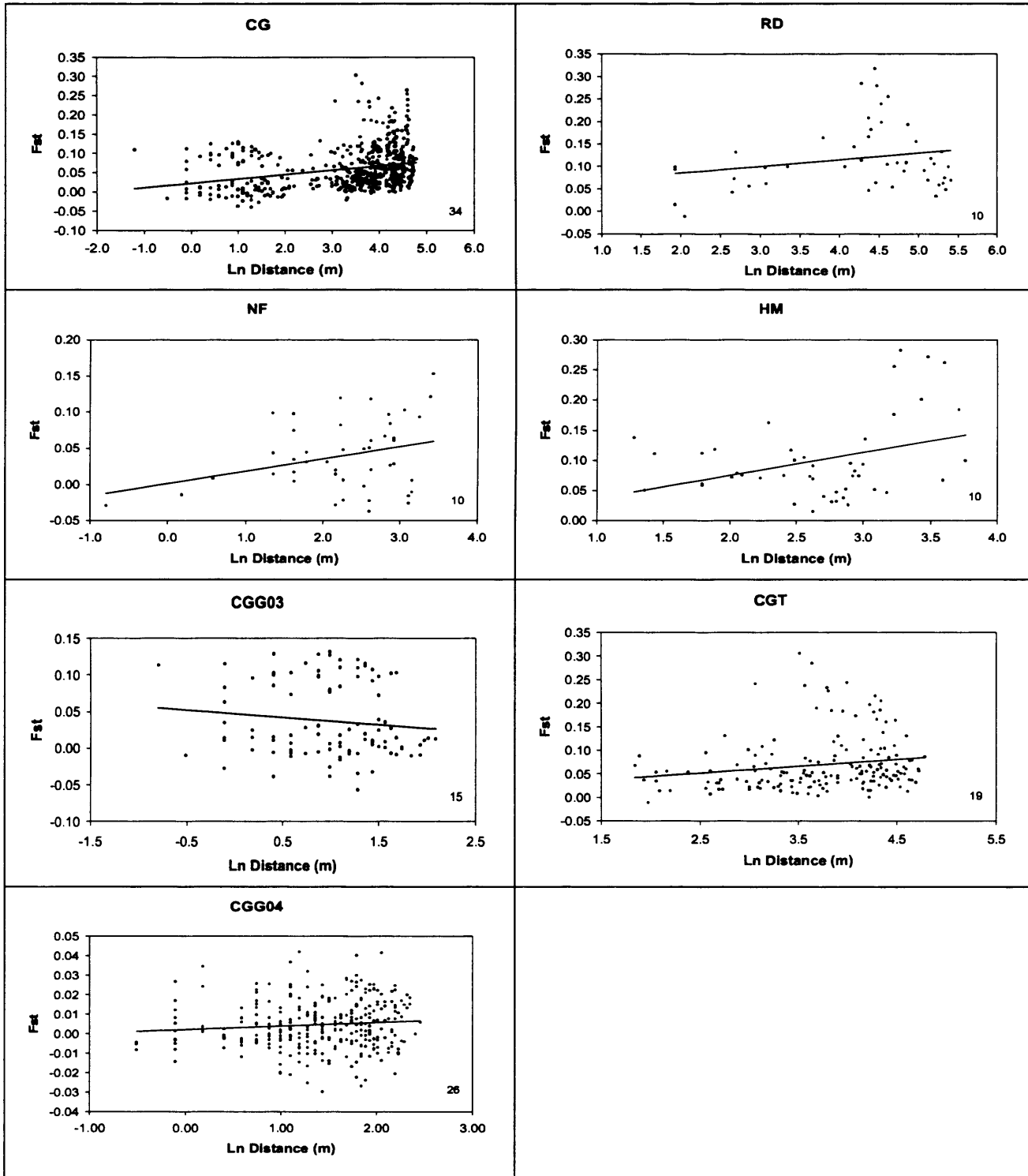
Genetic structure of population Cors Goch and its component sectors, within and between years (using individuals from Cors Goch Transect, Cors Goch Grid 03 and Cors Goch Grid 04). Putative number of populations (K) plotted against the likelihood of that value for K , given the observed multi-locus genotypes X ($\ln \Pr(X/K)$); results obtained from the program STRUCTURE version 2 (Pritchard et al. 2000). Error bars represent ± 1 SE, calculated over 3 repeated runs for each putative value of K . Number at the top centre of each graph represents the re-sampled data set used.



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Figure 4.5

Analysis of isolation by distance within all populations and sectors. CG = Cors Goch (comprising CGG03 and CGT), RD = Rhossili Down, NF = New Forest, HM = Hartland Moor, CGG03 = Cors Goch Grid 03, CGT = Cors Goch Transect, CGG04 = Cors Goch Grid 04. Numbers within chart refer to sample size (number of nests per population/sector).



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Figure 4.6

Successfully sequenced 972 bp of the Cytochrome Oxidase subunit 1 mitochondrial gene, from 4 UK populations of *F. picea* (Cors Goch Llanllwch (CG), Rhossili Down (RD), the New Forest (NF) and Hartland Moor (HM)) and from a related species of ant (*F. cunicularia*; (Hasegawa et al. 2002)). The start codon for all sequences is ATT, coding for the amino acid Isoleucine. Only one nucleotide substitution (at position 582 from the start of the sequence) revealed between English and Welsh *F. picea* populations; GCC (English populations) to GCT (Welsh populations) both codons coding for the amino acid Alanine (codon highlighted in sequence).

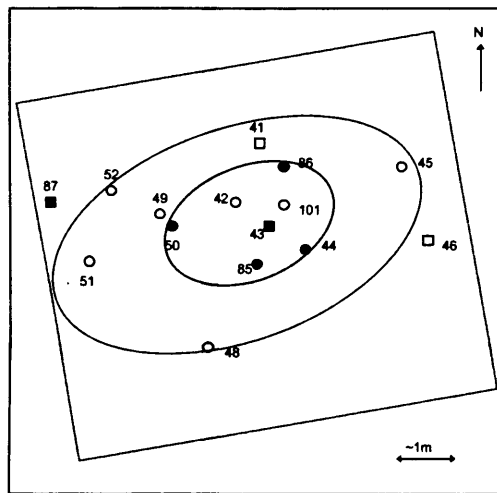
	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160
F.picea CG	attcctttaa	tattaggatc	tcagacata	gcttatcttc	gtataataa	tataagatt	tgacttttac	ccctctctat	cactctttaa	cttttaaga	atthtatta	tgatggtaca	ggaacaggat	gaactattta	cccccttta	gcttcaata
F.picea RD
F.picea NF
F.picea HM
F.cuniculariac.....c.t.g.....gc.....c.....g.....c.....
	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320
F.picea CG	tttttcataa	tggtccttct	gtagacttaa	caatttttcc	tottctattt	gcaggtatat	cctcaatttt	aggagcaatt	aatttttatt	caacaattct	taataacat	cataaaaatt	ttctatcga	tanaattccc	ttacttgat	gatcaattt
F.picea RD
F.picea NF
F.picea HM
F.cunicularia	a.....	a.a.g.....g.....g.....a.....g.....t.....tc.....g.....
	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480
F.picea CG	aatcacagct	atcttacttc	tattatctct	accagtttta	gcaggagcta	tcactatact	attaactgat	cgaattttaa	atcttctatt	ttttgatccc	tcaggagggg	gcgatccaat	tttatatcaa	catttatttt	gattttttgg	ccatccaga
F.picea RD
F.picea NF
F.picea HM
F.cunicularia	..ga.....gaa.....a.ct.....t.....t.....t.....t.....c.....
	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640
F.picea CG	gtatatattt	taattttacc	aggatttggg	ttaattttcc	atattttat	aatgaaaga	ggaaaaaag	aaacttttgg	agcttttagg	ataatttatg	gctttatagc	aattggattt	ttaggatttg	ttggttgagc	tcactatata	ttacaattg
F.picea RD
F.picea NF
F.picea HM
F.cunicularia	..t.....t.....
	650	660	670	680	690	700	710	720	730	740	750	760	770	780	790	800
F.picea CG	gtttagatgt	tgatacacga	gcttatttta	cttctgcaac	tatgattatt	gctatcccta	ccggaattaa	aatttttaga	tgaattacaa	ctctacatgg	aacaaaaatc	aataaatt	catctcttgg	atgatctata	ggatttattt	ttttattac
F.picea RD
F.picea NF
F.picea HM
F.cunicularia	..c.....c.....
	810	820	830	840	850	860	870	880	890	900	910	920	930	940	950	960
F.picea CG	tataggagga	ttaaccggtg	taactatttc	taattcatca	atgatatta	ttttcatgga	tacatattat	gtttagcttc	atttccatta	tgttttatct	ataggagcgg	tttttgcatt	tattgcaagt	tttatctatt	gatttccttt	ataactgga
F.picea RD
F.picea NF
F.picea HM
F.cunicularia	a.....g.....t.a.....
	970															
F.picea CG	ttctcattaa	at														
F.picea RD														
F.picea NF														
F.picea HM														
F.cuniculariaa.....														

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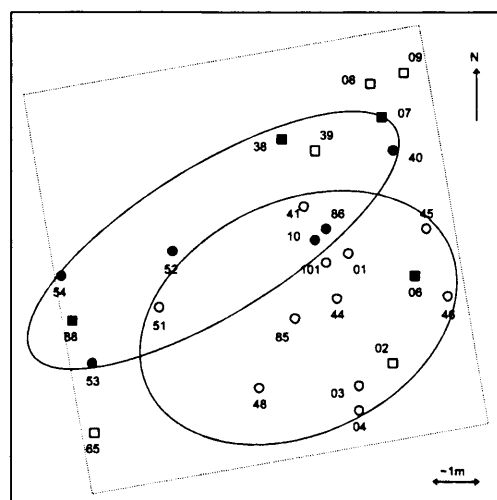
Figure 4.7

Putative polydomous colonies in (a) Cors Goch Grid 03 and (b) Cors Goch Grid 04, revealed by genetic analysis of con-coloniality. ○ = colony A, ● = colony B, ■ = colony C and □ = nests not assigned to a colony. Colony boundaries of colonies A and B represented by ovals. Colony boundaries of colony C not shown for visual simplicity. Dotted, tilted rectangles represent the areas sampled. A number of nests appear to change colonies between years. This may be due to the recruitment of queens into a nest from a different polydomous colony, or it may reflect a lack of precision in the technique used to detect putative polydomous colony boundaries.

(a)



(b)



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Discussion

In the current study I have conducted the first integrated research on the conservation genetics and ecology of a UK ant species. Throughout its range, *Formica picea* is a little-studied species. The only previous genetic investigations conducted on *F. picea* (Pamilo 1982a; Pamilo 1982b; Pamilo 1983), carried out before the advent of highly polymorphic microsatellite markers, were restricted to the use of relatively invariable allozyme markers. This precluded a detailed analysis of the social and genetic structure of colonies and populations, as presented here. In addition, work on the ecological requirements of *F. picea* is absent from published literature, with the exception of a recent study by Mabelis and Chardon (2005). However, this study analyses the fragmentation of *F. picea* populations based on a pre-defined definition of habitat suitability, addresses between-site differences and does not address micro-geographical ecological parameters in the immediate vicinity of the nest. In my study micro-geographical ecological parameters are measured making no assumptions about the type of habitat that may be suitable for *F. picea*.

Scientific conclusions

Ecological parameters

Investigating the micro-geographic ecological parameters in the immediate vicinity of *F. picea* nests at Cors Goch Llanllwch, Carmarthenshire, revealed what initially appeared to be conflicting results (Chapter 2). Two plant

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species, Bell Heather (*Erica tetralix*) and Deer Grass (*Trichophorum cespitosum*), which seemed to contribute to a favourable nest site within the southern, inhabited area of the bog, were more prevalent in the northern area of the bog which appeared to be completely uninhabited by *F. picea*. *Erica tetralix* has been previously associated with *F. picea* (North 2000) and I frequently noted workers upon its flowers, presumably feeding upon nectar, whereas *T. cespitosum* may be a favoured material for the construction of solaria, although this has not been previously demonstrated. It was therefore surprising that an area with a high percentage cover of these species did not appear to contain *F. picea* nests. A possible explanation for the observed distribution of *F. picea* nests with respect to these two plant species is that another parameter, such as water table level, is preventing the colonisation of the northern section of the bog. This is supported by the findings, in 2003, that both nest-level relative humidity and vegetation height, two parameters that could feasibly be linked to the availability of water in the soil, were significantly lower in the northern, apparently uninhabited section of the bog, compared to the southern, inhabited section. In addition, sexual cocoons were only observed within solaria in 2004, which was noticeably wetter than 2003, reinforcing the suggestion that water level may be conducive to suitable habitat for *F. picea*.

In contrast to *E. tetralix* and *T. cespitosum*, Heather (*Calluna vulgaris*), which seemed to contribute to an unfavourable nest site within the inhabited southern section of the bog, was less prevalent in the apparently uninhabited northern section of the bog (Chapter 2). *Calluna vulgaris* is frequently associated with unsuitable habitat for *F. picea*, probably due to the shading of solaria proving detrimental to brood incubation (North 1998a). A link between *C. vulgaris* and shading is supported in the present study by the fact that the relationship between vegetational shading and site type followed the same pattern as did percentage cover of *C. vulgaris*; both of these parameters were greater in the southern section of the bog compared to the northern section

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and greater at inhabited area controls compared to nest sites, although this latter relationship narrowly missed significance for vegetational shading. However, if *C. vulgaris* produces shade detrimental to brood incubation, a logical assumption would be that this species would have a greater percentage cover in the northern, uninhabited section of the bog. The observed results, which contradict this assumption, make more biological sense combined with the observation that *C. vulgaris*, despite its apparent effect on solaria insolation, appears to be an important food source for *F. picea*. Workers of this species have been frequently noted upon the flowers of *C. vulgaris*, both in this study and previous investigations (North 1998b). It therefore seems likely that the presence of *C. vulgaris* in the immediate vicinity of *F. picea* nests provides too much shade for efficient brood incubation within the solaria, but that the presence of *C. vulgaris* in the general locality of the nest provides an important food source.

Results from the investigation of small scale ecological parameters suggest that *F. picea* has highly specific requirements, probably governed by the water table. In habitat where the water table level is acceptable, the occurrence of three plant species appear to contribute to favourable habitat for this species; high percentage cover of *E. tetralix* and *T. cespitosum* in the immediate vicinity of the nest and a low density of *C. vulgaris* in the local occupied area. *Erica tetralix* and *C. vulgaris* are sources of nectar for *F. picea*, whereas *T. cespitosum* may be a favoured material for constructing solaria. A recent study investigating the fragmentation of *F. picea* habitat in The Netherlands (Mabelis and Chardon 2005), which revealed that this species was present in only 33% of habitat patches assumed to be suitable, suggests that the ecological requirements of *F. picea* are highly specific and poorly understood throughout its range.

Chapter 5

Social and genetic structure of colonies and populations

i) Within-population socio-genetic structure

The use of highly polymorphic microsatellite markers in this study to analyse nestmate worker relatedness has revealed that UK populations of *F. picea* are highly polygynous, with many reproducing queens per colony (Chapter 3). This is a form of social structure common among *Formica* species, including *F. lugubris* (Gyllenstrand and Seppä 2003), *F. paralugubris* (Chapuisat et al. 1997), *F. exsecta* (Seppä et al. 2004), *F. truncorum* (Sundström 1993) *F. cinerea* (Lindstrom et al. 1996) and *F. aquilonia* (Maki-Petays et al. 2005). Sundstrom et al. (2005) have shown that inbreeding is associated with low relatedness throughout the genus *Formica*, a finding that is supported by results from the present study as two of the four populations studied showed significant levels of inbreeding (Chapter 3). Correcting nestmate worker relatedness values for inbreeding, which can artificially inflate relatedness estimates, showed that within-colony relatedness was not significantly greater than zero in any population. These same estimates were all significantly lower than 0.25, the lowest relatedness possible under monogyny with multiple mating, reinforcing the conclusion that polygyny is indeed present in all populations.

A lack of relatedness and aggression between nestmate workers, as seen between *F. picea* workers within Cors Goch (Chapter 4), suggests a social organisation termed unicoloniality, where individuals can be exchanged freely between nests (Hölldobler and Wilson 1990; Bourke and Franks 1995). Although not a common phenomenon, unicoloniality has been discovered in populations of two other *Formica* species, *F. paralugubris* (Chapuisat and Keller 1999) and *F. truncorum* (Elias et al. 2005). There are two main possible explanations for the lack of aggression between nestmate workers that is essential for the development of unicoloniality. The first is a loss of genetic

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variation at recognition cues (Chapman and Bourke 2001), so that individuals cannot distinguish between relative and non-relatives. This is frequently associated with invasive species, which often undergo a population bottleneck (Tsutsui et al. 2000). However, *F. picea* is not an invasive species, no population bottleneck was revealed in the Cors Goch population (Chapter 4) and a recent study has demonstrated that unicoloniality may be present, and aggression absent, even when individuals recognise non-relatives (Chapuisat et al. 2005). A more likely explanation for the lack of aggression seen between *F. picea* workers within Cors Goch, especially given this species' apparent specific ecological requirements (Chapter 2), is connected to habitat saturation (Chapman and Bourke 2001), which may also account for the presence of polygyny in the studied populations. When the cost of colony foundation is high, possibly due to a lack of available suitable habitat, new queens may instead be accepted back into the natal colony, increasing the level of polygyny. Colony reproduction by budding (Rosengren and Pamilo 1983) is selected for, with new nests being established a short distance away from the natal nest. If the exchange of individuals between natal and bud nests ceases, an independent, although genetically similar, colony is formed. However, if individuals continue to move freely between nests then polydomy arises, with colonies consisting of multiple nests (Hölldobler and Wilson 1990). Extremely high levels of polygyny and polydomy are thought to lead to unicoloniality. I have presented some evidence for the presence of polydomy within one sector of Cors Goch, further supporting the theory that this population is largely unicolonial.

The detailed analysis of social and genetic structure within Cors Goch revealed a degree of social polymorphism between two sectors of this population (Chapters 3 and 4). A permanent monitoring grid containing a high density of nests displayed polydomy and inbreeding-corrected nestmate worker relatedness values not significantly greater than zero. Outside this area nest density was noticeably lower, worker nestmate relatedness,

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although low, was significantly greater than zero, and there was no evidence of polydomy. Intra-specific social polymorphism is common within the genus *Formica* (Sundström 1993; Goropashnaya et al. 2001; Gyllenstrand and Seppa 2003; Seppä et al. 2004). At its most extreme, colonies within a single population can vary between monogyny and high levels of polygyny, as seen in a population of *F. selysi* (Chapuisat et al. 2004). The differences in social and genetic structure between the two sectors of Cors Goch could be caused by varying availability of suitable habitat. Although ecological parameters were not measured within the monitoring grid, the high density of nests suggests that this area represents a relatively large patch of suitable habitat for *F. picea*, enabling the foundation of bud nests in the immediate vicinity of the natal nest. If suitable habitat is more scarce and patchy in the area of the transect, this may prevent the formation of large aggregations of polydomous colonies. Whereas nestmate worker relatedness estimates for colonies within the permanent grid reflect the number of reproducing queens as well as individuals moving freely between nests of polydomous colonies, the lack of polydomy detected in the area of the transect suggests that here the same estimates may reflect only the level of polygyny. If this is the case then there may be no difference in the level of polygyny between the two sectors, the apparent social polymorphism being due to the exploitation of a relatively large patch of suitable habitat by colonies within the grid. The almost total lack of aggression between workers from both sectors of Cors Goch (Chapter 4) suggests that the exchange of workers between all nests and colonies is possible, so that the whole population approximates unicolonality. However, the current results suggest that patchy availability of suitable habitat (Chapter 2) and the relatively large distance between colonies in the area of the transect prevent such exchange in this sector. Given the relatively low nest densities observed within Rhossili Down, the New Forest and Hartland Moor, it is unlikely that colonies sampled within these populations are polydomous. It may be, however, that polydomous colonies exist elsewhere in these populations, but were not sampled. This may be especially true at Rhossili

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Down, where only a small part of the area occupied by *F. picea* was sampled (Orledge et al. 1998). As inbreeding-corrected nestmate worker relatedness estimates were not significantly greater than zero in any of these populations, it is possible that aggression between non-relatives is absent and that exchange of workers between colonies could take place, making these populations also potentially unicolonial.

ii) Between-population genetic structure and dispersal

Both analyses of population structure implemented in this study revealed that all populations were genetically distinct from one another, with the exception of the New Forest and Hartland Moor (Chapter 4). This result made geographical sense, as these populations were the physically closest pair. However, intra-population genetic analysis showed significant isolation by distance, whereby colonies are more genetically similar to physically closer colonies, within Cors Goch and the New Forest (Chapter 4). Both Rhossili Down and Hartland Moor showed similar trends, although these narrowly missed statistical significance. Isolation by distance within a population suggests colony reproduction by budding, as expected in a polygynous population, and poor dispersal, as even a small amount of gene flow within the population could homogenise allele frequencies and prevent its detection. Given the trend towards isolation by distance and the high level of polygyny shown, it is likely that all populations here studied display colony reproduction by budding and poor dispersal. Low dispersal ability seems to contradict the finding that the New Forest and Hartland Moor are not genetically differentiated from one another, as this would suggest a level of gene flow between the two populations. If dispersal is not great enough to prevent the detection of isolation by distance within a population, then it is highly unlikely that individuals would be able to disperse to any neighbouring population, especially one over 30 km distant - the approximate distance between the New Forest and Hartland Moor. Within this study it was not possible to

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partition gene flow by sex, as the maternally inherited mitochondrial marker investigated proved relatively invariable (Chapter 4). However, as microsatellites are nuclear markers, the observed intra-population isolation by distance suggests that both sexes have limited dispersal. This correlates with a study (Mabelis and Chardon 2005) showing that *F. picea* in The Netherlands has limited dispersal, as there was no correlation between the occupancy of a patch and the distance to the nearest occupied patch,.

A more likely explanation for the observed pattern of gene flow between these two populations is that there are, or have been in the recent past, other populations bridging the gap between the New Forest and Hartland Moor. However, given the apparently extremely low dispersal ability of *F. picea* within the UK, this may not be enough to promote gene flow between the two English populations. It is possible that, at some point in the recent past, the dispersal ability of *F. picea* was greater, enabling gene flow, via intervening populations, between the New Forest and Hartland Moor. The colonisation of the UK by *F. picea*, some time after the last glaciation, circa 10000 years ago, probably occurred via the land bridge which once connected the UK and what is now continental Europe. This event itself suggests that queens of *F. picea* within the UK once had a level of dispersal ability and given the often observed dispersal polymorphism within the ants (Sundström 1995), it is possible that males had even greater dispersal ability. An estimated 80% of lowland heath (including wet heath) and 94% of raised bog habitats within the UK have been lost over the last two centuries (www.ukbap.org.uk). If the current high level of polygyny has been promoted by the lack of available suitable habitat, then it is feasible that this social structure, along with an associated drop in dispersal ability, is a relatively recent occurrence.

Conservation conclusions

This study has shown that relatedness between *F. picea* nestmate workers is low, suggesting multiple queens per nest. Genetic structure is present within populations, detected over distances as small as any in previous studies of *Formica* ants (Sundstrom et al. 2005). Both of these factors suggest that *F. picea* has extremely limited dispersal, which has obvious conservation implications. If queens are unable to colonise new habitat then the current small number of populations may never increase. It also greatly increases the risk to UK populations of *F. picea*, as any detrimental change to currently occupied habitat would be more likely to result in population extinction rather than migration. The apparent specificity of ecological requirements in *F. picea* may also affect our ability to facilitate the persistence of populations, as the change from favourable to unfavourable habitat may appear relatively minor, remaining undetected without detailed ecological measurements. To further investigate these requirements within Cors Goch, a study of the water table, specifically a comparison between the southern, inhabited area and the northern, apparently uninhabited area would prove invaluable and may help to determine if this is the main ecological parameter preventing the latter area's colonisation.

A number of factors inherently associated with the social organisation of ants can increase the potential risk to their populations, including haplo-diploidy, worker sterility and complementary sex determination (discussed in Chapter 1). This latter factor means that inbreeding can lead to the production of diploid males, which are usually sterile and impose a genetic load upon the colony. Given that significant levels of inbreeding were revealed at Rhossili Down and Hartland Moor, it is possible that diploid males are produced in these two populations. As inbreeding within a population can increase the risk of extinction (Saccheri et al. 1998) even in species without the added complication of complementary sex determination, any production of diploid

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males at both Rhossili Down and Hartland Moor could potentially have a large negative effect upon the persistence of these populations.

Although the limited dispersal ability associated with polygyny could increase the extinction risk of a population as previously discussed, multiple reproducing queens within a single colony increases the effective population size relative to a monogynous population. The high level of polygyny displayed by all the studied populations would therefore be expected to equate to relatively large effective population sizes, especially in the densely colonised grid area of Cors Goch. However, the polydomy detected in this area confounds estimates of effective population size, as each nest cannot be treated as a separate colony. Sampling multiple nests of a polydomous colony is essentially sampling the same colony multiple times. Any estimate of effective queen number gained from genetic analyses of these samples applies to the whole colony, not each nest individually. For example, if a polydomous colony consists of 5 nests and genetic analyses reveal an effective queen number of 10, then this is the total number of effectively reproducing queens in the colony and each nest contains an average of 2 queens. However, if polydomy was absent and each nest was a separate colony then each colony would contain 10 queens, bringing the total number of effectively reproducing queens to 50. Although estimates of nestmate worker relatedness within the grid section at Cors Goch were not significantly greater than zero, suggesting a large number of reproducing queens, the effect of polydomy should be taken into account when making assumptions about the effective population size at Cors Goch and its long term stability. The density of nests observed at Rhossili Down, Hartland Moor and the New Forest (personal observation) make it unlikely that high levels of polydomy are present in these populations, where effective population size will correlate with the number of nests in a more linear fashion.

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In conclusion, despite the increased effective population size conferred by high levels of polygyny, limited dispersal, inbreeding, polydomy (within Cors Goch) and the specificity of ecological requirements suggest that *F. picea* populations within the UK are in need of active conservation management, in addition to the current monitoring. As discussed, analysis of the effect of water table level on the distribution of *F. picea* nests within Cors Goch would help to reveal whether, as seems likely, this is the main factor influencing its distribution within this population. It is possible that some populations of *F. picea* remain undiscovered, as evidenced by the recent discovery of this species at Bisley Ranges in Surrey (Baldock 2003), and further searching for these populations could play a key role in the conservation of *F. picea* within the UK. Given the lack of genetic differentiation between Hartland Moor and the New Forest, suggesting that intervening populations either currently exist or have done in the recent past, it may be worthwhile concentrating future searches in the area between these two populations. Relocation of colonies to apparently suitable habitat, following detailed ecological analysis, may also prove fruitful, as extremely limited dispersal would otherwise prevent colonisation of new habitat, even if it were suitable and in the vicinity of established populations. Any of the four populations studied should make suitable donor populations as long as the population size is large enough, although it may be prudent to try and match the habitat of the donor population as closely as possible to the recipient site. Relocation of colonies to other, genetically distinct current populations may also be useful to increase genetic diversity and counteract inbreeding, especially at Rhossili Down and Hartland Moor, where significant inbreeding was detected. The observed genetic differentiation between populations suggests that any population would be suitable as a donor population for Rhossili Down, but that the New Forest would not be suitable as a donor population for Hartland Moor as they are not genetically distinct. Given the lack of aggression observed at Cors Goch, even between workers from nests that are probably out of dispersal range, it seems unlikely that aggression between colonies will

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prove detrimental to *F. picea* relocation. The results of this study suggest that conservation action as mentioned above should be implemented. If currently occupied habitat is stable there may be little immediate threat of extinction to UK populations. Although significant levels of inbreeding were detected at Rhossili Down and Hartland Moor, these estimates were low. Combined with the relatively high levels of genetic diversity observed, this suggests that the studied populations are genetically healthy. If the number of *F. picea* sites within the UK are not increased by relocation of colonies, however, then the current small number of populations may never increase. Due to habitat specificity and poor dispersal ability, any future stochastic damage to currently occupied habitat could have devastating consequences for the persistence of *F. picea* within the UK.

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Appendix; Table of Genotypes

Appendix; Table of genotypes

Cors Goch Grid 03

Both alleles are shown for all six loci in all successfully genotyped individuals. Within parentheses in sample names, numbers refer to the nest name and letters refer to the individual sampled from that nest.

Sample	Loci											
	FE19		FE38		FE49		FE51		FL12		FE21	
CGG(41a)	180	182	122	138	108	116	104	104	106	110	206	208
CGG(41b)	182	182	138	144	108	108	104	104	104	104	206	206
CGG(41c)	182	182	130	130	108	108	104	104	104	106	206	208
CGG(41d)	182	182	122	130	108	108	104	104	104	106	206	208
CGG(41e)	182	182	130	142	108	108	104	104	104	110	206	208
CGG(41f)	182	182	128	138	108	108	104	104	106	106	206	208
CGG(41g)	182	182	078	138	108	116	104	104	104	106	206	208
CGG(41h)	180	182	122	138	108	116	104	106	106	110	206	208
CGG(41i)	182	182	122	136	108	108	104	104	106	110	208	208
CGG(41j)	182	182	122	136	108	108	104	104	106	110	206	208
CGG(41k)	182	182	128	128	108	108	104	106	100	110	208	208
CGG(41l)	182	182	078	128	108	108	104	104	104	104	206	208
CGG(41m)	182	182	078	128	108	108	104	104	104	110	206	206
CGG(41n)	182	182	122	124	108	108	104	106	106	110	208	208
CGG(41o)	182	182	078	078	108	108	104	104	104	104	206	208
CGG(41p)	182	182	122	130	108	116	104	106	104	104	208	208
CGG(41q)	182	182	130	138	108	108	104	104	106	110	206	208
CGG(41r)	182	182	130	130	108	108	104	104	106	110	208	208
CGG(41s)	182	182	128	130	108	116	104	106	104	106	206	208
CGG(41t)	182	182	138	140	108	108	104	104	104	110	206	206
CGG(41u)	182	182	128	130	108	108	104	106	104	110	206	208
CGG(41v)	182	182	128	130	108	108	104	104	110	110	206	208
CGG(41w)	182	182	122	138	108	108	104	106	104	106	206	208
CGG(41x)	182	182	078	128	108	116	104	104	104	110	208	208
CGG(41y)	182	182	078	128	108	108	104	104	110	110	208	208
CGG(42a)	182	182	078	134	108	116	102	104	098	110	206	206
CGG(42b)	182	182	122	136	108	108	104	104	106	110	206	208
CGG(42c)	182	182	124	138	108	108	104	106	106	110	208	208
CGG(42d)	182	182	078	130	108	108	104	104	110	110	206	206
CGG(42e)	182	182	078	142	108	108	104	106	106	110	206	208
CGG(42f)	182	182	078	140	108	116	104	104	106	106	206	206
CGG(42g)	182	182	078	142	108	108	104	104	104	106	206	208
CGG(42h)	182	182	078	142	108	108	104	104	110	110	206	208
CGG(42i)	182	182	130	138	108	108	102	102	098	102	208	208
CGG(42j)	182	182	078	122	108	108	104	106	106	110	208	208
CGG(42k)	182	182	130	136	108	108	104	104	104	110	206	208
CGG(42l)	182	182	078	130	108	116	104	104	104	104	208	208

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CGG(42m)	182	182	136	136	108	108	104	104	104	110	206	208
CGG(42n)	182	182	128	132	108	116	104	106	104	110	206	208
CGG(42o)	180	182	130	136	108	116	104	106	100	104	208	208
CGG(42p)	180	182	140	140	108	108	106	106	110	110	208	208
CGG(42q)	182	182	128	130	108	116	104	104	104	110	208	208
CGG(42r)	182	182	078	078	108	108	104	104	104	106	206	208
CGG(42s)	182	182	122	130	108	108	102	104	102	104	206	208
CGG(42t)	182	182	078	130	108	108	104	104	104	110	208	208
CGG(42u)	182	182	078	136	108	108	104	104	104	104	206	208
CGG(42v)	182	182	136	142	108	108	104	104	106	110	206	206
CGG(42w)	182	182	124	134	108	108	102	102	098	110	208	208
CGG(42x)	182	182	078	134	108	108	104	104	110	110	208	208
CGG(42y)	182	182	122	130	108	108	102	102	104	110	208	208
CGG(43a)	180	182	130	130	108	108	104	104	104	110	206	206
CGG(43b)	182	182	078	138	108	108	104	104	106	106	208	208
CGG(43c)	182	182	122	130	108	108	104	104	100	106	208	208
CGG(43d)	182	182	078	130	108	108	104	104	104	106	206	208
CGG(43e)	182	182	130	132	108	108	104	104	110	110	208	208
CGG(43f)	182	182	126	126	106	106	100	102	100	100	208	208
CGG(43g)	182	182	128	130	106	106	100	100	108	108	208	208
CGG(43h)	182	182	122	140	108	108	104	106	100	110	206	208
CGG(43i)	182	182	078	140	108	116	104	104	104	110	208	208
CGG(43j)	182	182	128	128	108	108	104	104	104	110	206	208
CGG(44a)	180	182	078	140	108	116	104	104	104	110	208	208
CGG(44b)	182	182	130	142	108	108	104	104	100	104	208	208
CGG(44c)	182	182	078	078	108	108	104	104	104	110	206	208
CGG(44d)	182	182	126	130	106	106	100	102	100	100	206	206
CGG(44e)	182	182	132	134	106	106	100	100	096	108	208	208
CGG(44f)	182	182	078	138	106	106	100	100	108	108	206	206
CGG(44g)	182	182	078	140	108	108	104	104	104	110	206	208
CGG(44h)	182	182	078	126	106	106	100	100	100	108	208	208
CGG(44i)	182	182	122	128	108	116	106	106	104	110	208	208
CGG(44j)	182	182	122	130	108	108	104	104	100	106	208	208
CGG(44l)	182	182	078	128	108	108	104	104	104	110	206	208
CGG(44m)	182	182	078	078	108	108	104	104	104	110	208	208
CGG(44n)	182	182	134	140	108	108	104	104	104	110	206	208
CGG(44o)	182	182	122	122	108	108	104	106	100	104	208	208
CGG(44p)	182	182	078	128	106	106	100	100	100	102	208	208
CGG(44q)	182	182	078	138	106	106	100	100	096	100	208	208
CGG(44r)	182	182	122	122	108	108	106	106	104	110	208	208
CGG(44s)	182	182	120	138	106	106	100	100	100	100	208	208
CGG(44t)	182	182	078	130	108	108	104	106	104	106	206	208
CGG(44u)	182	182	120	136	106	106	100	100	102	108	208	208
CGG(44v)	182	182	130	140	108	108	104	104	104	110	208	208
CGG(44w)	182	182	078	078	108	108	104	104	104	110	206	208
CGG(44x)	180	182	122	132	108	108	104	104	104	104	206	206
CGG(44y)	182	182	126	138	106	106	100	100	100	100	206	208
CGG(45a)	182	182	078	078	108	108	104	104	104	104	206	208
CGG(45b)	182	182	078	136	108	108	104	104	104	104	206	208

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CGG(45c)	182	182	130	130	108	108	104	104	104	110	208	208
CGG(45d)	182	182	078	140	108	108	104	104	110	110	206	208
CGG(45e)	182	182	138	140	108	108	104	104	104	104	206	208
CGG(45f)	182	182	078	130	108	108	104	106	106	106	206	206
CGG(45g)	182	182	122	130	108	108	102	104	098	102	208	208
CGG(45h)	182	182	128	140	108	108	102	102	102	110	206	208
CGG(45i)	182	182	136	138	108	116	104	106	104	106	206	208
CGG(45j)	182	182	134	140	108	108	104	104	110	110	206	208
CGG(45k)	182	182	130	136	108	108	104	104	104	104	208	208
CGG(45l)	180	182	128	128	108	108	104	106	104	104	208	208
CGG(45m)	182	182	132	140	108	116	104	104	106	110	206	208
CGG(45n)	182	182	128	138	108	108	104	104	106	106	206	206
CGG(45o)	182	182	078	078	108	108	104	106	110	110	206	208
CGG(45p)	182	182	078	122	108	108	104	104	104	110	208	208
CGG(45q)	182	182	078	078	108	108	104	104	104	110	206	208
CGG(45r)	182	182	078	138	108	116	104	104	104	104	206	206
CGG(45s)	182	182	078	142	108	116	102	102	098	098	206	208
CGG(45t)	182	182	078	078	108	108	104	104	104	106	206	208
CGG(45u)	182	182	124	130	108	108	104	104	100	106	208	208
CGG(45v)	182	182	130	130	108	116	104	104	104	104	208	208
CGG(45w)	182	182	122	136	108	108	104	104	104	110	208	208
CGG(45x)	182	182	130	130	108	108	104	104	110	110	206	208
CGG(45y)	182	182	078	078	108	108	106	106	104	106	208	208
CGG(46a)	182	182	122	142	108	108	104	104	110	110	208	208
CGG(46b)	182	182	128	130	108	108	104	106	104	104	208	208
CGG(46c)	182	182	078	130	108	108	104	104	104	106	206	208
CGG(46d)	182	182	078	128	108	108	104	104	104	110	206	208
CGG(46e)	182	182	078	130	108	108	104	104	104	106	206	208
CGG(46f)	182	182	128	128	108	108	102	104	098	110	208	208
CGG(46h)	182	182	078	130	108	108	102	102	104	110	206	208
CGG(46i)	182	182	078	130	108	116	102	102	098	104	208	208
CGG(46j)	182	182	078	128	108	108	104	104	104	104	206	208
CGG(46k)	182	182	128	130	108	116	104	106	106	110	206	208
CGG(46l)	182	182	138	144	108	108	104	104	104	104	206	206
CGG(46m)	182	182	130	140	108	108	104	106	104	106	206	208
CGG(46n)	182	182	120	130	108	108	104	104	104	106	206	208
CGG(46o)	182	182	078	130	108	116	104	106	106	110	206	208
CGG(46p)	182	182	128	134	108	108	104	104	106	110	208	208
CGG(46q)	182	182	128	130	108	108	104	104	104	106	206	208
CGG(46r)	182	182	078	120	108	108	102	102	104	110	208	208
CGG(46s)	182	182	078	138	108	116	102	102	098	104	206	206
CGG(46t)	182	182	140	142	108	116	104	106	104	106	206	208
CGG(46u)	182	182	136	138	108	108	104	106	104	106	208	208
CGG(46y)	182	182	140	142	108	108	104	104	104	104	206	206
CGG(48a)	180	182	078	122	108	116	104	104	104	110	208	208
CGG(48e)	182	182	078	142	108	108	104	106	104	110	206	208
CGG(48f)	182	182	078	130	108	108	104	104	104	110	208	208
CGG(48g)	182	182	078	138	116	116	104	104	110	110	206	206
CGG(48h)	182	182	130	136	108	108	102	104	102	110	206	208

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CGG(48i)	182	182	122	136	108	108	104	104	104	106	208	208
CGG(48j)	182	182	134	136	108	108	104	104	104	110	206	208
CGG(48k)	182	182	128	142	108	108	104	104	106	110	208	208
CGG(48l)	182	182	078	138	108	108	104	104	110	110	208	208
CGG(48m)	182	182	122	144	108	108	104	104	104	110	208	208
CGG(48n)	182	182	130	140	108	108	104	104	110	110	206	208
CGG(48o)	182	182	078	078	108	108	104	104	104	104	206	208
CGG(48p)	180	182	136	138	108	116	104	106	106	106	206	208
CGG(48q)	180	180	128	136	108	108	106	106	110	110	208	208
CGG(48r)	182	182	130	132	108	108	104	104	110	110	206	208
CGG(48s)	182	182	078	122	108	108	104	104	104	106	206	208
CGG(48t)	182	182	078	134	108	108	104	104	104	110	208	208
CGG(48u)	182	182	122	128	108	108	104	104	104	106	206	206
CGG(48v)	182	182	136	138	108	108	104	104	104	104	208	208
CGG(48w)	182	182	078	138	108	108	104	104	104	110	208	208
CGG(48x)	182	182	132	134	108	116	104	104	110	110	208	208
CGG(48y)	182	182	130	140	108	108	102	102	098	104	208	208
CGG(49a)	182	182	078	138	108	108	104	104	104	110	206	208
CGG(49b)	182	182	122	130	108	108	104	104	104	106	206	208
CGG(49c)	182	182	078	128	108	108	104	104	104	106	206	208
CGG(49d)	182	182	128	138	108	116	104	104	104	106	206	206
CGG(49e)	182	182	078	130	108	116	102	102	104	104	208	208
CGG(49f)	182	182	122	140	108	108	104	106	104	104	206	208
CGG(49g)	182	182	128	130	108	108	104	104	104	106	206	206
CGG(49h)	182	182	078	124	108	108	104	104	104	110	208	208
CGG(49i)	182	182	128	132	108	108	106	106	106	110	208	208
CGG(49j)	182	182	130	142	108	108	104	106	104	110	208	208
CGG(49k)	182	182	124	142	108	108	104	104	104	106	208	208
CGG(49l)	182	182	078	120	108	108	104	104	104	110	208	208
CGG(49m)	182	182	078	138	108	116	104	106	106	110	206	208
CGG(49n)	182	182	078	142	108	108	104	106	104	110	206	208
CGG(49o)	182	182	078	078	108	108	104	104	104	110	206	208
CGG(49p)	182	182	078	078	108	108	104	106	104	104	208	208
CGG(49q)	182	182	078	128	108	108	104	104	104	104	206	206
CGG(49r)	180	182	140	146	108	108	106	106	104	110	206	208
CGG(49s)	182	182	078	130	108	108	104	104	106	110	206	208
CGG(49t)	182	182	078	130	108	108	104	104	104	110	208	208
CGG(49u)	182	182	140	142	108	108	104	104	106	110	206	208
CGG(49v)	182	182	130	142	108	108	104	104	104	104	208	208
CGG(49w)	182	182	078	138	108	108	104	104	106	110	208	208
CGG(49x)	182	182	122	128	108	108	104	106	104	110	208	208
CGG(49y)	182	182	128	138	108	108	104	104	104	110	208	208
CGG(50a)	182	182	078	130	108	116	104	104	106	106	208	208
CGG(50b)	182	182	124	134	108	108	104	106	104	110	208	208
CGG(50c)	182	182	132	140	106	114	100	100	100	108	208	208
CGG(50d)	182	182	078	138	106	114	100	100	100	102	206	208
CGG(50e)	182	182	078	130	106	106	100	100	100	100	208	208
CGG(50f)	182	182	078	120	106	106	100	100	108	108	206	208
CGG(50g)	182	182	078	122	108	108	104	104	104	106	206	208

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CGG(50h)	182	182	078	142	108	108	106	106	106	110	206	208
CGG(50i)	182	182	078	130	108	116	104	104	100	106	208	208
CGG(50j)	182	182	078	138	106	106	100	100	100	100	208	208
CGG(50k)	182	182	078	138	108	108	104	104	104	104	208	208
CGG(50l)	182	182	078	140	108	108	104	104	104	104	206	208
CGG(51a)	182	182	078	136	108	108	104	104	104	110	206	206
CGG(51b)	182	182	122	130	108	108	104	104	104	104	208	208
CGG(51c)	182	182	078	128	108	108	104	106	106	110	208	208
CGG(51d)	182	182	078	138	108	108	104	104	106	110	208	208
CGG(51e)	182	182	078	130	108	108	104	106	106	110	206	206
CGG(51f)	180	182	132	142	108	108	104	104	110	110	206	208
CGG(51g)	182	182	078	130	108	108	104	104	104	104	206	206
CGG(51h)	182	182	078	132	108	108	104	104	104	110	208	208
CGG(51i)	182	182	128	130	108	108	104	104	104	110	208	208
CGG(51j)	182	182	078	140	108	108	102	104	104	110	206	208
CGG(51k)	182	182	122	140	108	108	104	104	104	106	206	208
CGG(51l)	180	182	136	144	108	108	104	106	110	110	208	208
CGG(51m)	182	182	128	128	108	108	104	104	104	110	206	206
CGG(51n)	180	182	000	000	108	108	104	104	104	110	206	208
CGG(51o)	182	182	078	130	108	108	106	106	104	104	208	208
CGG(51p)	182	182	078	130	108	116	104	104	104	104	208	208
CGG(51q)	182	182	078	078	108	116	104	104	110	110	208	208
CGG(51r)	182	182	078	138	108	108	104	104	106	110	208	208
CGG(51s)	182	182	078	142	108	108	104	104	104	104	206	206
CGG(51t)	182	182	128	130	108	108	104	104	106	110	206	208
CGG(51u)	182	182	078	142	106	106	100	100	100	100	206	208
CGG(51v)	182	182	078	128	108	108	104	106	106	110	208	208
CGG(51w)	182	182	138	142	108	108	104	104	104	106	206	208
CGG(51x)	182	182	138	140	108	108	104	104	104	110	208	208
CGG(51y)	182	182	078	138	108	108	104	106	106	110	208	208
CGG(52a)	182	182	078	128	108	108	104	104	104	110	206	208
CGG(52b)	182	182	078	128	108	108	104	104	110	110	208	208
CGG(52c)	182	182	130	142	108	108	104	104	104	110	206	208
CGG(52d)	182	182	138	142	108	108	104	104	104	104	206	208
CGG(52e)	182	182	136	142	108	108	104	104	104	106	206	208
CGG(52f)	182	182	122	128	108	116	104	104	104	110	206	208
CGG(52g)	182	182	130	132	108	108	106	106	106	110	206	206
CGG(52h)	182	182	128	130	108	108	104	104	104	110	206	208
CGG(52i)	182	182	128	140	108	108	104	104	106	110	208	208
CGG(52j)	182	182	078	140	108	108	104	104	104	106	206	206
CGG(52k)	182	182	078	130	108	108	104	104	102	102	208	208
CGG(52l)	182	182	078	120	108	108	104	104	104	110	206	208
CGG(52m)	182	182	078	130	108	108	104	104	104	104	208	208
CGG(52n)	182	182	078	138	116	116	104	104	104	110	206	208
CGG(52o)	182	182	078	130	108	116	104	106	104	110	206	206
CGG(52p)	182	182	136	138	108	116	104	106	104	104	206	208
CGG(52q)	182	182	078	130	108	108	104	104	104	110	206	208
CGG(85a)	182	182	132	138	106	106	100	102	100	108	206	206
CGG(85b)	182	182	078	130	108	116	104	104	106	110	208	208

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CGG(85c)	182	182	120	128	106	106	100	100	100	102	206	208
CGG(85d)	182	182	126	142	106	106	100	100	102	108	206	208
CGG(85e)	182	182	120	128	106	106	100	100	096	102	208	208
CGG(85f)	182	182	078	130	108	116	104	104	104	106	206	208
CGG(85g)	180	182	078	136	108	116	104	104	104	110	208	208
CGG(85h)	182	182	122	136	108	108	104	104	106	110	208	208
CGG(85i)	182	182	138	140	108	108	104	104	104	106	208	208
CGG(85j)	182	182	138	140	108	116	104	104	104	110	206	208
CGG(85k)	182	182	138	138	108	116	104	104	106	110	206	206
CGG(85l)	182	182	078	078	108	108	104	104	104	110	208	208
CGG(85m)	182	182	078	078	106	106	100	100	100	108	206	208
CGG(85n)	182	182	078	142	108	116	104	106	104	110	208	208
CGG(85o)	182	182	130	132	108	108	104	104	110	110	208	208
CGG(85p)	182	182	078	128	108	116	104	106	104	110	208	208
CGG(85q)	180	180	078	078	108	108	104	104	104	110	208	208
CGG(85r)	182	182	128	142	106	106	100	100	100	108	206	208
CGG(85s)	180	182	120	128	106	106	100	100	100	100	206	208
CGG(85t)	182	182	078	138	106	106	100	100	096	108	206	206
CGG(85u)	182	182	136	138	106	114	100	102	100	100	206	208
CGG(85v)	182	182	078	122	106	106	100	100	100	108	208	208
CGG(85w)	182	182	078	126	106	106	100	100	108	108	206	208
CGG(85x)	182	182	122	130	108	116	104	106	100	104	208	208
CGG(86a)	182	182	122	130	108	108	106	106	106	110	208	208
CGG(86b)	182	182	128	138	108	116	104	106	110	110	206	206
CGG(86c)	182	182	134	140	106	106	100	108	108	108	208	208
CGG(86d)	182	182	078	126	106	106	100	100	102	108	208	208
CGG(86e)	180	182	124	128	108	108	104	104	104	104	208	208
CGG(86f)	182	182	078	128	106	106	100	100	100	108	206	208
CGG(86g)	182	182	124	142	108	108	104	104	100	104	208	208
CGG(86h)	180	182	140	140	108	108	106	106	110	110	206	208
CGG(86i)	180	182	128	138	108	108	104	104	104	106	208	208
CGG(86j)	182	182	132	142	108	116	104	104	104	110	208	208
CGG(86k)	182	182	122	130	108	108	104	104	104	106	206	208
CGG(86l)	182	182	138	140	106	106	100	100	100	100	206	208
CGG(86m)	182	182	128	128	106	114	100	100	100	102	208	208
CGG(86n)	182	182	132	140	108	116	104	104	104	110	206	208
CGG(86o)	182	182	078	138	106	106	100	100	096	108	208	208
CGG(86p)	182	182	078	140	106	106	100	100	102	108	206	208
CGG(86q)	182	182	128	128	106	106	100	100	096	108	208	208
CGG(86r)	182	182	078	128	106	106	100	100	100	102	208	208
CGG(86s)	182	182	078	120	106	114	100	100	100	108	206	208
CGG(86t)	180	182	140	140	108	108	104	106	110	110	208	208
CGG(86u)	182	182	142	142	108	108	104	104	104	106	206	208
CGG(86v)	182	182	130	138	108	108	104	104	104	110	208	208
CGG(86w)	182	182	138	138	108	108	104	104	106	110	208	208
CGG(86x)	182	182	078	130	108	108	104	106	104	104	206	208
CGG(86y)	182	182	120	140	106	114	100	100	100	108	206	206
CGG(87a)	180	182	078	142	108	108	104	104	110	110	206	208
CGG(87b)	182	182	128	140	108	116	104	104	104	106	206	206

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CGG(87c)	182	182	134	138	108	116	104	106	104	106	206	208
CGG(87d)	182	182	130	130	108	108	104	106	104	106	208	208
CGG(87e)	182	182	140	142	108	108	104	104	106	110	206	208
CGG(87f)	182	182	078	140	106	114	100	100	102	108	208	208
CGG(87g)	182	182	078	134	108	108	104	106	104	104	206	208
CGG(87h)	182	182	078	138	108	108	104	104	104	110	206	208
CGG(87i)	182	182	122	128	108	108	104	104	106	110	206	208
CGG(87j)	182	182	078	130	108	108	104	106	100	106	208	208
CGG(87k)	182	182	128	138	108	108	106	106	104	104	208	208
CGG(87l)	182	182	122	122	108	108	104	104	110	110	206	208
CGG(87m)	182	182	130	130	108	108	104	104	104	110	208	208
CGG(87n)	182	182	128	140	106	106	100	100	100	108	206	208
CGG(87o)	182	182	126	128	106	106	100	100	100	102	206	208
CGG(87p)	182	182	128	136	106	106	100	100	100	108	206	208
CGG(87q)	182	182	078	078	108	116	104	104	104	104	206	208
CGG(87r)	182	182	136	136	108	108	104	104	104	104	206	206
CGG(87s)	182	182	126	140	106	114	100	100	100	108	206	206
CGG(87t)	182	182	130	138	108	108	104	104	104	106	208	208
CGG(87u)	182	182	078	130	108	108	104	104	104	110	208	208
CGG(87v)	182	182	142	142	108	108	104	104	104	104	206	208
CGG(87w)	182	182	078	128	108	108	106	106	104	104	206	206
CGG(87y)	182	182	078	078	108	108	104	106	110	110	206	208
CGG(101a)	182	182	000	000	108	108	104	106	110	110	206	208
CGG(101b)	182	182	130	130	108	116	104	104	110	110	206	208
CGG(101c)	182	182	078	124	108	108	104	104	104	106	208	208
CGG(101d)	182	182	000	000	108	116	104	104	104	106	208	208
CGG(101e)	182	182	128	140	108	108	104	104	104	110	206	206
CGG(101f)	182	182	078	138	108	116	104	104	104	110	206	208
CGG(101i)	180	182	078	130	108	108	104	104	104	104	206	208

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Cors Goch Transect

Both alleles are shown for all six loci in all successfully genotyped individuals. Within parentheses in sample names, numbers refer to the nest name and letters refer to the individual sampled from that nest.

Sample	Loci											
	FE19		FE38		FE49		FE51		FL12		FE21	
CGT(1A)	182	182	140	142	108	108	104	104	104	110	208	208
CGT(1B)	182	182	128	142	108	108	106	106	104	110	208	208
CGT(1C)	182	182	136	144	108	108	104	106	104	106	208	208
CGT(1D)	182	182	124	142	108	108	104	106	104	110	208	208
CGT(1E)	182	182	078	130	108	108	104	104	100	110	208	208
CGT(1F)	182	182	140	142	108	108	104	104	104	110	206	208
CGT(1G)	182	182	128	136	108	108	106	106	104	110	208	208
CGT(1H)	180	182	122	136	108	108	106	106	104	110	206	208
CGT(1H)	182	182	130	130	108	108	102	104	098	098	208	208
CGT(1I)	182	182	078	128	108	108	104	106	104	104	206	208
CGT(1J)	182	182	130	142	108	108	104	104	104	104	206	208
CGT(1K)	182	182	136	138	000	000	104	106	110	110	208	208
CGT(1L)	182	182	122	140	108	108	104	106	106	110	208	208
CGT(1M)	182	182	140	144	108	108	104	106	104	106	208	208
CGT(1N)	182	182	130	140	108	108	104	104	100	110	208	208
CGT(1O)	182	182	130	130	108	116	104	106	100	104	206	208
CGT(1Q)	182	182	078	128	108	108	104	106	104	106	206	208
CGT(1R)	182	182	130	144	108	108	104	106	104	110	208	208
CGT(1S)	182	182	130	130	108	108	104	104	100	104	206	208
CGT(1T)	182	182	078	130	108	108	104	104	110	110	208	208
CGT(1U)	182	182	140	142	108	108	104	104	104	110	208	208
CGT(1V)	182	182	078	130	108	108	104	104	100	110	208	208
CGT(1W)	182	182	140	144	108	108	104	106	106	110	208	208
CGT(1X)	182	182	140	144	108	108	104	106	104	106	208	208
CGT(1Y)	182	182	140	142	108	116	104	106	100	110	208	208
CGT(2A)	182	182	128	128	108	108	104	104	104	110	206	208
CGT(2B)	182	182	128	130	108	108	104	104	104	110	206	208
CGT(2C)	182	182	078	136	108	116	104	104	104	104	206	208
CGT(2D)	182	182	140	144	108	108	104	104	104	110	208	208
CGT(2E)	182	182	078	128	108	108	104	104	104	110	208	208
CGT(2F)	182	182	130	144	108	108	104	106	106	110	206	208
CGT(2G)	180	182	078	122	108	108	104	104	104	110	206	206
CGT(2H)	182	182	128	130	108	108	104	104	104	104	206	208
CGT(2I)	182	182	136	142	108	108	104	104	104	104	208	208
CGT(2J)	182	182	128	136	108	108	104	104	100	104	206	208
CGT(2K)	182	182	128	140	108	108	104	106	104	106	206	208
CGT(2L)	182	182	128	142	108	108	104	104	104	110	206	206

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CGT(2M)	182	182	078	142	108	108	104	104	104	104	208	208
CGT(2N)	182	182	130	130	108	108	104	106	104	106	206	208
CGT(2P)	182	182	130	144	108	108	104	106	104	104	208	208
CGT(2Q)	182	182	122	138	108	108	104	104	104	104	206	208
CGT(2R)	182	182	130	138	108	108	104	104	104	110	206	208
CGT(2S)	182	182	130	136	108	108	104	106	104	106	206	208
CGT(2T)	180	182	078	122	108	108	104	104	104	110	206	206
CGT(2U)	182	182	122	128	108	108	104	106	104	110	206	208
CGT(2V)	182	182	124	144	108	108	104	104	104	106	208	208
CGT(2W)	182	182	122	128	108	108	104	106	104	110	208	208
CGT(2X)	182	182	130	140	108	116	104	104	104	110	208	208
CGT(2Y)	180	182	136	136	108	116	104	104	104	104	206	208
CGT(3A)	180	182	078	122	108	108	104	106	104	104	206	208
CGT(3C)	180	182	078	144	108	108	104	106	104	104	208	208
CGT(3D)	182	182	136	144	108	108	104	104	100	104	208	208
CGT(3E)	180	182	078	124	108	108	104	104	104	106	208	208
CGT(3F)	182	182	128	140	108	108	104	106	104	104	208	208
CGT(3G)	182	182	078	136	108	108	104	104	104	106	206	208
CGT(3H)	182	182	078	130	108	108	104	106	104	104	206	208
CGT(3I)	182	182	130	136	108	116	104	106	104	104	208	208
CGT(3J)	182	182	128	130	108	108	104	106	104	110	206	208
CGT(3K)	182	182	078	144	108	108	104	106	104	104	208	208
CGT(3L)	182	182	078	130	108	108	104	104	104	110	208	208
CGT(3M)	180	182	078	130	000	000	106	106	106	110	208	208
CGT(3N)	182	182	130	140	108	108	106	106	104	110	208	208
CGT(3O)	000	000	124	136	108	108	104	104	104	106	208	208
CGT(3P)	182	182	128	136	108	108	104	104	104	104	208	208
CGT(3Q)	180	182	078	136	108	108	104	104	104	110	208	208
CGT(3R)	182	182	130	136	108	116	104	104	104	106	206	206
CGT(3S)	182	182	078	144	108	108	104	106	104	110	206	208
CGT(3T)	182	182	128	136	108	108	104	104	104	104	206	208
CGT(3U)	182	182	136	136	108	108	104	104	100	110	208	208
CGT(3V)	182	182	140	140	108	116	104	106	104	110	208	208
CGT(3W)	180	182	078	122	108	108	104	104	100	110	206	208
CGT(3X)	180	182	136	136	108	108	104	104	104	104	206	208
CGT(3Y)	182	182	128	128	108	108	104	106	104	104	206	208
CGT(4A)	182	182	130	140	108	108	104	104	104	106	208	208
CGT(4B)	182	182	130	136	108	108	104	106	104	110	208	208
CGT(4C)	182	182	130	130	108	108	104	104	110	110	208	208
CGT(4E)	182	182	122	128	108	108	104	106	104	106	208	208
CGT(4G)	180	182	128	140	000	000	104	104	104	104	000	000
CGT(4H)	182	182	128	130	108	108	104	106	100	100	206	208
CGT(4I)	182	182	130	130	108	108	104	106	110	110	208	208
CGT(4K)	182	182	122	144	108	108	104	106	100	106	208	208
CGT(4L)	000	000	078	130	108	116	104	104	104	104	206	208
CGT(4M)	000	000	128	130	000	000	104	106	100	110	206	208
CGT(4N)	182	182	130	140	108	116	104	106	104	110	208	208
CGT(4O)	182	182	124	128	108	108	104	106	104	110	208	208

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CGT(4P)	180	182	122	130	108	108	104	104	104	104	206	208
CGT(4Q)	182	182	078	122	108	108	104	104	104	110	208	208
CGT(4R)	182	182	122	140	108	116	104	104	104	110	208	208
CGT(4S)	182	182	132	136	108	116	104	104	104	104	206	208
CGT(4T)	180	182	078	130	108	108	104	104	104	110	208	208
CGT(4U)	182	182	124	136	108	108	104	106	104	110	208	208
CGT(4V)	182	182	130	136	108	108	104	106	104	106	206	208
CGT(4W)	182	182	078	138	108	108	104	104	104	110	206	208
CGT(4X)	182	182	078	136	108	110	104	106	100	104	206	208
CGT(4Y)	182	182	128	136	110	110	104	104	100	100	208	208
CGT(6A)	182	182	130	136	000	000	104	104	110	110	000	000
CGT(6B)	182	182	078	078	000	000	104	106	104	104	000	000
CGT(6C)	182	182	122	140	108	116	104	106	104	106	208	208
CGT(6D)	182	182	078	132	108	116	104	106	104	110	208	208
CGT(6F)	182	182	122	128	108	108	104	106	106	106	208	208
CGT(6G)	182	182	078	136	108	108	106	106	104	106	206	208
CGT(6H)	182	182	078	128	108	108	104	106	110	110	206	208
CGT(6I)	182	182	124	128	108	108	104	106	104	110	206	206
CGT(6J)	182	182	132	132	108	116	104	106	104	104	206	208
CGT(6K)	182	182	078	132	108	116	104	106	104	104	208	208
CGT(6L)	182	182	078	140	108	108	104	106	106	110	206	208
CGT(6M)	182	182	078	142	108	116	104	106	106	110	206	208
CGT(6N)	182	182	136	136	108	116	104	104	104	110	208	208
CGT(6O)	182	182	124	128	108	108	106	106	104	104	208	208
CGT(6P)	182	182	128	132	108	108	106	106	110	110	208	208
CGT(6Q)	182	182	130	136	108	108	104	104	110	110	208	208
CGT(6R)	182	182	128	132	108	108	106	106	110	110	206	208
CGT(6S)	180	182	078	144	108	108	104	106	104	104	206	208
CGT(6T)	182	182	128	132	108	108	104	106	104	110	208	208
CGT(6U)	182	182	122	140	108	116	104	106	104	106	208	208
CGT(6V)	182	182	128	150	106	108	106	106	100	110	206	208
CGT(6W)	182	182	122	140	108	116	104	106	104	106	208	208
CGT(6X)	182	182	128	136	108	108	104	106	110	110	206	208
CGT(6Y)	182	182	122	128	108	108	104	106	104	110	208	208
CGT(7B)	182	182	128	144	108	108	104	106	110	110	206	208
CGT(7E)	182	182	124	144	108	108	104	106	110	110	206	208
CGT(7G)	182	182	078	078	108	110	104	104	106	110	206	208
CGT(7H)	182	182	124	128	108	108	104	106	110	110	208	208
CGT(7I)	182	182	078	122	108	116	104	104	104	104	206	208
CGT(7J)	182	182	122	128	108	108	104	106	104	106	208	208
CGT(7K)	182	182	124	128	108	108	106	106	104	110	206	208
CGT(7L)	182	182	128	130	108	108	104	104	106	110	206	208
CGT(7M)	182	182	122	136	000	000	104	104	104	104	208	208
CGT(7P)	182	182	078	128	108	108	104	106	110	110	206	208
CGT(7Q)	182	182	132	140	108	116	104	106	104	104	206	208
CGT(7R)	182	182	128	128	108	108	104	106	104	110	208	208
CGT(7S)	182	182	078	136	108	108	104	106	104	104	206	208
CGT(7T)	182	182	078	144	108	116	104	106	104	110	206	208

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CGT(7U)	182	182	136	140	106	106	100	102	100	100	206	208
CGT(7V)	182	182	078	122	108	108	106	106	104	104	208	208
CGT(7W)	182	182	132	140	108	116	104	106	104	104	206	208
CGT(7X)	182	182	122	124	108	108	104	104	104	110	206	208
CGT(7Y)	180	182	078	132	108	108	106	106	104	106	206	208
CGT(8A)	182	182	122	136	108	108	104	104	104	110	208	208
CGT(8C)	182	182	128	140	108	108	104	106	106	110	208	208
CGT(8E)	182	182	078	142	108	108	104	104	100	104	206	208
CGT(8F)	182	182	128	140	108	116	104	106	104	104	208	208
CGT(8G)	182	182	122	132	106	106	100	102	100	100	206	208
CGT(8H)	182	182	124	138	108	108	106	106	104	110	208	208
CGT(8I)	182	182	122	144	108	108	104	106	104	104	208	208
CGT(8J)	182	182	128	128	108	116	106	106	104	104	208	208
CGT(8K)	182	182	078	136	108	108	104	106	104	110	208	208
CGT(8L)	182	182	124	130	108	108	104	106	104	106	208	208
CGT(8M)	182	182	078	128	108	108	104	104	104	104	206	208
CGT(8N)	182	182	124	130	108	108	104	106	104	106	208	208
CGT(8O)	182	182	128	144	108	116	106	106	104	104	208	208
CGT(8P)	182	182	122	144	108	108	106	106	104	104	208	208
CGT(8Q)	182	182	128	132	108	108	104	106	104	104	206	208
CGT(8R)	182	182	128	144	108	116	106	106	104	104	208	208
CGT(8S)	182	182	128	144	108	116	104	104	102	102	208	208
CGT(8T)	182	182	130	130	108	108	104	106	104	104	208	208
CGT(8U)	182	182	128	132	108	108	106	106	104	104	206	208
CGT(8V)	182	182	124	142	108	108	104	106	104	104	208	208
CGT(8W)	182	182	124	132	108	108	104	106	104	104	206	208
CGT(8X)	182	182	078	144	108	108	104	104	110	110	208	208
CGT(8Y)	182	182	122	122	108	116	104	104	110	110	208	208
CGT(9A)	182	182	078	144	108	108	106	106	104	110	000	000
CGT(9C)	180	182	130	140	108	108	104	106	104	106	206	208
CGT(9D)	182	182	078	130	108	108	106	106	104	110	206	208
CGT(9F)	180	182	124	130	108	108	104	106	104	106	206	208
CGT(9G)	180	182	130	140	108	108	104	106	106	106	206	208
CGT(9H)	180	182	130	140	108	108	104	106	106	106	206	208
CGT(9I)	182	182	078	140	108	108	104	106	106	110	206	208
CGT(9J)	182	182	130	140	108	108	104	106	106	106	206	208
CGT(9K)	180	182	130	140	108	108	104	106	106	106	206	208
CGT(9L)	180	182	136	144	108	108	104	106	104	110	208	208
CGT(9M)	182	182	078	136	108	108	104	106	106	110	206	208
CGT(9N)	000	000	078	128	000	000	104	106	104	110	000	000
CGT(9O)	182	182	078	140	108	108	104	106	104	110	206	208
CGT(9P)	180	180	136	144	108	108	104	106	104	110	208	208
CGT(9Q)	180	182	078	144	108	108	106	106	104	110	206	208
CGT(9R)	180	182	124	130	108	108	104	106	106	106	208	208
CGT(9S)	182	182	128	136	108	108	104	106	104	110	208	208
CGT(9T)	180	182	078	130	108	108	106	106	104	110	206	208
CGT(9U)	182	182	124	130	108	108	104	106	104	106	206	208
CGT(9V)	180	182	124	130	108	108	106	106	106	106	206	208

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CGT(9W)	182	182	130	140	108	108	104	106	106	106	206	208
CGT(9X)	182	182	124	130	108	108	104	106	106	106	206	208
CGT(9Y)	180	182	124	130	108	108	104	106	104	106	206	208
CGT(10A)	182	182	136	138	108	108	104	104	104	110	206	208
CGT(10C)	182	182	122	130	108	116	104	104	110	110	208	208
CGT(10D)	182	182	078	128	108	108	104	106	106	110	208	208
CGT(10E)	182	182	122	122	108	108	104	104	110	110	208	208
CGT(10F)	182	182	130	138	108	108	104	104	104	106	208	208
CGT(10G)	182	182	078	122	108	108	106	106	100	104	206	208
CGT(10H)	182	182	078	140	108	116	104	104	104	110	206	208
CGT(10I)	182	182	132	142	108	116	104	106	100	106	206	208
CGT(10J)	180	182	128	142	108	108	104	106	106	110	206	208
CGT(10K)	182	182	122	140	108	108	106	106	104	106	206	208
CGT(10L)	000	000	128	130	108	108	104	104	104	110	206	208
CGT(10M)	182	182	136	138	108	108	104	104	100	106	206	208
CGT(10N)	182	182	130	136	108	108	104	106	100	110	206	208
CGT(10O)	182	182	138	142	108	108	104	104	106	110	206	208
CGT(10P)	182	182	078	122	108	108	104	104	110	110	208	208
CGT(10Q)	182	182	140	142	108	116	104	104	106	110	208	208
CGT(10R)	182	182	138	142	108	108	104	104	110	110	206	208
CGT(10S)	182	182	122	130	108	116	104	104	104	110	208	208
CGT(10T)	182	182	138	142	108	108	104	104	106	110	206	208
CGT(10U)	182	182	142	142	108	108	104	104	106	110	206	208
CGT(10V)	182	182	138	142	108	108	104	104	106	110	206	208
CGT(10W)	182	182	138	142	108	108	104	104	106	110	206	208
CGT(10X)	182	182	078	140	108	108	104	106	106	106	206	208
CGT(10Y)	182	182	078	078	108	116	104	104	104	106	206	206
CGT(11A)	182	182	136	140	108	108	104	104	104	104	208	208
CGT(11B)	182	182	130	140	108	108	104	104	104	110	206	208
CGT(11C)	182	182	130	140	108	108	104	106	106	110	208	208
CGT(11D)	182	182	078	136	108	116	104	104	104	104	208	208
CGT(11E)	182	182	078	078	108	116	104	104	104	104	208	208
CGT(11F)	182	182	078	122	000	000	104	104	106	110	206	208
CGT(11G)	182	182	078	078	108	108	104	104	104	104	206	208
CGT(11H)	182	182	122	122	108	108	104	106	106	110	208	208
CGT(11I)	182	182	130	132	108	108	104	104	104	106	206	208
CGT(11J)	182	182	078	078	108	108	104	106	104	104	208	208
CGT(11K)	182	182	078	140	108	108	104	106	104	104	208	208
CGT(11L)	180	180	078	128	108	108	106	106	104	110	208	208
CGT(11M)	182	182	078	130	108	108	104	104	104	110	208	208
CGT(11N)	182	182	140	140	108	108	104	106	106	110	206	208
CGT(11O)	000	000	122	130	108	108	104	106	104	110	206	206
CGT(11P)	180	182	130	130	108	108	104	106	104	106	208	208
CGT(11Q)	182	182	140	140	108	108	104	106	104	110	208	208
CGT(11R)	182	182	130	142	108	116	104	104	106	110	206	208
CGT(11S)	182	182	078	130	108	108	104	104	104	110	208	208
CGT(11T)	182	182	078	128	108	108	104	104	104	110	206	208
CGT(11U)	182	182	122	122	108	108	104	106	106	110	206	206

Appendix; Table of Genotypes

CGT(11V)	182	182	122	134	108	108	106	106	100	106	206	208
CGT(11W)	182	182	078	140	108	108	104	104	104	104	208	208
CGT(11X)	180	182	128	130	108	108	104	106	106	110	208	208
CGT(12A)	182	182	078	130	108	108	104	104	106	110	206	208
CGT(12B)	182	182	078	140	108	108	104	106	104	106	208	208
CGT(12C)	182	182	078	078	108	108	104	106	104	106	208	208
CGT(12D)	182	182	122	142	108	108	104	106	104	104	208	208
CGT(12E)	182	182	078	130	108	116	104	104	106	110	206	206
CGT(12F)	182	182	078	140	108	108	104	104	106	106	206	208
CGT(12G)	182	182	078	130	108	116	106	106	106	110	208	208
CGT(12H)	182	182	078	078	108	116	104	106	104	106	206	208
CGT(12I)	182	182	140	142	108	108	104	106	104	104	206	208
CGT(12J)	182	182	130	140	108	108	104	106	104	106	208	208
CGT(12K)	182	182	124	140	108	108	106	106	104	104	206	208
CGT(12L)	182	182	078	140	108	108	104	104	104	106	206	208
CGT(12M)	000	000	122	130	108	108	104	106	104	110	206	206
CGT(12N)	182	182	130	130	000	000	104	106	106	110	000	000
CGT(12O)	182	182	078	130	108	108	104	104	110	110	206	206
CGT(12P)	182	182	130	140	108	108	104	106	104	110	206	206
CGT(12Q)	182	182	078	078	108	108	104	104	104	106	208	208
CGT(12R)	182	182	122	140	108	108	106	106	106	106	208	208
CGT(12S)	182	182	138	140	108	108	104	104	104	106	206	208
CGT(12T)	182	182	078	142	108	108	104	104	106	110	206	206
CGT(12U)	182	182	138	140	108	108	104	104	104	110	208	208
CGT(12V)	182	182	130	140	108	108	104	104	106	110	206	206
CGT(12W)	182	182	140	140	108	108	106	106	104	106	208	208
CGT(12X)	182	182	130	140	108	108	104	104	106	110	206	208
CGT(12Y)	182	182	130	140	108	108	106	106	106	110	206	208
CGT(13A)	182	182	130	138	108	108	104	104	100	110	206	208
CGT(13C)	180	182	078	136	000	000	104	104	100	104	000	000
CGT(13E)	182	182	122	138	108	108	104	104	104	110	208	208
CGT(13F)	182	182	130	138	108	108	104	104	100	106	208	208
CGT(13G)	180	182	130	132	108	108	104	104	104	110	206	208
CGT(13H)	180	182	122	130	108	108	104	104	106	110	206	208
CGT(13I)	182	182	078	142	108	108	104	104	104	110	208	208
CGT(13J)	182	182	136	142	108	108	104	106	104	110	208	208
CGT(13K)	182	182	122	130	108	108	104	106	104	104	206	208
CGT(13L)	180	182	128	130	108	108	104	106	104	110	206	206
CGT(13M)	182	182	130	142	108	108	104	106	104	104	208	208
CGT(13N)	182	182	078	138	108	108	104	106	104	110	206	208
CGT(13O)	182	182	078	128	108	108	104	106	106	110	208	208
CGT(13P)	182	182	130	132	108	108	104	104	100	104	208	208
CGT(13Q)	182	182	136	142	108	108	104	106	104	110	208	208
CGT(13R)	182	182	130	142	108	108	104	104	100	100	208	208
CGT(13S)	182	182	128	130	108	116	104	106	100	100	206	206
CGT(13T)	182	182	078	078	108	108	104	106	104	110	208	208
CGT(13U)	182	182	124	142	108	108	104	104	110	110	208	208
CGT(13V)	182	182	132	136	108	108	104	104	100	110	206	208

Appendix; Table of Genotypes

CGT(13W)	182	182	136	138	108	116	104	104	100	104	206	208
CGT(13X)	182	182	138	140	108	108	104	104	104	106	208	208
CGT(13Y)	182	182	122	142	108	108	104	104	106	110	206	208
CGT(14A)	182	182	128	130	108	108	102	104	098	110	206	206
CGT(14B)	182	182	078	130	108	108	104	104	104	110	206	208
CGT(14C)	182	182	132	144	000	000	104	104	104	110	208	208
CGT(14D)	182	182	130	132	108	116	104	104	104	110	208	208
CGT(14E)	182	182	130	138	108	116	104	104	104	110	206	208
CGT(14F)	182	182	078	130	108	108	104	104	106	110	208	208
CGT(14G)	182	182	078	078	108	116	104	106	106	110	206	206
CGT(14I)	182	182	078	130	108	108	104	104	104	110	000	000
CGT(14K)	182	182	000	000	108	108	104	104	104	110	206	208
CGT(14L)	182	182	078	128	108	108	102	102	102	104	206	208
CGT(14M)	182	182	130	130	108	108	102	104	098	102	208	208
CGT(14N)	182	182	128	130	108	108	102	104	098	102	208	208
CGT(14O)	182	182	078	132	108	108	104	104	104	110	208	208
CGT(14P)	182	182	078	078	108	116	104	104	106	110	206	208
CGT(14Q)	182	182	128	142	108	108	104	104	100	104	208	208
CGT(14R)	182	182	078	128	108	116	104	106	106	110	206	206
CGT(14S)	182	182	136	136	108	108	104	104	100	110	206	208
CGT(14T)	182	182	078	132	108	116	104	106	104	110	208	208
CGT(14U)	182	182	128	130	108	108	104	104	106	110	206	208
CGT(14V)	182	182	130	130	108	108	104	106	100	104	208	208
CGT(14W)	182	182	130	130	108	108	104	104	100	106	208	208
CGT(14X)	182	182	128	144	106	106	100	100	102	108	206	208
CGT(14Y)	182	182	126	128	106	106	100	100	100	108	206	208
CGT(15A)	182	182	078	078	108	108	104	104	104	110	206	208
CGT(15B)	182	182	078	078	108	108	102	102	110	110	206	208
CGT(15C)	182	182	078	078	108	108	102	104	102	110	206	208
CGT(15D)	182	182	078	078	108	108	104	104	000	000	206	208
CGT(15E)	182	182	078	078	108	108	104	104	104	110	206	208
CGT(15F)	182	182	078	078	108	108	104	106	104	110	206	208
CGT(15G)	182	182	078	078	108	108	104	104	104	110	206	208
CGT(15H)	182	182	078	078	108	108	104	106	104	110	206	208
CGT(15I)	182	182	078	078	108	108	102	104	102	110	206	208
CGT(15J)	182	182	078	078	108	108	104	104	110	110	000	000
CGT(15K)	182	182	078	078	108	108	104	104	110	110	206	208
CGT(15L)	182	182	078	078	108	108	102	104	102	110	206	208
CGT(15M)	182	182	078	078	108	108	102	104	102	110	206	208
CGT(15N)	182	182	078	078	108	108	104	106	110	110	206	208
CGT(15O)	182	182	078	078	108	108	104	106	104	110	206	208
CGT(15P)	182	182	078	078	108	108	102	104	110	110	206	208
CGT(15Q)	182	182	078	078	108	108	104	104	104	110	206	208
CGT(15R)	182	182	078	078	108	108	104	104	104	110	206	208
CGT(15S)	182	182	078	078	108	108	104	104	110	110	206	208
CGT(15T)	182	182	078	078	108	108	104	106	110	110	206	208
CGT(15V)	182	182	078	078	108	108	104	104	110	110	206	208
CGT(15W)	182	182	122	130	108	108	104	104	104	106	208	208

Appendix; Table of Genotypes

CGT(15X)	182	182	078	078	108	108	104	104	110	110	206	208
CGT(15Y)	182	182	078	078	108	108	104	104	104	110	206	208
CGT(16A)	182	182	122	142	108	108	102	102	098	098	208	208
CGT(16B)	182	182	078	130	116	116	104	104	106	106	208	208
CGT(16C)	180	182	122	122	108	108	102	104	098	110	000	000
CGT(16D)	182	182	132	142	108	108	102	104	098	102	208	208
CGT(16E)	182	182	078	122	108	108	104	104	110	110	000	000
CGT(16F)	182	182	130	142	108	108	106	106	100	104	208	208
CGT(16G)	182	182	122	128	108	108	104	104	110	110	208	208
CGT(16H)	182	182	122	122	108	108	104	104	104	110	000	000
CGT(16I)	182	182	078	130	108	108	104	104	100	106	206	208
CGT(16J)	182	182	078	122	108	108	104	106	106	110	208	208
CGT(16K)	180	182	132	144	108	108	104	104	104	104	208	208
CGT(16L)	182	182	078	140	108	108	104	104	106	110	206	206
CGT(16M)	182	182	132	142	108	108	104	106	104	104	208	208
CGT(16N)	180	182	122	142	108	108	104	104	104	110	208	208
CGT(16O)	182	182	122	130	108	108	104	104	104	110	206	208
CGT(16P)	180	182	078	122	108	108	104	106	110	110	206	208
CGT(16Q)	182	182	136	140	108	108	104	104	100	106	208	208
CGT(16R)	182	182	078	132	108	116	104	104	104	110	206	208
CGT(16S)	182	182	122	138	108	108	104	104	104	110	208	208
CGT(16T)	182	182	078	122	108	108	104	104	110	110	206	208
CGT(16U)	182	182	122	128	108	108	104	106	104	110	206	208
CGT(16V)	182	182	122	142	108	108	104	106	100	104	208	208
CGT(16W)	182	182	122	122	108	108	104	104	110	110	206	208
CGT(16X)	182	182	078	122	108	108	104	104	106	106	208	208
CGT(16Y)	182	182	078	130	108	108	104	106	110	110	208	208
CGT(17A)	182	182	128	132	108	108	104	106	106	110	208	208
CGT(17B)	182	182	128	132	108	108	104	106	104	106	208	208
CGT(17C)	182	182	130	130	108	108	104	104	104	104	208	208
CGT(17D)	182	182	128	132	108	116	104	104	106	110	206	208
CGT(17E)	182	182	078	078	108	116	104	106	104	110	206	208
CGT(17F)	182	182	128	132	108	116	104	104	104	106	208	208
CGT(17G)	182	182	122	130	108	116	104	104	106	110	208	208
CGT(17H)	182	182	128	132	108	116	104	106	104	106	208	208
CGT(17I)	182	182	078	132	116	116	104	106	104	110	208	208
CGT(17J)	182	182	078	130	108	108	104	104	104	110	000	000
CGT(17K)	182	182	078	138	108	116	106	106	110	110	206	208
CGT(17L)	182	182	124	130	108	108	104	104	110	110	208	208
CGT(17M)	182	182	130	132	108	116	104	106	104	106	208	208
CGT(17N)	182	182	078	118	108	108	102	104	098	110	208	208
CGT(17O)	180	182	124	140	108	108	104	104	104	110	206	208
CGT(17P)	182	182	132	140	108	108	104	104	104	110	208	208
CGT(17Q)	182	182	122	130	108	108	104	106	110	110	206	208
CGT(17R)	182	182	128	130	108	116	104	106	106	110	208	208
CGT(17S)	182	182	078	128	108	116	104	106	106	106	208	208
CGT(17T)	182	182	078	132	108	116	104	106	104	106	206	208
CGT(17U)	180	182	078	130	108	108	104	104	100	110	208	208

Appendix; Table of Genotypes

CGT(17V)	182	182	132	136	108	108	106	106	100	104	208	208
CGT(17W)	182	182	122	138	108	108	104	104	104	110	206	208
CGT(17X)	182	182	132	138	108	116	104	106	110	110	206	208
CGT(17Y)	182	182	122	130	108	116	104	106	104	106	208	208
CGT(18B)	182	182	078	120	106	106	100	100	100	108	000	000
CGT(18D)	182	182	122	122	108	108	104	104	104	110	208	208
CGT(18E)	182	182	078	122	108	108	104	104	104	104	000	000
CGT(18F)	182	182	078	128	108	116	104	106	104	106	206	208
CGT(18G)	180	182	078	078	108	108	104	106	100	110	000	000
CGT(18H)	182	182	078	078	108	108	104	106	106	110	000	000
CGT(18I)	182	182	138	138	108	108	104	104	104	104	208	208
CGT(18J)	182	182	078	136	108	108	104	104	100	110	208	208
CGT(18K)	182	182	122	138	108	108	104	104	104	110	208	208
CGT(18L)	182	182	122	138	108	108	104	104	104	110	208	208
CGT(18M)	182	182	122	142	108	108	104	104	106	110	208	208
CGT(18O)	182	182	078	078	108	108	104	104	104	110	208	208
CGT(18P)	182	182	078	122	108	108	104	104	104	104	208	208
CGT(18Q)	182	182	078	138	108	108	104	106	104	110	208	208
CGT(18R)	182	182	078	078	108	108	104	104	104	110	208	208
CGT(18S)	182	182	078	122	108	108	104	106	104	104	208	208
CGT(18T)	182	182	078	124	108	116	104	104	104	104	206	206
CGT(18U)	182	182	122	122	108	108	104	104	106	110	208	208
CGT(18V)	182	182	078	138	108	108	104	106	110	110	208	208
CGT(18W)	182	182	122	138	108	108	104	104	104	110	208	208
CGT(18X)	182	182	122	138	108	108	104	104	104	110	206	208
CGT(18Y)	182	182	078	122	108	108	104	106	106	110	208	208
CGT(19A)	182	182	078	130	108	108	102	104	098	110	206	208
CGT(19B)	182	182	132	132	108	108	102	104	098	110	208	208
CGT(19C)	182	182	124	128	108	108	104	106	106	110	208	208
CGT(19D)	182	182	078	132	108	116	102	104	098	110	206	208
CGT(19E)	182	182	122	122	108	108	104	104	110	110	208	208
CGT(19F)	182	182	122	132	108	108	104	104	110	110	208	208
CGT(19G)	180	182	136	140	108	108	106	106	104	110	206	208
CGT(19H)	182	182	132	140	108	108	106	106	106	106	206	208
CGT(19I)	180	182	132	140	108	108	104	106	106	110	000	000
CGT(19J)	182	182	078	132	108	108	104	106	106	110	208	208
CGT(19K)	182	182	132	140	108	116	102	104	098	110	206	208
CGT(19L)	182	182	128	132	108	108	104	104	106	110	000	000
CGT(19M)	180	182	122	128	108	116	106	106	104	110	000	000
CGT(19N)	182	182	078	130	108	108	104	104	110	110	208	208
CGT(19O)	182	182	132	140	108	108	104	106	106	110	208	208
CGT(19P)	182	182	140	142	108	108	104	106	106	106	208	208
CGT(19Q)	182	182	122	136	108	108	104	106	106	110	206	206
CGT(19R)	182	182	128	132	108	108	104	104	106	110	208	208
CGT(19S)	182	182	078	128	108	108	104	104	110	110	206	208
CGT(19T)	182	182	078	078	108	108	104	106	110	110	206	206
CGT(19U)	182	182	122	136	108	108	104	106	106	110	206	208
CGT(19V)	182	182	078	142	108	108	104	104	100	106	208	208

Appendix; Table of Genotypes

CGT(19W)	182	182	130	136	108	108	104	106	104	110	208	208
CGT(19X)	182	182	128	130	108	108	104	106	110	110	206	208
CGT(19Y)	182	182	122	138	108	108	106	106	110	110	206	208
CGT(20A)	182	182	078	130	108	116	104	104	104	110	206	208
CGT(20B)	182	182	078	122	108	108	104	104	110	110	208	208
CGT(20C)	182	182	122	130	108	108	104	104	110	110	000	000
CGT(20D)	182	182	078	122	108	108	104	106	104	110	208	208
CGT(20E)	180	180	122	146	108	108	104	104	104	110	206	208
CGT(20F)	180	182	078	130	108	116	104	106	104	110	206	208
CGT(20G)	180	182	122	122	108	108	104	104	104	110	206	208
CGT(20H)	182	182	122	130	108	108	104	104	110	110	206	208
CGT(20I)	182	182	126	126	106	114	100	100	100	100	206	208
CGT(20J)	180	182	078	136	108	116	104	104	106	110	206	208
CGT(20K)	182	182	122	144	108	108	104	106	110	110	208	208
CGT(20M)	182	182	078	130	108	116	104	106	104	110	206	208
CGT(20N)	180	182	122	122	108	108	104	104	106	110	208	208
CGT(20O)	182	182	132	136	108	108	104	104	100	104	206	206
CGT(20P)	180	182	078	122	108	116	104	104	104	110	206	208
CGT(20Q)	182	182	122	130	108	108	104	104	104	110	206	208
CGT(20R)	182	182	078	130	108	116	104	104	104	110	206	208
CGT(20S)	182	182	078	122	108	108	104	104	110	110	208	208
CGT(20T)	180	182	130	136	108	108	104	104	104	110	206	208
CGT(20U)	182	182	078	130	108	116	104	104	104	104	206	208
CGT(20V)	182	182	122	144	108	108	104	106	104	110	208	208
CGT(20W)	182	182	078	122	108	108	104	104	104	110	208	208
CGT(20X)	180	182	130	136	108	108	104	104	110	110	206	206

Appendix; Table of Genotypes

Cors Goch Grid 04

Both alleles are shown for all six loci in all successfully genotyped individuals. Within parentheses in sample names, numbers refer to the nest name and letters refer to the individual sampled from that nest.

Sample	Loci											
	FE19		FE38		FE49		FE51		FL12		FE21	
CGG04(01a)	182	182	078	136	108	108	104	104	104	106	206	208
CGG04(01b)	182	182	078	130	108	108	104	106	104	106	206	208
CGG04(01c)	180	182	078	132	108	108	104	106	100	110	208	208
CGG04(01d)	182	182	078	130	108	108	104	104	104	110	208	208
CGG04(01e)	182	182	078	136	108	108	104	104	100	110	206	208
CGG04(01f)	182	182	078	130	108	108	104	104	104	106	208	208
CGG04(01g)	182	182	078	130	108	116	104	104	106	110	208	208
CGG04(01h)	182	182	078	136	108	108	104	104	104	110	206	208
CGG04(01i)	182	182	130	130	108	108	104	106	104	110	206	206
CGG04(01j)	182	182	078	130	108	108	104	104	104	106	208	208
CGG04(01k)	182	182	140	142	108	108	104	106	106	110	206	208
CGG04(01l)	182	182	130	130	108	108	104	104	104	110	208	208
CGG04(01m)	182	182	138	140	108	108	104	104	104	104	206	208
CGG04(01n)	182	182	078	128	108	108	104	104	104	110	208	208
CGG04(01o)	182	182	078	138	108	108	104	106	104	106	208	208
CGG04(01p)	182	182	132	138	108	108	104	104	104	110	206	206
CGG04(01q)	182	182	130	138	108	108	102	102	098	102	208	208
CGG04(01r)	182	182	128	142	108	116	104	104	104	110	206	208
CGG04(01s)	182	182	122	128	000	000	104	104	104	104	000	000
CGG04(01t)	182	182	130	130	108	108	104	104	110	110	206	208
CGG04(02a)	182	182	078	138	108	108	104	106	104	110	206	208
CGG04(02b)	182	182	078	130	108	108	104	104	106	110	208	208
CGG04(02c)	000	000	000	000	000	000	104	106	104	106	000	000
CGG04(02d)	182	182	078	134	108	108	102	102	098	102	208	208
CGG04(02e)	182	182	122	124	108	108	104	104	106	110	208	208
CGG04(02f)	182	182	078	142	108	108	104	104	104	110	206	208
CGG04(02h)	182	182	122	136	108	108	104	104	106	110	206	208
CGG04(02i)	182	182	078	134	108	108	102	104	098	110	206	206
CGG04(02j)	182	182	078	138	108	116	102	102	098	102	206	206
CGG04(02k)	182	182	128	128	108	108	104	104	106	108	206	208
CGG04(02l)	182	182	128	130	108	116	104	106	104	106	206	208
CGG04(02m)	182	182	132	138	108	116	104	104	110	110	206	208
CGG04(02n)	182	182	130	134	108	108	104	104	104	110	208	208
CGG04(02p)	182	182	078	138	108	116	104	104	106	110	208	208
CGG04(02q)	180	182	078	136	000	000	100	100	100	100	208	208
CGG04(03a)	182	182	122	128	108	108	104	104	104	110	206	206
CGG04(03b)	182	182	130	130	108	108	104	104	104	110	208	208
CGG04(03c)	182	182	128	128	108	108	104	106	104	104	208	208
CGG04(03d)	182	182	078	122	108	108	104	106	104	110	208	208
CGG04(03e)	182	182	136	138	108	116	104	106	104	104	208	208
CGG04(03f)	182	182	078	142	108	116	104	106	104	110	206	208
CGG04(03g)	182	182	132	134	108	108	104	106	104	110	206	208

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CGG04(03h)	182	182	128	132	108	116	104	104	104	110	206	208
CGG04(03i)	182	182	078	138	108	116	104	104	104	104	206	206
CGG04(03j)	182	182	130	130	108	116	104	106	104	106	206	208
CGG04(03k)	182	182	122	128	108	108	102	104	098	102	208	208
CGG04(03l)	182	182	130	136	108	116	104	104	104	110	208	208
CGG04(03m)	182	182	078	138	108	108	104	104	104	106	208	208
CGG04(03n)	182	182	078	130	108	116	104	104	104	110	206	208
CGG04(03o)	182	182	078	130	108	108	104	104	000	000	206	208
CGG04(03q)	182	182	078	128	108	108	104	104	104	110	208	208
CGG04(03r)	182	182	078	132	108	108	104	104	110	110	206	208
CGG04(03s)	182	182	138	140	108	116	104	104	104	106	206	206
CGG04(04a)	182	182	122	138	108	116	104	104	110	110	206	206
CGG04(04b)	182	182	078	130	108	108	104	104	106	110	206	208
CGG04(04c)	182	182	078	078	108	108	104	104	104	110	000	000
CGG04(04d)	182	182	078	138	108	108	104	104	104	106	208	208
CGG04(04e)	182	182	128	138	108	108	104	104	104	104	208	208
CGG04(04f)	182	182	078	138	108	108	104	104	104	110	206	208
CGG04(04g)	182	182	078	136	108	108	104	104	104	110	206	208
CGG04(04i)	182	182	078	140	108	116	104	106	104	110	206	208
CGG04(04j)	182	182	078	122	108	116	104	106	106	110	208	208
CGG04(04k)	000	000	000	000	108	108	104	106	106	110	206	208
CGG04(04l)	182	182	122	130	108	108	104	104	106	110	206	208
CGG04(04m)	000	000	078	130	108	108	104	106	104	106	000	000
CGG04(06a)	182	182	128	130	108	116	104	106	104	106	206	208
CGG04(06b)	182	182	078	122	108	108	104	104	104	106	206	208
CGG04(06c)	180	182	078	130	108	108	104	104	104	104	208	208
CGG04(06d)	182	182	134	140	108	108	104	104	104	110	206	208
CGG04(06e)	182	182	078	078	108	108	104	106	106	110	206	208
CGG04(06f)	182	182	136	138	108	116	104	106	104	106	208	208
CGG04(06g)	182	182	078	128	108	108	104	104	106	110	206	208
CGG04(06h)	182	182	120	138	108	108	102	104	098	102	208	208
CGG04(06i)	182	182	128	130	108	108	104	104	106	106	206	208
CGG04(06j)	182	182	128	136	108	116	104	106	104	104	206	208
CGG04(06k)	182	182	078	122	108	108	104	104	104	104	206	208
CGG04(06l)	182	182	136	136	108	116	104	106	104	104	208	208
CGG04(06m)	182	182	128	130	108	108	104	104	106	106	206	208
CGG04(06n)	182	182	122	142	108	108	104	104	104	106	206	208
CGG04(06o)	182	182	078	142	108	108	104	106	106	110	206	208
CGG04(06p)	182	182	128	138	108	108	104	104	104	106	206	208
CGG04(06q)	182	182	122	134	108	108	104	106	104	110	206	208
CGG04(07a)	182	182	122	140	108	108	104	106	104	110	206	208
CGG04(07b)	182	182	130	136	108	116	104	104	104	104	208	208
CGG04(07c)	180	182	078	128	108	108	104	104	104	104	208	208
CGG04(07d)	182	182	138	142	108	116	104	104	104	110	206	206
CGG04(07e)	182	182	128	128	108	108	104	104	104	110	206	206
CGG04(07f)	182	182	138	142	108	108	104	104	106	106	206	208
CGG04(07g)	182	182	128	134	108	108	104	104	104	104	206	208
CGG04(07h)	182	182	132	136	108	108	104	104	104	104	206	206
CGG04(07i)	182	182	122	128	108	108	104	104	106	106	206	208
CGG04(07j)	182	182	130	132	108	108	104	104	100	110	208	208
CGG04(07k)	182	182	130	138	108	108	104	104	106	110	206	208
CGG04(07l)	180	182	128	134	108	108	104	106	104	104	206	206
CGG04(07m)	182	182	128	130	108	108	104	104	104	110	206	208
CGG04(07n)	182	182	122	128	108	108	104	104	104	110	208	208
CGG04(07o)	182	182	122	128	108	108	104	104	104	104	000	000

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CGG04(07p)	182	182	130	132	108	108	106	106	104	106	206	208
CGG04(07q)	182	182	128	128	108	108	102	104	098	110	206	208
CGG04(07r)	182	182	078	140	108	108	104	104	104	106	208	208
CGG04(07s)	182	182	128	132	108	108	104	104	106	110	206	206
CGG04(08a)	182	182	128	130	108	108	104	104	104	110	206	208
CGG04(08b)	182	182	142	142	108	108	104	106	110	110	206	208
CGG04(08c)	182	182	124	128	108	116	104	106	104	110	208	208
CGG04(08d)	182	182	128	138	108	108	104	104	110	110	206	206
CGG04(08e)	182	182	128	132	108	108	104	104	104	110	206	208
CGG04(08f)	182	182	122	132	108	108	104	106	104	110	206	206
CGG04(08g)	182	182	078	140	108	108	104	104	104	110	206	206
CGG04(08h)	182	182	078	128	108	116	104	104	104	110	206	206
CGG04(08i)	182	182	130	134	108	108	104	104	104	104	206	208
CGG04(08j)	182	182	128	130	108	108	104	104	104	110	206	206
CGG04(08k)	182	182	122	128	108	108	104	104	104	104	208	208
CGG04(08l)	180	182	078	128	108	116	104	106	104	104	208	208
CGG04(08m)	182	182	122	128	108	108	104	104	106	110	206	208
CGG04(08n)	182	182	128	142	108	108	102	102	098	098	208	208
CGG04(08o)	182	182	130	130	108	108	104	104	104	106	208	208
CGG04(08p)	182	182	142	142	108	108	104	104	104	110	206	208
CGG04(08q)	182	182	078	130	108	108	104	104	106	110	206	208
CGG04(08r)	182	182	130	138	108	108	104	106	106	110	206	208
CGG04(08s)	180	182	122	128	108	108	104	106	104	106	206	208
CGG04(08t)	182	182	078	134	108	108	104	104	104	110	208	208
CGG04(08u)	182	182	078	120	108	116	104	104	104	110	206	208
CGG04(08v)	182	182	078	128	108	108	102	102	098	098	208	208
CGG04(08w)	182	182	130	140	108	108	104	104	110	110	208	208
CGG04(09a)	182	182	128	140	108	108	104	104	104	110	206	208
CGG04(09b)	182	182	128	138	108	108	104	106	106	110	206	208
CGG04(09c)	182	182	138	140	108	108	104	104	106	110	206	208
CGG04(09d)	182	182	078	144	108	108	104	104	104	106	208	208
CGG04(09e)	182	182	078	138	108	108	104	104	104	110	206	206
CGG04(09f)	182	182	140	140	108	108	104	104	104	104	206	208
CGG04(09g)	182	182	078	136	108	108	104	104	104	110	206	208
CGG04(09h)	182	182	078	130	108	108	104	104	104	106	208	208
CGG04(09i)	182	182	130	138	108	108	104	104	104	104	206	208
CGG04(09j)	182	182	078	138	108	108	104	106	104	110	206	208
CGG04(09k)	182	182	078	138	108	108	104	104	106	110	208	208
CGG04(09l)	182	182	078	140	108	108	104	104	104	110	208	208
CGG04(09m)	182	182	078	128	108	108	104	104	104	104	206	208
CGG04(09n)	182	182	122	138	108	116	104	104	104	106	208	208
CGG04(09o)	182	182	138	142	108	108	104	104	106	110	208	208
CGG04(09p)	182	182	078	142	108	108	104	104	104	110	208	208
CGG04(09q)	182	182	078	120	108	108	104	104	104	106	206	206
CGG04(09r)	182	182	130	142	108	108	104	104	104	106	208	208
CGG04(09s)	180	182	128	130	108	108	104	104	104	106	208	208
CGG04(09t)	182	182	124	138	108	108	104	104	104	110	206	208
CGG04(09u)	180	182	078	140	108	116	104	104	104	110	208	208
CGG04(09v)	182	182	078	138	108	108	104	104	106	106	208	208
CGG04(09w)	182	182	128	128	108	108	102	104	098	110	208	208
CGG04(09x)	182	182	124	128	000	000	104	104	110	110	206	208
CGG04(101a)	182	182	130	138	108	116	104	104	104	104	206	206
CGG04(101b)	182	182	122	128	108	108	104	104	104	110	206	206
CGG04(101c)	182	182	078	130	108	116	104	104	104	106	206	208
CGG04(101d)	182	182	078	134	108	108	102	102	102	110	208	208

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CGG04(101e)	182	182	132	140	108	116	104	104	104	110	206	208
CGG04(101f)	182	182	132	138	108	108	104	104	106	110	206	206
CGG04(101g)	182	182	078	134	108	108	104	104	104	110	208	208
CGG04(101h)	182	182	078	138	108	108	102	102	110	110	206	208
CGG04(101i)	182	182	078	138	108	108	104	104	104	110	206	208
CGG04(101j)	180	182	128	140	108	108	104	104	104	110	208	208
CGG04(101k)	182	182	078	138	108	108	104	104	106	110	208	208
CGG04(101l)	182	182	130	140	108	108	104	104	104	106	206	208
CGG04(101m)	182	182	130	130	108	108	104	104	104	106	208	208
CGG04(101n)	182	182	078	078	108	116	104	104	100	104	206	206
CGG04(101o)	182	182	078	130	108	108	104	104	106	106	208	208
CGG04(10a)	182	182	078	078	108	108	104	104	106	110	208	208
CGG04(10b)	182	182	078	136	108	108	104	104	104	110	206	208
CGG04(10c)	182	182	122	124	108	108	104	106	106	106	208	208
CGG04(10d)	180	182	078	146	108	108	104	104	110	110	206	208
CGG04(10e)	182	182	128	132	108	108	104	106	104	106	206	206
CGG04(10f)	182	182	078	142	108	108	104	104	104	110	206	208
CGG04(10g)	182	182	128	138	108	108	104	106	106	110	206	208
CGG04(10h)	182	182	078	142	108	108	104	104	106	110	206	208
CGG04(10i)	182	182	078	134	108	108	104	104	110	110	208	208
CGG04(10j)	182	182	122	124	108	108	104	104	106	110	208	208
CGG04(10k)	182	182	078	130	108	108	104	106	104	104	206	208
CGG04(10l)	180	182	078	122	108	108	104	104	104	104	206	208
CGG04(10m)	182	182	078	136	116	116	104	104	100	104	208	208
CGG04(10n)	182	182	122	138	108	108	104	104	110	110	206	208
CGG04(10o)	182	182	130	130	108	116	104	104	104	110	208	208
CGG04(10p)	180	182	078	136	106	114	100	100	100	100	206	208
CGG04(10q)	182	182	128	130	108	108	104	104	104	110	206	206
CGG04(10r)	182	182	130	138	108	108	104	104	106	110	208	208
CGG04(10s)	180	182	130	142	108	108	104	106	110	110	206	208
CGG04(10t)	182	182	078	138	108	108	104	104	106	110	208	208
CGG04(38a)	182	182	130	140	108	108	104	104	104	104	206	208
CGG04(38b)	182	182	128	140	108	108	102	104	098	110	206	208
CGG04(38c)	182	182	138	140	108	108	104	104	104	106	208	208
CGG04(38d)	182	182	128	140	108	108	104	106	104	110	208	208
CGG04(38e)	182	182	122	142	108	108	104	104	104	110	208	208
CGG04(38f)	182	182	122	130	108	108	104	106	106	106	206	208
CGG04(38g)	182	182	130	138	108	116	104	104	104	110	208	208
CGG04(38h)	182	182	136	142	108	108	104	104	104	110	208	208
CGG04(38i)	182	182	122	128	108	108	104	104	106	106	206	206
CGG04(38j)	182	182	134	142	108	108	104	104	104	106	206	206
CGG04(38k)	182	182	122	124	108	108	104	106	104	110	208	208
CGG04(38l)	182	182	130	138	116	116	104	104	104	110	206	206
CGG04(38m)	182	182	122	132	108	108	104	106	104	106	206	206
CGG04(38n)	182	182	130	140	108	108	104	104	110	110	208	208
CGG04(38o)	182	182	078	078	108	108	104	104	104	106	206	208
CGG04(38p)	182	182	138	140	108	108	104	104	106	110	208	208
CGG04(38q)	182	182	078	130	108	116	104	104	104	110	206	208
CGG04(38r)	182	182	128	130	108	108	104	106	104	110	206	208
CGG04(38t)	182	182	130	138	108	116	104	106	104	110	206	206
CGG04(38u)	182	182	078	142	108	116	104	104	104	110	206	208
CGG04(38v)	182	182	078	134	108	108	104	104	104	106	206	206
CGG04(38w)	182	182	130	134	108	108	104	104	104	106	206	208
CGG04(38x)	182	182	122	138	108	116	104	106	106	106	206	208
CGG04(38y)	182	182	122	130	108	108	104	106	106	110	208	208

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CGG04(39a)	182	182	078	142	108	108	104	104	104	106	208	208
CGG04(39b)	182	182	122	140	108	108	104	104	106	110	206	208
CGG04(39c)	182	182	078	134	108	108	104	104	104	110	208	208
CGG04(39d)	182	182	122	138	108	116	104	104	104	104	208	208
CGG04(39e)	182	182	122	128	108	108	104	104	106	110	206	208
CGG04(39f)	182	182	078	134	108	116	104	104	104	104	206	206
CGG04(39g)	182	182	078	134	108	108	104	104	104	110	206	208
CGG04(39h)	182	182	078	136	108	108	104	104	100	106	206	208
CGG04(39i)	182	182	122	134	108	108	104	106	104	110	208	208
CGG04(39k)	182	182	078	142	108	108	104	104	106	110	208	208
CGG04(39m)	182	182	134	138	108	108	104	106	106	110	206	206
CGG04(39n)	182	182	138	140	108	116	104	104	106	110	208	208
CGG04(39o)	182	182	078	138	108	116	104	104	104	110	208	208
CGG04(39p)	182	182	130	134	108	108	104	104	104	110	206	208
CGG04(39q)	182	182	132	134	108	108	104	104	104	110	206	208
CGG04(39r)	182	182	132	134	108	108	104	104	104	104	206	208
CGG04(39s)	180	182	128	134	108	108	104	104	106	110	208	208
CGG04(39t)	182	182	130	134	108	108	104	104	104	104	206	208
CGG04(39u)	182	182	134	138	108	108	104	104	104	106	206	208
CGG04(39v)	182	182	078	120	108	108	104	104	104	104	206	208
CGG04(39w)	182	182	078	130	108	108	104	104	104	106	208	208
CGG04(39x)	182	182	130	140	108	108	104	104	104	104	206	206
CGG04(39y)	182	182	128	130	108	108	104	104	104	110	206	208
CGG04(40a)	182	182	078	138	108	116	104	106	104	110	206	208
CGG04(40b)	182	182	138	140	108	116	104	104	106	110	206	206
CGG04(40c)	182	182	130	138	108	108	104	104	106	110	206	208
CGG04(40d)	182	182	122	132	108	108	104	106	104	110	206	208
CGG04(40e)	182	182	130	138	108	108	104	104	106	110	206	208
CGG04(40f)	182	182	078	078	108	108	104	104	106	110	206	206
CGG04(40g)	182	182	130	142	108	108	104	104	104	110	206	208
CGG04(40h)	182	182	130	140	108	108	104	104	104	110	206	208
CGG04(40i)	182	182	078	138	108	108	104	104	106	106	208	208
CGG04(40j)	182	182	078	134	108	108	104	104	104	110	206	208
CGG04(40k)	182	182	122	132	108	108	106	106	104	110	206	208
CGG04(40l)	182	182	078	142	108	116	104	104	106	110	206	208
CGG04(40m)	182	182	130	140	108	108	104	104	104	110	206	208
CGG04(40n)	182	182	078	140	108	116	104	104	104	110	206	208
CGG04(41a)	182	182	078	128	108	116	104	104	104	110	208	208
CGG04(41b)	182	182	132	140	108	116	104	104	110	110	208	208
CGG04(41c)	182	182	120	136	108	108	104	104	104	106	208	208
CGG04(41d)	182	182	078	122	108	108	104	104	104	110	206	206
CGG04(41e)	182	182	122	124	108	108	104	104	104	106	208	208
CGG04(41f)	182	182	122	130	108	108	104	106	106	110	206	208
CGG04(41g)	182	182	078	128	108	116	104	106	104	104	206	208
CGG04(41h)	180	182	078	128	108	108	104	104	104	106	208	208
CGG04(41i)	182	182	078	138	108	116	104	106	106	110	208	208
CGG04(41j)	182	182	122	140	108	108	104	104	104	106	208	208
CGG04(41k)	180	182	078	128	108	108	104	104	104	106	208	208
CGG04(41m)	182	182	140	142	108	108	104	104	104	104	206	208
CGG04(41n)	182	182	078	130	108	108	104	104	104	104	206	206
CGG04(41o)	182	182	130	140	108	108	104	104	110	110	206	208
CGG04(41p)	182	182	078	138	108	108	104	106	104	110	206	208
CGG04(41q)	182	182	128	130	108	116	104	104	104	110	208	208
CGG04(41r)	182	182	140	140	108	108	104	104	104	104	206	208
CGG04(41s)	182	182	128	130	108	108	104	104	104	110	206	206

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CGG04(41t)	182	182	078	142	108	108	104	104	104	106	206	208
CGG04(41u)	182	182	078	140	108	108	104	104	104	110	208	208
CGG04(41v)	182	182	128	128	108	108	104	106	104	104	208	208
CGG04(41w)	182	182	122	130	108	108	104	106	100	104	208	208
CGG04(41x)	182	182	130	138	116	116	104	104	104	110	206	206
CGG04(41y)	182	182	078	142	108	108	106	106	106	110	206	208
CGG04(44a)	182	182	078	142	108	116	104	106	110	110	208	208
CGG04(44b)	182	182	078	138	108	116	104	104	104	104	206	206
CGG04(44c)	182	182	078	132	108	108	106	106	104	110	206	206
CGG04(44d)	182	182	132	134	108	108	104	106	104	110	206	208
CGG04(44e)	182	182	128	136	108	116	104	104	104	106	206	208
CGG04(44f)	182	182	132	142	108	108	106	106	104	110	206	208
CGG04(44g)	180	182	140	146	108	108	104	106	106	106	208	208
CGG04(44i)	182	182	120	122	108	108	104	106	106	110	208	208
CGG04(44j)	182	182	122	124	108	108	104	104	104	106	208	208
CGG04(44k)	182	182	122	130	108	116	104	106	100	104	208	208
CGG04(44l)	182	182	078	130	108	108	104	104	100	110	206	208
CGG04(44m)	182	182	130	140	108	108	104	104	104	110	206	208
CGG04(44o)	182	182	138	140	108	108	104	104	104	110	206	206
CGG04(44p)	182	182	078	122	108	108	104	104	100	104	206	208
CGG04(44q)	182	182	130	138	108	116	104	104	100	104	206	206
CGG04(44r)	182	182	078	138	108	108	104	104	104	110	206	206
CGG04(44s)	182	182	078	130	108	116	104	104	104	106	206	208
CGG04(44t)	182	182	078	134	108	108	104	104	106	110	206	208
CGG04(44u)	182	182	130	140	108	108	104	104	104	106	206	208
CGG04(44v)	182	182	122	124	108	108	104	104	104	110	208	208
CGG04(44w)	182	182	138	140	108	108	104	104	104	104	206	206
CGG04(44x)	182	182	134	136	108	108	104	104	106	110	206	208
CGG04(44y)	180	182	078	078	108	108	104	104	104	104	208	208
CGG04(45a)	180	182	128	128	108	108	106	106	104	104	208	208
CGG04(45b)	182	182	078	128	108	116	104	104	110	110	208	208
CGG04(45c)	180	182	078	130	108	108	104	104	104	104	206	208
CGG04(45d)	182	182	078	134	108	108	104	104	104	106	208	208
CGG04(45e)	182	182	078	132	108	108	104	106	110	110	208	208
CGG04(45f)	182	182	122	130	108	108	104	104	100	106	208	208
CGG04(45g)	182	182	122	132	108	108	104	104	104	110	208	208
CGG04(45h)	182	182	140	142	108	108	104	104	106	110	206	208
CGG04(45i)	182	182	078	124	108	108	104	104	104	106	208	208
CGG04(45j)	182	182	078	078	108	108	104	104	104	106	208	208
CGG04(45k)	182	182	136	136	108	108	104	104	104	110	206	208
CGG04(45l)	182	182	000	000	108	108	102	104	098	102	206	208
CGG04(45m)	182	182	130	140	108	108	104	104	104	104	206	208
CGG04(45n)	182	182	134	140	108	108	104	104	104	110	206	208
CGG04(45o)	182	182	078	138	108	108	104	106	104	110	206	208
CGG04(45p)	182	182	078	130	108	116	104	106	106	110	208	208
CGG04(45q)	182	182	120	122	108	108	104	106	106	110	206	208
CGG04(45r)	180	182	078	130	108	108	104	104	104	110	206	208
CGG04(45s)	182	182	128	136	108	116	104	104	104	106	208	208
CGG04(45t)	182	182	078	134	108	108	104	104	104	110	208	208
CGG04(45u)	182	182	130	140	108	108	104	104	104	104	206	208
CGG04(45v)	182	182	130	142	108	108	104	104	104	110	206	208
CGG04(46a)	182	182	128	130	108	108	104	104	104	106	206	208
CGG04(46b)	182	182	078	132	108	108	104	106	110	110	206	208
CGG04(46c)	182	182	078	142	108	108	104	104	104	104	206	208
CGG04(46d)	182	182	128	130	108	116	104	104	104	110	208	208

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CGG04(46e)	182	182	130	136	108	108	104	104	100	104	206	208
CGG04(46f)	182	182	078	130	108	116	104	104	104	110	206	208
CGG04(46g)	182	182	128	142	108	116	104	104	104	110	206	208
CGG04(46h)	182	182	128	130	108	108	104	104	104	110	208	208
CGG04(46i)	182	182	078	128	108	116	104	106	104	104	206	208
CGG04(46j)	182	182	132	134	108	116	104	104	110	110	206	208
CGG04(46k)	182	182	078	134	108	108	104	104	104	110	206	208
CGG04(46l)	182	182	128	128	108	108	106	106	104	104	208	208
CGG04(46m)	182	182	078	138	108	108	104	104	100	104	208	208
CGG04(46n)	182	182	078	130	108	108	104	104	104	104	206	208
CGG04(46o)	180	182	130	138	108	108	104	104	104	106	208	208
CGG04(46q)	182	182	122	128	108	108	104	106	104	106	206	206
CGG04(46r)	182	182	078	078	108	108	104	104	104	106	208	208
CGG04(46s)	182	182	130	136	108	108	104	106	104	110	206	208
CGG04(48a)	182	182	078	128	108	108	104	104	104	106	208	208
CGG04(48b)	182	182	078	140	108	116	104	104	104	110	206	206
CGG04(48c)	182	182	078	140	108	108	104	104	104	110	208	208
CGG04(48d)	182	182	078	140	108	116	104	104	106	110	208	208
CGG04(48e)	180	182	128	142	108	108	104	104	106	110	208	208
CGG04(48f)	182	182	122	128	108	108	104	104	104	110	208	208
CGG04(48g)	182	182	130	140	108	108	104	104	104	106	206	208
CGG04(48h)	182	182	078	122	108	108	104	104	104	104	208	208
CGG04(48i)	182	182	078	140	108	108	106	106	104	104	206	206
CGG04(48j)	182	182	132	136	108	108	104	104	104	110	206	206
CGG04(48k)	182	182	078	140	108	108	104	104	110	110	208	208
CGG04(48l)	182	182	078	142	108	108	104	104	104	104	208	208
CGG04(48m)	182	182	078	136	108	108	104	104	104	104	206	206
CGG04(48n)	182	182	132	136	108	108	104	104	104	110	206	208
CGG04(48o)	180	182	130	142	108	108	104	106	110	110	206	208
CGG04(48q)	182	182	130	142	108	116	104	104	104	104	206	208
CGG04(48r)	182	182	130	140	108	108	104	104	104	106	206	208
CGG04(48s)	182	182	120	130	108	108	104	104	110	110	208	208
CGG04(48t)	182	182	078	130	108	108	104	104	104	110	208	208
CGG04(48u)	182	182	078	138	108	116	104	104	104	110	206	208
CGG04(48v)	180	182	078	128	108	108	104	104	104	104	208	208
CGG04(48w)	182	182	136	136	108	108	104	104	104	104	206	206
CGG04(48x)	182	182	138	140	108	116	104	104	104	104	206	208
CGG04(48y)	182	182	078	078	108	108	104	104	104	110	208	208
CGG04(51a)	182	182	126	138	108	108	104	104	110	110	206	208
CGG04(51b)	182	182	078	130	108	108	104	104	104	110	206	208
CGG04(51c)	182	182	078	122	108	108	104	104	106	106	208	208
CGG04(51d)	182	182	122	130	108	108	104	106	100	104	208	208
CGG04(51e)	182	182	078	138	108	108	104	104	104	104	208	208
CGG04(51f)	182	182	078	138	108	116	104	104	106	110	208	208
CGG04(51g)	182	182	130	136	108	108	104	104	110	110	206	208
CGG04(51h)	182	182	078	138	108	108	104	106	104	106	208	208
CGG04(51i)	182	182	128	142	108	108	104	104	104	110	206	206
CGG04(51j)	182	182	130	138	108	108	104	106	110	110	206	208
CGG04(51k)	182	182	078	128	108	108	104	104	110	110	206	206
CGG04(51l)	182	182	078	130	108	108	104	104	104	104	206	208
CGG04(51m)	182	182	078	130	108	108	104	104	104	110	208	208
CGG04(51n)	182	182	078	140	108	108	104	104	104	104	206	208
CGG04(51o)	182	182	142	146	108	108	104	104	106	110	206	208
CGG04(51p)	182	182	128	130	108	108	104	104	104	110	208	208
CGG04(51q)	182	182	130	136	108	108	104	104	104	110	206	208

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CGG04(51r)	182	182	078	078	108	116	104	106	104	106	206	208
CGG04(51s)	182	182	132	136	108	108	104	104	104	110	206	206
CGG04(51t)	182	182	078	128	108	108	104	104	104	110	208	208
CGG04(51u)	182	182	130	136	108	108	104	104	104	104	206	206
CGG04(51v)	182	182	078	130	108	108	106	106	106	110	208	208
CGG04(51w)	182	182	130	140	108	108	106	106	104	104	206	208
CGG04(51x)	182	182	132	136	108	108	104	104	104	104	206	206
CGG04(51y)	182	182	078	136	108	108	104	104	104	110	208	208
CGG04(52a)	182	182	138	142	108	108	104	104	110	110	206	208
CGG04(52b)	182	182	078	140	108	108	106	106	104	104	206	208
CGG04(52c)	182	182	122	130	108	108	104	104	104	106	206	208
CGG04(52e)	182	182	078	140	108	108	104	104	106	110	206	208
CGG04(52f)	182	182	078	128	108	108	104	104	110	110	206	208
CGG04(52g)	180	182	078	142	108	108	104	104	104	104	206	208
CGG04(52h)	182	182	078	130	108	108	104	104	104	104	206	206
CGG04(52i)	182	182	078	140	108	108	104	104	104	110	208	208
CGG04(52j)	182	182	078	138	108	108	104	104	104	110	206	208
CGG04(52k)	182	182	078	136	108	108	104	104	110	110	206	208
CGG04(52l)	182	182	078	130	108	108	104	104	100	110	208	208
CGG04(52m)	182	182	130	142	108	108	106	106	110	110	206	208
CGG04(52o)	182	182	130	136	108	108	104	106	106	110	206	208
CGG04(52p)	182	182	122	122	108	108	104	104	106	110	206	208
CGG04(52q)	182	182	128	128	108	108	104	104	110	110	206	206
CGG04(52r)	182	182	078	130	108	108	104	104	106	110	208	208
CGG04(52t)	182	182	078	134	108	108	104	104	104	110	208	208
CGG04(52u)	182	182	130	142	108	108	104	106	104	110	206	206
CGG04(52v)	182	182	078	134	108	116	104	106	104	110	206	208
CGG04(52w)	182	182	078	138	108	108	104	106	106	110	206	208
CGG04(52x)	182	182	132	138	108	108	104	104	106	110	206	208
CGG04(52y)	182	182	128	138	108	108	104	104	106	110	206	208
CGG04(53a)	182	182	130	136	108	108	104	104	100	106	208	208
CGG04(53b)	182	182	136	138	108	108	104	104	104	104	206	206
CGG04(53c)	182	182	132	134	108	116	104	104	104	110	208	208
CGG04(53d)	182	182	078	122	108	108	104	104	104	110	206	206
CGG04(53e)	182	182	078	122	108	108	104	104	104	106	208	208
CGG04(53f)	182	182	078	134	108	108	104	106	110	110	208	208
CGG04(53g)	182	182	078	142	108	108	104	104	104	106	206	208
CGG04(53h)	182	182	128	140	108	108	104	104	110	110	206	208
CGG04(53i)	182	182	078	122	108	108	104	104	106	110	208	208
CGG04(53j)	182	182	140	140	108	108	104	106	104	104	206	208
CGG04(53k)	182	182	078	128	108	108	104	106	100	104	206	206
CGG04(53l)	182	182	138	146	108	116	104	104	106	110	206	208
CGG04(53m)	182	182	078	130	108	108	104	104	106	110	208	208
CGG04(53n)	182	182	130	142	108	108	104	104	106	110	206	208
CGG04(53o)	182	182	078	138	108	116	104	104	104	104	206	206
CGG04(53p)	182	182	132	138	108	116	104	104	110	110	206	208
CGG04(53q)	182	182	078	140	108	116	104	106	104	110	206	208
CGG04(53r)	182	182	128	138	108	116	104	104	104	110	206	206
CGG04(53s)	182	182	130	138	108	108	104	106	110	110	206	208
CGG04(53t)	182	182	118	138	108	108	102	102	098	104	206	206
CGG04(53u)	182	182	078	128	108	108	104	104	104	106	206	208
CGG04(53v)	182	182	122	132	108	116	104	104	106	110	208	208
CGG04(53w)	182	182	130	130	108	108	106	106	104	110	206	206
CGG04(53x)	182	182	078	130	108	108	106	106	106	110	206	208
CGG04(53y)	180	182	128	132	108	108	104	104	110	110	206	208

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CGG04(54a)	182	182	122	130	108	108	104	106	106	110	208	208
CGG04(54b)	182	182	138	142	108	108	104	104	104	110	208	208
CGG04(54c)	182	182	140	142	108	108	104	106	110	110	206	208
CGG04(54d)	182	182	138	142	108	110	104	104	110	110	206	208
CGG04(54e)	180	182	130	138	108	108	104	104	106	110	206	208
CGG04(54f)	182	182	078	130	108	108	106	106	106	110	208	208
CGG04(54g)	182	182	130	142	108	108	104	106	104	110	206	206
CGG04(54h)	182	182	128	136	108	108	104	104	104	110	206	208
CGG04(54i)	182	182	078	078	108	108	104	104	110	110	208	208
CGG04(54j)	182	182	078	136	108	108	104	104	110	110	206	208
CGG04(54k)	182	182	078	138	108	108	104	104	104	110	206	208
CGG04(54l)	182	182	128	142	108	108	104	104	104	110	206	208
CGG04(54m)	182	182	124	128	108	108	104	104	104	106	206	208
CGG04(54n)	182	182	078	130	108	108	104	104	104	110	208	208
CGG04(54o)	182	182	078	130	108	108	104	104	106	110	208	208
CGG04(54p)	182	182	128	134	108	108	106	106	110	110	206	208
CGG04(54q)	182	182	128	130	108	108	104	104	106	110	206	206
CGG04(54r)	182	182	130	140	108	108	104	104	104	106	206	208
CGG04(54s)	182	182	078	132	108	108	106	106	104	110	206	206
CGG04(54t)	182	182	128	130	108	108	104	104	104	106	206	206
CGG04(54u)	182	182	078	144	108	108	104	104	104	110	208	208
CGG04(54v)	182	182	128	130	108	108	104	104	104	106	206	206
CGG04(54w)	182	182	124	130	108	108	104	104	104	106	208	208
CGG04(54x)	182	182	078	138	108	108	104	104	106	110	208	208
CGG04(54y)	182	182	078	138	108	108	104	104	104	110	206	208
CGG04(65a)	182	182	128	142	108	108	104	104	104	110	206	208
CGG04(65b)	182	182	078	128	108	116	104	106	104	110	208	208
CGG04(65c)	182	182	078	130	108	108	104	104	104	110	208	208
CGG04(65d)	182	182	078	140	108	116	104	106	104	106	206	206
CGG04(65e)	180	182	078	130	108	108	104	104	106	110	206	208
CGG04(65f)	180	182	130	130	108	108	104	104	104	110	206	208
CGG04(65g)	182	182	078	130	108	108	104	104	104	110	206	208
CGG04(65h)	182	182	078	128	108	108	106	106	104	104	206	208
CGG04(65i)	180	182	078	130	108	108	104	104	106	110	206	208
CGG04(65j)	182	182	128	130	108	116	104	104	104	106	206	208
CGG04(65k)	182	182	078	142	108	108	104	106	104	110	208	208
CGG04(65l)	180	182	142	142	108	108	104	104	104	110	208	208
CGG04(65m)	182	182	128	138	108	108	104	104	104	106	206	208
CGG04(65n)	182	182	128	138	108	116	104	104	106	106	208	208
CGG04(65o)	182	182	120	142	106	114	100	100	100	102	206	208
CGG04(65p)	182	182	124	142	108	116	104	104	104	106	208	208
CGG04(85a)	182	182	078	078	108	108	104	106	104	110	206	208
CGG04(85b)	182	182	078	130	108	108	104	106	104	104	206	208
CGG04(85c)	182	182	078	078	108	108	104	104	110	110	208	208
CGG04(85d)	182	182	122	128	108	116	104	104	104	110	206	208
CGG04(85e)	182	182	078	078	108	108	104	104	104	110	208	208
CGG04(85f)	182	182	078	130	108	108	104	104	104	104	206	208
CGG04(85g)	182	182	078	128	108	108	104	104	104	110	206	206
CGG04(85h)	182	182	078	138	108	108	104	104	104	104	206	208
CGG04(85i)	182	182	078	130	108	108	104	104	110	110	208	208
CGG04(85j)	182	182	130	136	108	108	104	104	104	110	206	208
CGG04(85k)	182	182	134	140	108	108	104	104	104	110	208	208
CGG04(85l)	182	182	078	128	108	108	104	104	104	110	206	206
CGG04(85m)	182	182	128	132	108	108	104	106	104	106	206	206
CGG04(85n)	180	182	122	140	108	108	104	106	106	110	208	208

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CGG04(85p)	182	182	130	136	108	108	104	104	104	110	206	208
CGG04(85q)	182	182	130	130	108	108	104	104	110	110	206	208
CGG04(85r)	000	000	078	130	108	108	106	106	104	110	208	208
CGG04(85s)	182	182	078	138	108	116	104	104	104	106	208	208
CGG04(86a)	182	182	078	124	108	108	104	104	104	106	206	208
CGG04(86b)	182	182	122	130	108	108	104	104	104	106	208	208
CGG04(86c)	182	182	124	130	108	108	104	104	104	106	208	208
CGG04(86d)	182	182	122	138	108	116	104	104	106	110	206	208
CGG04(86e)	182	182	122	130	108	108	104	106	106	106	208	208
CGG04(86f)	182	182	130	140	108	108	104	104	104	104	206	208
CGG04(86g)	182	182	130	138	108	108	104	106	104	110	208	208
CGG04(86h)	182	182	122	128	108	108	104	106	100	110	206	208
CGG04(86i)	182	182	130	138	108	108	104	106	110	110	206	208
CGG04(86j)	182	182	130	140	108	108	104	104	110	110	206	208
CGG04(86k)	182	182	130	132	108	108	104	104	104	106	206	208
CGG04(86l)	182	182	078	138	108	108	104	104	104	106	206	208
CGG04(86p)	182	182	078	130	108	108	104	104	104	110	208	208
CGG04(86q)	182	182	132	140	108	108	104	104	104	110	206	208
CGG04(86r)	182	182	128	130	108	108	102	102	102	110	208	208
CGG04(86s)	182	182	078	128	108	108	104	104	106	110	206	208
CGG04(86t)	182	182	122	136	108	108	104	104	106	110	208	208
CGG04(86u)	182	182	078	078	108	108	106	106	106	106	206	208
CGG04(86v)	182	182	078	078	108	108	104	104	104	104	206	208
CGG04(86w)	182	182	078	132	108	108	104	104	104	110	206	208
CGG04(86x)	182	182	078	078	108	108	104	104	104	110	208	208
CGG04(86y)	182	182	128	130	108	108	104	104	106	110	206	208
CGG04(86g)	182	182	122	132	108	108	104	104	106	110	206	208
CGG04(88h)	182	182	136	140	108	108	104	106	104	110	206	208
CGG04(88i)	180	182	124	142	108	108	104	106	104	104	206	208
CGG04(88j)	182	182	128	130	108	108	104	104	104	106	206	208
CGG04(88l)	182	182	078	132	108	108	104	104	104	104	206	206
CGG04(88m)	182	182	122	122	108	108	104	104	104	104	206	208
CGG04(88o)	182	182	128	130	108	108	104	104	104	106	206	208
CGG04(88p)	182	182	124	142	108	108	106	106	104	110	206	208
CGG04(88q)	182	182	078	138	108	108	104	104	104	104	208	208
CGG04(88r)	182	182	122	132	108	116	104	104	106	110	208	208
CGG04(88s)	182	182	078	128	108	116	104	104	104	106	206	208
CGG04(88t)	182	182	128	132	108	108	104	104	104	104	206	206
CGG04(88u)	182	182	078	130	108	116	104	104	104	106	206	208
CGG04(88v)	182	182	128	130	108	108	104	104	104	106	206	208
CGG04(88w)	182	182	078	142	108	108	104	104	104	110	208	208
CGG04(88x)	182	182	078	142	000	000	104	104	104	106	000	000

Appendix; Table of Genotypes

Rhossili Down

Both alleles are shown for all six loci in all successfully genotyped individuals. Within parentheses in sample names, numbers refer to the nest name and letters refer to the individual sampled from that nest.

Sample	Loci											
	FE19		FE38		FE49		FE51		FL12		FE21	
RD(1a)	180	182	080	128	110	116	104	104	104	104	206	206
RD(1b)	182	182	118	128	110	118	102	104	098	102	206	206
RD(1c)	182	182	000	000	110	118	102	102	098	098	206	206
RD(1d)	180	182	098	126	108	108	102	104	098	102	206	206
RD(1e)	182	182	124	126	110	118	102	102	098	098	206	206
RD(1f)	180	182	118	128	108	108	104	106	104	104	206	206
RD(1g)	180	182	118	136	108	108	104	104	104	104	206	206
RD(1h)	180	182	118	128	108	110	104	104	104	104	206	206
RD(1i)	180	182	118	136	108	110	104	104	104	104	206	206
RD(1j)	182	182	124	126	110	110	102	102	098	102	206	206
RD(1k)	182	182	118	128	110	110	102	104	098	098	206	206
RD(1l)	180	182	100	126	108	118	104	104	104	104	206	206
RD(2a)	180	182	080	128	108	116	104	106	104	104	206	206
RD(2b)	182	182	122	128	110	110	106	106	104	104	206	206
RD(2c)	180	182	000	000	108	110	104	104	104	104	206	206
RD(2d)	182	182	126	136	108	118	104	106	104	104	206	206
RD(2e)	180	182	116	136	108	108	102	102	098	102	206	206
RD(2f)	180	182	118	136	108	110	104	104	104	104	206	206
RD(2g)	180	182	080	128	110	116	102	104	098	102	206	206
RD(2h)	182	182	122	128	110	110	104	106	104	104	206	206
RD(2i)	180	182	080	128	110	116	104	106	104	104	206	206
RD(2j)	180	182	116	128	108	108	102	104	098	102	206	206
RD(2k)	182	182	124	128	110	118	104	104	104	104	206	206
RD(2l)	182	182	122	136	110	110	106	106	104	104	206	206
RD(3a)	182	182	126	136	108	118	104	104	098	110	206	206
RD(3b)	182	182	116	118	110	116	102	102	098	102	206	206
RD(3c)	182	182	116	118	110	110	102	104	098	102	206	206
RD(3d)	180	182	000	000	110	110	102	104	098	102	206	206
RD(3e)	182	182	128	136	110	118	104	106	104	110	206	206
RD(3f)	180	182	116	126	110	118	104	104	098	098	206	208
RD(3g)	182	182	126	132	108	118	104	104	098	110	206	206
RD(3h)	182	182	126	128	108	110	104	106	104	110	206	206
RD(3i)	182	182	122	132	108	118	102	102	098	110	206	208
RD(3j)	182	182	120	128	110	110	102	102	098	102	206	206
RD(3k)	182	182	120	128	110	110	098	102	098	102	206	206
RD(3l)	182	182	128	132	110	118	104	106	100	104	206	206
RD(4a)	182	182	126	128	108	110	102	104	098	102	206	206
RD(4b)	182	182	120	132	110	118	104	106	100	104	206	206
RD(4c)	182	182	124	126	108	110	102	102	098	110	206	206
RD(4d)	182	182	124	126	108	110	102	102	098	102	206	206
RD(4e)	180	182	116	126	108	118	102	104	098	098	206	206
RD(4f)	182	182	136	136	108	116	104	104	104	110	206	208

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RD(4g)	182	182	116	132	110	116	102	102	098	098	206	208
RD(4h)	182	182	126	136	110	110	102	104	098	102	206	206
RD(4i)	182	182	126	132	108	116	104	104	098	098	206	206
RD(4j)	180	182	116	132	110	116	102	102	098	110	206	206
RD(4k)	182	182	118	132	110	116	102	104	098	110	206	206
RD(4l)	182	182	128	132	110	116	104	104	098	110	206	206
RD(5a)	182	182	118	132	108	110	102	102	098	102	206	206
RD(5b)	182	182	098	132	110	116	102	102	098	102	206	208
RD(5c)	182	182	080	118	110	116	102	102	098	098	206	206
RD(5d)	182	182	118	132	110	116	102	102	098	098	206	206
RD(5e)	180	182	120	132	108	116	104	104	100	104	208	208
RD(5f)	182	182	098	132	110	110	102	102	098	102	208	208
RD(5g)	182	182	080	118	110	116	102	102	098	098	206	206
RD(5h)	182	182	118	132	110	116	102	102	098	102	206	206
RD(5i)	182	182	080	118	108	110	102	102	098	098	206	208
RD(5j)	180	182	118	132	108	116	102	102	098	102	208	208
RD(5k)	180	182	126	132	116	118	102	102	098	102	206	208
RD(5l)	180	182	118	132	108	116	102	102	098	098	208	208
RD(6a)	182	182	098	134	108	116	102	102	098	098	206	208
RD(6b)	182	182	098	126	108	116	102	102	098	102	206	206
RD(6c)	182	182	132	134	108	116	102	102	098	102	206	208
RD(6d)	182	182	126	132	108	118	102	102	098	102	206	208
RD(6e)	182	182	126	132	108	118	102	102	098	102	206	206
RD(6f)	182	182	098	134	108	118	102	102	098	098	206	208
RD(6g)	182	182	098	134	108	116	102	102	098	102	206	208
RD(6h)	182	182	126	132	108	118	104	104	100	100	000	000
RD(6i)	182	182	098	134	108	116	102	102	098	098	206	208
RD(6j)	182	182	126	132	108	116	102	102	098	102	206	208
RD(6k)	182	182	126	132	108	118	102	102	098	102	206	206
RD(6l)	182	182	098	126	108	118	102	102	098	098	206	206
RD(7a)	182	182	118	126	108	108	102	102	098	098	206	206
RD(7b)	180	182	098	128	110	110	102	102	098	102	206	206
RD(7c)	180	182	122	126	110	116	102	102	098	098	206	208
RD(7d)	182	182	126	134	108	108	102	104	098	098	206	206
RD(7e)	180	182	126	134	108	118	102	104	098	098	206	206
RD(7f)	180	182	080	128	110	110	104	104	100	104	206	206
RD(7g)	182	182	098	128	110	110	102	102	098	102	206	206
RD(7h)	182	182	126	134	108	118	102	104	098	098	206	206
RD(7i)	180	182	080	128	110	110	102	102	098	102	206	206
RD(7j)	180	182	098	128	110	110	102	102	098	102	206	206
RD(7k)	182	182	080	126	108	108	102	102	098	098	206	206
RD(7l)	182	182	122	126	108	110	102	102	098	098	206	208
RD(8a)	182	182	128	128	110	116	102	102	098	102	206	206
RD(8b)	182	182	128	128	110	116	102	102	098	110	206	206
RD(8c)	182	182	126	128	000	000	104	104	104	110	206	206
RD(8d)	182	182	128	128	110	116	104	104	100	110	206	206
RD(8e)	182	182	126	128	110	110	104	104	104	110	206	206
RD(8f)	182	182	128	132	108	116	104	106	104	104	206	206
RD(8g)	182	182	126	128	110	110	104	104	110	110	206	206
RD(8h)	182	182	128	130	108	116	104	106	104	104	206	206
RD(8i)	182	182	128	132	108	116	104	106	104	104	206	206
RD(8j)	182	182	128	128	110	116	102	102	098	102	206	206
RD(8k)	182	182	128	128	110	116	102	102	098	102	206	206
RD(8l)	182	182	128	128	110	116	102	102	098	102	206	206
RD(9a)	180	180	128	132	000	000	102	104	098	110	206	206

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RD(9b)	182	182	116	124	108	108	104	104	104	106	206	208
RD(9c)	182	182	120	120	108	110	102	102	098	104	206	206
RD(9d)	182	182	112	128	108	108	102	102	098	102	206	206
RD(9e)	182	182	116	134	108	108	104	104	100	104	206	208
RD(9f)	182	182	124	128	108	116	104	104	098	110	206	206
RD(9g)	180	182	132	132	108	110	102	102	098	102	206	206
RD(9h)	182	182	114	122	108	116	102	102	098	102	206	206
RD(9i)	180	180	128	132	108	110	102	104	098	110	206	208
RD(9j)	180	182	128	128	108	116	102	102	098	102	206	206
RD(9k)	180	182	128	128	110	116	102	102	098	110	206	206
RD(9l)	182	182	128	132	108	116	102	102	098	110	206	206
RD(10a)	182	182	122	126	108	116	104	106	106	110	206	206
RD(10b)	182	182	120	122	108	110	102	102	098	102	206	206
RD(10c)	180	182	124	126	110	110	102	104	098	110	206	206
RD(10d)	182	182	122	124	108	110	102	102	098	102	206	206
RD(10e)	180	182	122	130	110	110	102	104	098	098	206	208
RD(10f)	180	180	122	124	108	110	102	104	098	102	206	208
RD(10g)	180	182	122	128	110	110	102	102	098	102	206	208
RD(10h)	182	182	120	122	108	108	104	104	104	110	206	206
RD(10i)	180	180	122	124	110	118	102	104	098	102	206	208
RD(10j)	180	182	122	124	110	118	104	106	104	110	206	208
RD(10k)	182	182	120	122	108	108	104	104	104	110	206	206
RD(10l)	180	182	122	124	108	110	104	106	104	110	206	208

Appendix; Table of Genotypes

Hartland Moor

Both alleles are shown for all six loci in all successfully genotyped individuals. Within parentheses in sample names, numbers refer to the nest name and letters refer to the individual sampled from that nest.

Sample	Loci											
	FE19		FE38		FE49		FE51		FL12		FE21	
HM(1a)	180	182	082	082	110	116	106	106	106	110	206	206
HM(1b)	180	182	082	082	108	116	106	106	106	110	206	206
HM(1c)	180	182	080	082	108	110	106	108	110	110	206	208
HM(1d)	180	182	082	082	108	116	106	106	106	110	206	206
HM(1e)	180	182	082	082	108	116	106	106	110	110	206	206
HM(1f)	180	182	082	082	110	116	106	106	106	110	206	206
HM(1g)	180	182	082	082	110	116	106	106	106	110	206	206
HM(1h)	180	182	082	082	110	116	106	106	106	110	206	206
HM(1i)	180	182	082	082	108	116	106	106	110	110	206	206
HM(1j)	180	182	082	082	108	116	106	106	110	110	206	206
HM(1k)	180	182	082	082	108	116	106	106	110	110	206	206
HM(1l)	180	182	082	082	108	116	106	106	106	110	206	206
HM(2a)	180	182	082	082	110	116	104	106	110	110	206	206
HM(2b)	180	182	082	082	108	116	104	108	110	110	206	206
HM(2c)	182	182	080	082	108	116	104	106	098	110	206	206
HM(2d)	182	182	082	082	108	110	102	102	098	110	206	206
HM(2e)	182	182	080	112	108	116	102	106	098	110	206	208
HM(2f)	182	182	082	120	116	116	102	106	098	110	206	208
HM(2g)	180	182	082	120	110	110	104	104	110	110	206	206
HM(2h)	180	182	082	082	110	116	104	104	110	110	206	208
HM(2i)	180	182	082	118	110	116	102	106	098	098	206	206
HM(2j)	182	182	080	096	108	110	104	106	110	110	206	206
HM(2k)	180	180	082	084	110	116	102	104	098	110	206	206
HM(2l)	182	182	082	118	110	110	102	102	098	110	206	206
HM(3a)	180	182	082	122	108	108	104	108	110	110	206	206
HM(3b)	182	182	080	080	108	110	104	104	110	110	206	206
HM(3c)	180	180	082	082	108	116	104	108	110	110	206	206
HM(3d)	182	182	080	082	108	116	104	104	110	110	206	206
HM(3e)	180	182	078	082	110	110	104	104	110	110	208	208
HM(3f)	180	180	082	082	116	116	104	108	110	110	206	206
HM(3g)	180	182	082	082	110	116	104	104	110	110	206	206
HM(3i)	182	182	082	084	108	108	104	106	110	110	206	206
HM(3j)	182	182	080	082	108	116	104	104	110	110	206	206
HM(3k)	182	182	080	120	108	108	104	106	110	110	206	206
HM(3l)	182	182	080	082	106	108	100	100	096	108	206	206
HM(4a)	182	182	082	082	108	110	102	104	098	110	206	208
HM(4b)	180	182	080	122	108	110	104	108	110	110	206	206
HM(4c)	182	182	082	082	108	110	102	104	098	110	206	206
HM(4d)	180	182	082	112	110	110	102	106	098	110	206	206
HM(4e)	182	182	082	082	110	110	104	104	098	110	206	206
HM(4f)	182	182	082	082	108	110	104	104	098	110	206	206

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HM(4g)	182	182	082	082	108	110	106	106	100	110	206	206
HM(4h)	182	182	080	118	110	110	102	102	098	110	206	206
HM(4i)	180	182	080	082	108	110	104	104	110	110	206	206
HM(4j)	180	182	080	122	108	110	104	104	110	110	206	206
HM(4k)	182	182	082	082	110	110	104	104	098	110	206	206
HM(4l)	182	182	082	082	110	116	104	106	110	110	206	206
HM(5a)	182	182	082	124	110	110	106	106	110	110	206	206
HM(5b)	180	182	114	122	108	110	106	108	110	110	206	206
HM(5c)	182	182	082	124	110	110	104	106	110	110	206	206
HM(5d)	180	182	110	120	108	110	104	106	098	110	206	206
HM(5e)	182	182	082	082	108	110	104	106	110	110	206	206
HM(5f)	180	182	112	122	108	108	106	108	110	110	206	206
HM(5g)	182	182	082	082	108	110	104	106	110	110	206	206
HM(5h)	180	182	112	120	108	108	104	106	098	110	206	206
HM(5i)	182	182	082	082	110	110	104	106	110	110	206	206
HM(5j)	180	182	112	122	108	110	106	108	110	110	206	206
HM(5k)	182	182	082	124	108	110	104	106	110	110	206	206
HM(5l)	182	182	082	082	108	110	104	106	110	110	206	206
HM(6a)	182	182	082	112	116	116	104	104	110	110	206	206
HM(6b)	180	182	082	120	108	110	104	104	110	110	206	206
HM(6c)	180	182	082	118	108	116	102	106	110	110	206	206
HM(6d)	182	182	082	112	116	116	104	108	110	110	206	206
HM(6e)	182	182	082	082	116	116	104	104	110	110	206	206
HM(6f)	182	182	082	110	116	116	102	106	098	110	206	206
HM(6g)	182	182	082	120	110	110	108	108	110	110	206	206
HM(6h)	180	182	082	120	108	110	104	104	110	110	206	206
HM(6i)	180	182	082	120	108	116	104	104	110	110	206	206
HM(6j)	180	182	082	120	108	110	104	104	110	110	206	206
HM(6k)	180	182	082	120	108	116	104	104	110	110	206	206
HM(6l)	180	182	082	082	116	116	104	108	100	110	206	206
HM(6m)	182	182	082	112	116	116	104	104	100	110	206	206
HM(7a)	180	180	080	122	108	116	106	106	106	110	206	206
HM(7b)	180	182	080	082	108	108	106	106	106	110	206	206
HM(7c)	180	182	082	082	110	116	106	108	110	110	206	206
HM(7d)	180	180	080	082	108	116	106	106	106	110	206	206
HM(7e)	180	180	082	082	108	108	106	108	110	110	206	206
HM(7f)	182	182	082	114	108	108	104	108	110	110	206	206
HM(7g)	182	182	082	082	116	116	104	106	110	110	206	206
HM(7h)	182	182	000	000	000	000	106	106	110	110	206	206
HM(7i)	182	182	082	082	108	116	104	108	110	110	206	206
HM(7j)	180	182	082	082	108	116	106	108	100	110	206	206
HM(7k)	182	182	114	122	108	116	104	108	110	110	206	206
HM(8a)	182	182	082	110	108	116	102	106	098	110	206	206
HM(8b)	182	182	082	112	108	116	104	108	110	110	206	206
HM(8c)	182	182	082	110	110	116	102	106	098	110	206	206
HM(8d)	182	182	082	112	110	116	104	108	110	110	206	206
HM(8e)	182	182	082	112	110	116	104	108	110	110	206	206
HM(8f)	182	182	082	112	108	116	104	108	110	110	206	206
HM(8g)	182	182	082	112	108	116	104	108	110	110	206	206
HM(8h)	182	182	082	110	110	116	102	106	098	110	206	206
HM(8i)	182	182	082	112	108	116	104	108	110	110	206	206
HM(8j)	182	182	082	110	110	116	102	106	098	110	206	206
HM(8k)	182	182	082	110	110	116	102	106	098	110	206	206
HM(9a)	182	182	082	082	110	116	104	104	110	110	206	206
HM(9b)	182	182	082	122	108	108	104	108	110	110	206	206

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HM(9c)	182	182	000	000	106	114	100	100	096	108	206	206
HM(9d)	182	182	082	082	108	110	104	106	100	110	206	206
HM(9e)	182	182	080	120	108	110	106	106	100	110	206	206
HM(9h)	182	182	084	112	108	116	104	104	110	110	206	206
HM(9i)	180	182	082	082	108	110	104	106	100	110	206	206
HM(9j)	180	182	082	122	116	116	104	104	106	110	208	208
HM(9k)	182	182	082	082	116	116	104	106	106	110	208	208
HM(9l)	180	182	082	082	116	116	104	106	106	110	208	208
HM(10a)	180	182	080	082	108	108	102	102	098	098	206	206
HM(10b)	180	182	080	080	116	116	106	106	110	110	206	206
HM(10c)	180	182	082	082	108	110	106	108	110	110	206	206
HM(10d)	182	182	082	082	108	110	106	108	110	110	206	206
HM(10e)	182	182	082	082	108	110	106	106	106	110	206	206
HM(10f)	180	182	080	082	110	116	102	104	098	110	206	208
HM(10g)	182	182	082	082	108	108	106	106	106	110	206	206
HM(10h)	182	182	082	082	108	110	106	106	106	110	206	206
HM(10i)	182	182	082	082	110	116	104	104	098	110	206	206
HM(10j)	182	182	080	082	110	110	104	106	098	110	206	206
HM(10k)	182	182	082	082	110	116	106	108	110	110	206	206
HM(10l)	182	182	082	082	108	108	104	106	106	110	206	206

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New Forest

Both alleles are shown for all six loci in all successfully genotyped individuals. Within parentheses in sample names, numbers refer to the nest name and letters refer to the individual sampled from that nest.

	FE19		FE38		FE49		FE51		FL12		FE21	
NF(10a)	182	182	082	082	108	110	104	104	106	110	206	206
NF(10c)	182	182	080	116	106	108	100	100	096	108	206	206
NF(10d)	182	182	080	082	106	114	100	100	108	108	206	206
NF(10e)	182	182	082	082	108	110	104	106	106	106	206	206
NF(10f)	182	182	080	082	108	110	104	106	106	110	206	208
NF(10g)	182	182	082	082	108	116	104	104	100	110	206	208
NF(10h)	182	182	080	082	110	116	104	104	106	106	206	206
NF(10i)	182	182	080	082	108	114	100	100	102	102	206	208
NF(10j)	182	182	080	082	108	116	104	106	106	106	206	208
NF(10k)	182	182	080	082	108	116	104	104	106	106	206	208
NF(10l)	182	182	080	082	108	110	104	104	106	106	206	206
NF(1a)	182	182	082	082	116	116	104	106	110	110	206	206
NF(1b)	182	182	082	082	110	110	104	106	098	110	206	208
NF(1c)	182	182	082	082	116	116	102	106	098	110	206	208
NF(1d)	182	182	080	082	116	116	104	104	110	110	206	208
NF(1e)	182	182	082	082	110	116	104	106	106	110	208	208
NF(1f)	182	182	082	082	108	108	104	106	110	110	206	206
NF(1g)	182	182	080	082	110	110	102	104	098	104	206	206
NF(1h)	182	182	080	082	108	110	102	104	098	110	206	208
NF(1i)	182	182	082	082	108	110	102	104	098	110	206	208
NF(1j)	182	182	082	082	110	110	104	104	110	110	208	208
NF(1l)	182	182	080	080	110	110	104	106	106	110	000	000
NF(1m)	182	182	080	082	108	116	106	106	110	110	206	206
NF(1q)	182	182	080	082	110	116	098	102	098	110	208	208
NF(2a)	182	182	080	082	116	116	102	104	098	110	206	206
NF(2b)	182	182	082	082	110	116	104	104	110	110	206	208
NF(2c)	182	182	080	082	110	110	104	104	098	110	206	208
NF(2d)	182	182	080	082	110	116	102	102	098	110	206	208
NF(2e)	182	182	082	082	108	116	104	106	110	110	206	206
NF(2f)	182	182	080	114	108	108	104	104	098	110	206	208
NF(2g)	182	182	082	082	110	110	104	104	110	110	206	208
NF(2h)	182	182	082	082	110	116	102	104	098	110	206	208
NF(2i)	182	182	080	082	116	116	102	104	098	110	206	208
NF(2j)	182	182	080	082	110	110	102	104	098	110	208	208
NF(2k)	182	182	080	082	110	116	102	104	098	110	206	206

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NF(2m)	182	182	080	082	110	116	104	104	110	110	208	208
NF(2n)	182	182	082	082	110	116	104	106	110	110	206	208
NF(3a)	182	182	082	082	110	110	104	108	106	110	206	208
NF(3b)	182	182	082	082	110	116	104	108	110	110	206	206
NF(3c)	182	182	082	120	108	110	104	106	100	106	206	206
NF(3d)	182	182	082	082	110	110	106	108	100	110	206	208
NF(3e)	182	182	080	082	116	116	106	106	100	100	206	206
NF(3f)	182	182	082	082	116	116	104	106	100	100	206	208
NF(3g)	182	182	082	120	108	108	106	108	106	110	206	208
NF(3h)	182	182	082	082	110	116	106	108	100	110	206	206
NF(3i)	182	182	082	082	108	110	104	104	110	110	208	208
NF(3j)	182	182	082	084	108	116	104	108	106	110	206	206
NF(4a)	182	182	080	082	116	116	104	108	106	110	206	208
NF(4b)	182	182	082	118	108	116	104	106	098	110	208	208
NF(4c)	182	182	080	084	110	116	104	104	110	110	206	208
NF(4d)	182	182	080	118	110	116	102	102	098	110	206	206
NF(4e)	182	182	082	082	110	116	102	106	098	110	206	206
NF(4f)	182	182	082	082	110	110	104	106	098	110	206	206
NF(5a)	182	182	082	082	108	116	102	104	098	110	206	206
NF(5b)	182	182	082	082	110	110	106	108	106	110	206	208
NF(5c)	182	182	082	082	110	116	104	106	098	098	206	208
NF(5d)	182	182	082	082	110	110	104	106	106	110	206	208
NF(5e)	182	182	082	082	108	110	104	106	098	110	206	206
NF(5f)	182	182	082	082	110	110	104	106	106	110	206	208
NF(5g)	182	182	080	082	110	116	104	104	106	110	206	208
NF(5h)	182	182	082	082	116	116	104	106	106	110	206	206
NF(5I)	182	182	080	082	108	110	104	106	106	110	206	208
NF(5J)	182	182	082	082	108	110	104	106	098	110	206	208
NF(6a)	182	182	082	120	108	110	104	106	110	110	206	206
NF(6b)	182	182	082	120	108	108	104	106	100	110	206	206
NF(6c)	182	182	080	082	114	114	100	100	096	108	206	208
NF(6d)	182	182	082	082	114	114	100	100	096	108	206	208
NF(6e)	182	182	080	080	116	116	104	106	100	110	206	208
NF(6f)	182	182	080	080	106	114	100	100	096	096	206	208
NF(6g)	182	182	082	082	110	116	104	106	110	110	206	206
NF(6h)	182	182	080	082	108	110	104	106	110	110	206	208
NF(6i)	182	182	080	080	108	116	108	108	100	100	206	206
NF(6j)	182	182	080	120	108	116	104	106	110	110	206	206
NF(6k)	182	182	080	082	116	116	104	106	106	110	206	208
NF(6l)	182	182	080	082	110	116	104	108	100	110	206	206
NF(7a)	182	182	082	084	116	116	104	106	100	110	208	208
NF(7b)	180	182	080	082	110	110	104	106	100	110	206	206
NF(7c)	182	182	080	080	108	110	106	106	100	110	206	208
NF(7d)	182	182	080	082	116	116	104	108	110	110	206	208
NF(7e)	182	182	080	082	110	116	106	106	110	110	208	208
NF(7f)	182	182	082	120	108	108	104	106	100	110	206	208
NF(7g)	182	182	080	082	110	116	104	106	110	110	206	208
NF(7h)	182	182	080	082	110	116	104	104	100	110	206	206

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NF(7i)	182	182	080	082	108	116	104	106	100	110	206	206
NF(7j)	182	182	082	082	108	116	106	108	100	110	206	206
NF(7k)	182	182	080	082	108	110	104	108	110	110	206	208
NF(7l)	182	182	080	082	108	108	104	108	100	110	206	208
NF(8c)	182	182	080	120	116	116	104	106	110	110	206	208
NF(8d)	182	182	082	122	108	110	104	104	110	110	206	206
NF(8e)	182	182	082	082	116	116	102	102	098	110	206	208
NF(8f)	182	182	082	118	108	116	102	104	098	110	206	208
NF(8i)	182	182	082	084	110	116	104	106	110	110	206	208
NF(8j)	182	182	080	082	116	116	104	106	110	110	206	208
NF(8k)	182	182	080	084	110	116	104	106	110	110	206	208
NF(8l)	182	182	082	082	108	110	102	104	098	104	206	208
NF(8m)	182	182	080	082	108	110	102	104	098	110	206	208
NF(8n)	182	182	080	082	108	110	104	104	098	110	206	208
NF(8o)	182	182	082	120	000	000	106	106	110	110	206	208
NF(8p)	182	182	080	080	108	110	104	108	106	110	206	208
NF(8q)	182	182	082	082	116	116	102	102	098	110	206	208
NF(8r)	182	182	082	122	108	110	104	104	110	110	206	206
NF(9c)	182	182	082	082	108	108	104	106	106	110	206	206
NF(9d)	182	182	080	082	108	110	104	104	110	110	206	208
NF(9e)	182	182	080	082	108	110	104	106	110	110	206	206
NF(9f)	182	182	080	082	108	116	102	102	098	110	206	206
NF(9i)	182	182	082	122	108	110	104	104	106	110	206	206
NF(9j)	182	182	082	120	110	116	106	106	110	110	208	208
NF(9k)	182	182	080	082	116	116	104	106	110	110	206	208
NF(9l)	182	182	082	082	110	110	102	104	098	110	206	208

