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Hannaleena Mäki-Petäys

CONSERVATION AND MANAGEMENT OF POPULATIONS IN A FRAGMENTED FOREST LANDSCAPE

BEHAVIOURAL ECOLOGY MEETS POPULATION GENETICS

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HANNALEENA MÄKI-PETÄYS

CONSERVATION AND MANAGEMENT OF POPULATIONS IN A FRAGMENTED FOREST LANDSCAPE

Behavioural ecology meets population genetics

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Abstract

The effects of habitat loss and fragmentation on the genetic structure and vulnerability of populations strongly depend on the behaviour of a particular species. In this thesis, I examined the effects of forest fragmentation on genetic population structure with the aim of identifying and evaluating the different genetic and behavioural factors important for species conservation and management on different geographical scales. The species studied were the mound building red wood ants *Formica lugubris* and *F. aquilonia*, and a lekking bird, the capercaillie, *Tetrao urogallus*.

Habitat loss and fragmentation affected the genetic structure in both wood ants and capercaillie. In general, the effects were related to the time since fragmentation and to the level of habitat loss and isolation from the other existing populations. The loss of genetic diversity due to population fragmentation was less observable than the differences in population structure. The response to habitat fragmentation was further dependent on species characteristics such as dispersal and mating behaviour. Sociality affected the genetic vulnerability of wood ant populations by decreasing gene diversity, increasing inbreeding depression and restricting gene flow between subpopulations. The results on the capercaillie in turn suggested that lekking behaviour restricts dispersal of both sexes, thus elevating the occurrence of inbreeding between individuals.

The present study provided important information on species conservation and management in terms of better understanding species' biology and behaviour, as well as increased knowledge concerning the genetic issues that should be taken into account when planning conservation actions. By examining the genetic structure of the species it was possible to clarify the conservation status including the effective population size, the question of origin, and the genetic vulnerability (genetic diversity, inbreeding and inbreeding depression) of the populations and/or species. Overall, the results emphasised the importance of preserving the effective population size and the connectivity of habitat patches when planning species specific management strategies. There were great differences in conservation needs among the species, which should be taken into account especially in local management actions.

Keywords: conservation genetics, genetic diversity, lekking behaviour, sociality

To my loving family

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Oulu, December 2006 Hannaleena Mäki-Petäys

Abbreviations

bp	base pair
IBD	Isolation by Distance
MtDNA	Mitochondrial DNA
PCR	Polymerase chain reaction
SSCP	Single stranded Conformation Polymorphism

List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Mäki-Petäys H, Zakharov A, Viljakainen L, Corander J & Pamilo P (2005) Genetic changes associated to declining populations of *Formica* ants in fragmented forest landscape. Mol Ecol 14: 733-742.

II Mäki-Petäys H & Breen J (2006) Genetic vulnerability of a remnant ant population (Conserv Genet, DOI10.1007/s10592-006-9182-1).

III Mäki-Petäys H, Corander J, Aalto J, Liukkonen T, Helle P & Orell M (2007) No genetic evidence of sex-biased dispersal in a lekking bird, the capercaillie (*Tetrao urogallus*) (J Evol Biol, Accepted).

IV Mäki-Petäys H, Liukkonen T, & Orell M (2007) Genetic structure and vulnerability of the lekking bird, the Capercaillie (*Tetrao urogallus*), in fragmented forest landscape (Manuscript).

Contents

1 Introduction

Resources for conservation are always limited. To maximise the benefits of any conservation action, it is worthwhile to focus on the highest conservation priorities. Thus, it is necessary to make judgments concerning the relative importance of different areas on a range of different scales (Sutherland 2000).

The conservation importance of an area is typically determined by assessing its biodiversity. Biodiversity is the popular term for the richness and diversity of life (Pullin 2002). It is simply biological diversity for short, having several different scientific definitions (Gaston 1998). Biodiversity covers both the number of different populations and species that exist, and the complex interactions that occur among them. Overall, biodiversity can be viewed as nested components of one or more organisational hierarchies. The most widely accepted is the hierarchy that distinguishes between ecological diversity, organism diversity and genetic diversity (Heywood 1995, Gaston 1998). Ecological diversity comprises populations, niches, habitats, ecosystems, landscapes, bioregions and biomes, whereas organism diversity comprises individuals, populations, subspecies, species, genera, families, phyla and kingdoms. Genetic diversity is commonly used to describe the heritable variation that is found within biological entities. It is reflected in the differences among individuals for many characters. Additionally, it encompasses the whole variety of alleles and genotypes present in a population or species (Frankham *et al.* 2002).

The maintenance of genetic diversity is a principal objective in the management of populations of threatened species (Frankham *et al.* 2002). The loss of genetic diversity, i.e. genetic erosion, is often associated with reduced reproductive fitness (Crnokrak & Roff 1999). Additionally, genetic diversity is the raw material for adaptive evolutionary change and thus, genetic erosion diminishes the capacity of populations to respond to environmental change and in the long term increases the risk of extinction (Keller & Weller 2002) (Fig. 1). The risk of genetic erosion is highest in small and isolated populations. This can particularly be the case where a single continuous population has been fragmented into smaller sub-populations.

1.1 Genetic consequences of habitat fragmentation

Habitat loss and fragmentation due to human land use are considered to be the most important deterministic threats to many species (Heywood 1995). When considering the fate of single species, a major consequence of habitat fragmentation is that populations are fragmented into smaller isolated units. The isolation can lead to reduced dispersal between remaining subpopulations, and the viability of subpopulations may become jeopardised by demographic, environmental or genetic stochasticity factors, which are amplified especially in small populations (Holsinger 2000, Keller & Weller 2002) (Fig.1).

From a genetic point of view, the main impact of population fragmentation is the loss of genetic diversity and increased genetic differentiation of remaining populations. The risk of genetic erosion is highest in a small and isolated population because of increased genetic stochastic and reduced interpopulation gene flow (Gilpin 1991, Raijmann *et al.* 1994, Hedrick 2001) (Fig. 1.).

Genetic stochasticity comprises random loss of genetic variation through genetic drift, and elevated inbreeding. Genetic drift describes random fluctuations in allele frequency. The effect of genetic drift is insignificant in large populations since the fluctuations tend to cancel each other out over the population as a whole. In small populations, in turn, genetic drift results in loss of genetic diversity, random changes in allele frequencies and diversification among populations. Inbreeding refers to the mating of individuals related by descent. Related individuals are more likely to share an allele in common than unrelated individuals, thus, there is an increased probability that alleles will occur in the homozygous state in a descendant. Gene pools of populations contain recessive alleles, which are deleterious if expressed in individual phenotypes. When a population is small, relatives are more likely to mate with each other (demographic stochasticity) and there is an increased probability that deleterious alleles will occur in the homozygous state and be expressed. This can lead to a reduction in reproduction and survival that is referred to as inbreeding depression (Lowe *et al.* 2004, Frankham *et al.* 2002, Pullin 2002).

The genetic consequences of fragmentation depend on the effective population size (N_E) rather than on the number of individuals in the population (N). The effective population size is the size of an idealised population that has the same genetic properties as observed for the actual population (Wright 1931). In nature, the effective size of a population is usually much smaller that the census size (N) (Frankham 1995).

In addition to small population size, the effects of fragmentation depend critically upon the level of gene flow among fragments. The amount of gene flow, in turn, is dependent on factors related to environmental and population patterns, such as the number and spatial structure of populations. There are several potential fragmented population structures that can be distinguished (e.g. Frankham *et al.* 2002), for example island models (migration is equal among equal sized islands), stepping stone models (neighbouring populations exchange migrants), source-sink models (migration from source to sink populations) and metapopulations (regular extinction and colonisation events) (Hanski & Gilpin 1997). The effects of fragmentation are also time-dependent, as well as being influenced by the dispersal ability of the species and associated migration rates between the remaining habitat fragments.

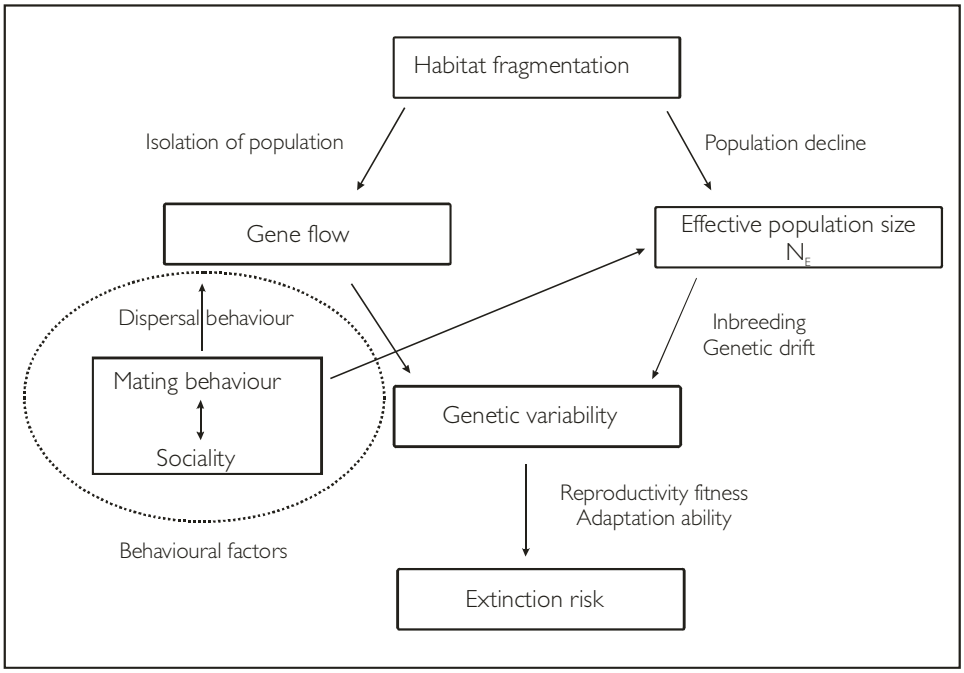

Fig. 1. The effects of habitat fragmentation on genetic structure and extinction risk of a population, associated with the behavioural factors studied.

1.2 Behavioural factors in conservation

There are several behavioural factors that affect the genetic vulnerability of a population or species to fragmentation. For example, dispersal behaviour is one of the main determinants of the level of gene flow between population fragments, whereas breeding behaviour also influences the effective population size of a species. Dispersal behaviour is further connected to breeding, mating and social behaviour, and altogether, behavioural factors create a complex interactive network of factors (Fig.1).

1.2.1 Dispersal and mating behaviour

There are great differences in dispersal behaviour between species. Some animals may range over extremely large distances whereas others remain in their natal site. There are some generalisations throughout a taxa, for example, marine mammals and birds can migrate thousands of kilometres in a season maintaining genetic connectivity of dispersed populations (Avise 1994). In genetic studies it has been observed that the genetic differentiation of populations tends to be lowest in birds and insects, and highest in

amphibians (Frankham *et al.* 2002). However, even if the dispersal ability of a species may offer potential gene flow between populations, there can be behavioural factors which may favour philopatry, i.e. reproducing at the birth site, leading to reduced gene flow and to a strong genetic differentiation of populations (Lowe *et al.* 2004). In addition to the level of gene flow, the breeding behaviour greatly affects the effective population size of the species.

One aspect of breeding behaviour which is potentially important for effective population size and gene flow is polygamy. In socially monogamous species, males and females form a pair bond that lasts at least a part of or a whole breeding season, and both parents often take care of the offspring. When an individual of one sex mates with several members of the other, the species is polygamous. A polygamous relationship most often involves a single male that mates with several females (called polygyny) while a female mates only with one male and usually provides parental care. There are also species in which a single female mates with several males (polyandry), where males mostly take care of the offspring. In promiscuous species, both males and females mate several times with different individuals and either sex may care for the eggs or young (Krebs & Davies 1993).

Mating behaviour is often connected to the dispersal behaviour of a species. In many species of birds and mammals, one sex disperses more than the other (reviewed by Clarke *et al.* 1997 and Greenwood 1980). The dispersing sex tends to be female in birds and male in mammals. It has been argued that the direction of sex bias in dispersal is connected to the type of mating system (Greenwood 1980). Many avian species are monogamous and the mating system is usually based on competition for territories. In this resource defence system, males benefit from philopatry because of familiarity with resources, and females benefit from dispersal, which allows them to choose among males and their defended resources. In a mate defence mating system, common for many mammalian species, males compete for a female or group of females, and polygyny is favoured (Greenwood 1980).

Restricted dispersal may lead to reduced gene flow between the populations causing high genetic structuring at the population level. Consequently, gender biased differences in dispersal behaviour can cause differences in the genetic structure of populations between the sexes (if individuals are sampled after dispersal), but also between differently inherited genomes (Prugnolle & de Meeus 2002) depending, for example, on the breeding characteristics and mating system (Chesser & Baker 1996) (see 2.4.4 and paper III).

The effect of mating system on genetic diversity and population structure is also connected to effective population size. Mating in an ideal population is very simplified; all individuals are potential breeders and mating is random with no selection involved (Frankham *et al.* 2002). In turn, in nature individuals can show great differences in their mating success as a consequence of sexual selection. One of the ultimate bases of mate choice is suggested to be genetic quality of the individual (e.g. Krebs & Davies 1993). This is likely to be important especially in polygynous species, in which males do not provide any parental care, and sperm is their only contribution to offspring. A good example of such a mating system is the polygynous lek mating system that occurs in a large number of taxa (reviewed by Höglung & Alatalo 1995).

1.2.1.1 The lek mating system

Leks consist of a group of males that display in small areas. Females visit the lek sites and choose among courting males. After mating, there is no further contact between males and females. As a result of strong selection on male traits, mating success is strongly skewed, with the majority of mating performed by a small proportion of males on the lek (Höglung & Alatalo 1995). The small proportion of reproducing males makes the effective size of the population much smaller than that of monogamous species.

Lekking behaviour is shown by many species of birds (Hjorth 1970, Beehler & Pruett-Jones 1983) and mammals (Lazenby-Cohen & Cockburn 1988, Pemberton & Balmford 1987), but also by several species of insects (Beani & Turilazzi 1990), fish (McKaye *et al.* 1990) and amphibians (Bourne 1992). Several hypotheses have been proposed to explain the aggregation of males into leks (reviewed by Höglung & Alatalo 1995), for example, males are suggested to aggregate on hotspots of females (Bradbury *et al.* 1986), to reduce predation (Hjorth 1970, Wiley 1974) and to increase female attraction (Queller 1987, Alatalo *et al.* 1992). It has been also suggested that males could enhance their inclusive fitness by increasing the lek size of related males (kin selection theory, Kokko & Lindström 1996) as females prefer to mate in large male aggregations rather than in small ones (Alatalo *et al.*1992).

In addition to small effective population size, lekking behaviour may increase the genetic vulnerability of a population or species to habitat fragmentation by decreasing the level of gene flow. It has been suggested that the dispersal of the lekking species can be very restricted and sex-biased with males being the philopatric sex as a result of a resource defence system and / or kin selection (see more in paper III and IV). However, only limited data are available for lekking species, and these studies suggest both female- and male-biased dispersal (e.g. Dunn & Braun 1985; Martin *et al.* 2001).

1.2.2 Importance of sociality for conservation

The social behaviour of some insects (Hymenoptera; ants, bees and wasps, Isoptera; termites) provides good examples of extreme altruism, where self-sacrifice reaches the point where a large number of individuals are completely sterile. These sterile individuals, workers, do not reproduce themselves but help care for the young of others, the reproductive queens (Krebs & Davies 1993). Social insects can be divided into monogynous and polygynous species depending on their social structure. Species that usually have just one queen per colony are called monogynous, whereas those with more than one queen are called polygynous (e.g. Pamilo & Crozier 1997). The number of queens in a nest can also vary among populations within the same species.

Social insects represent a very large fraction of animal biomass in terrestrial ecosystems worldwide, having a large impact on other species and on the structure and function of ecological communities (Chapman & Bourke 2001). However, their special features have received only little attention in conservation discussions (Pamilo & Crozier 1997). Social insects have several unique features that are important when evaluating the vulnerability of their populations (Pamilo & Crozier 1997, Chapman and Bourke 2001):

the ratio of effective population size to biomass is low; social structure may restrict the dispersal of individuals and promote spatial differentiation (Rosengren & Pamilo 1983, Seppä & Pamilo 1995); and the male haploid sex-determining system, ancestral in aculeate Hymenoptera (Crozier 1971, Cook and Crozier 1995), readily leads to inbreeding depression.

The number of queens per nest is a major determinant of effective population size (N_E) as workers are usually sterile and do not contribute to the breeding population. In Hymenoptera, the effective population size is still lower than that of diploids with the same census size, because a haploid male carries half the genetic material compared to diploid females. For example, one colony of army ant (*Eciton burchelli*) in Barro Colorado Island can consist of a single queen and ca. 400 000 sterile workers (Franks 1982). The total number of individuals on the island is approximately 20 million, but with single mating of queens, the effective population size is only 75 (Chapman & Bourke 2001).

Dispersal behaviour is connected to the number of queens in a nest (Seppä & Pamilo 1995, Gyllenstrand 2002). In polygynous species, i.e. species with many queens within the nest, dispersal is often restricted because females can stay in natal colonies without dispersing at all (Rosengren & Pamilo 1983, Chapuisat & Keller 1999) and new nests are often established in the neighbourhood by budding (Keller 1991). Conversely, females from monogynous (single queen) nests disperse by flight and establish new colonies independently. It has been suggested that monogynous species may even gain a competitive advantage from habitat fragmentation as a result of their better dispersal ability (Punttila *et al.* 1994). Restricted gene flow and a small effective population size, resulting from a low ratio of reproductive individuals to the biomass of a colony, may easily lead to inbreeding and inbreeding depression (Pamilo & Crozier 1997).

The genetic vulnerability of many social insects is further affected by their complementary sex determination system, which makes them sensitive to inbreeding depression (Crozier 1971, Cook & Crozier 1995). Diploid individuals homozygous at the sex-determining locus will develop into diploid males, which are normally sterile and/or unviable, thus increasing colony mortality and decreasing colony growth rate (Ratnieks 1990, Ross *et al.* 1993). Small populations are expected to have a low level of genetic diversity at the sex locus and thus a high level of diploid male production. However, little data exist on the frequency of diploid males in most social insects, especially in ants.

1.3 Study species

Many species are threatened by the loss and fragmentation of boreal forests (e.g. Anon 1992). The wood ant (*Formica rufa* group) and the capercaillie (*Tetrao urogallus*) represent native species of boreal coniferous forests, and typically occur in mature forest with mixed tree stands of medium density. The species are severely threatened by habitat loss due to human land use in southern and western parts of their distribution range, whereas in their continuous distribution range, they still form a tight network of populations. Even if they share the same problem, their response to habitat loss and fragmentation may vary greatly due to their special features in behaviour.

1.3.1 Wood ants

Mound building red wood ants play an important role in coniferous forests of the northern hemisphere, and their ecological effects extend over most trophic levels (*e.g.* Hölldobler & Wilson 1990, Rosengren & Sundström 1991, Elgmork & Kaasa 1992). However, all species are included on the World Conservation Union (IUCN) red list of threatened species (Hilton-Taylor 2000). The major threats are caused by human impact. In particular, forest cutting has had several negative impacts on the viability of ants: causing direct damage of nests; exposing nests by removing protective vegetation; destroying food resources (particularly the aphid colonies in the trees, Rosengren & Sundström 1991); and altering the visual cues used for canopy orientation (Rosengren & Pamilo 1978).

The study species, the Scottish wood ant (*Formica aquilonia)* and the hairy wood ant *(Formica lugubris*), are territorial and often locally ecologically dominant. Both species are widely distributed from Scandinavia, south to northern Italy and from Ireland, east to Russia. They are also found in coastal areas in eastern Siberia. The social structure of the species differs greatly. *Formica aquilonia* is typically highly polygynous having many reproductively active queens whereas *F. lugubris* is socially polymorphic. The populations of *F. lugubris* have been described as monogynous in Ireland (Breen 1976), Fennoscandia (Pamilo *et al.* 1994) and Switzerland (Bernasconi *et al.* 2005), and polygynous populations have been found in England (Gyllenstrand & Seppä 2003) and in the Swiss Alps (Bernasconi *et al.* 2005).

The ability of ants and other social insects to respond to habitat fragmentation has been suggested to depend on the social structure of the species. However, earlier studies of the effects of fragmentation on population structure of social insects, including studies with *F. lugubris* (Gyllenstrand & Seppä 2003) and *F. aquilonia* (Punttila 1996), have been conducted a few years after fragmentation or they lack details from the fragmentation process (e.g. Bestelmeyer & Wiens 1996, Carvalho & Wasconcelos 1999). Additionally, most of the studies on wood ant populations have been conducted in their continuous distribution range (e.g. Pamilo *et al.* 1994, Seppä *et al.* 2004), whereas little is known about the isolated populations under severe threat of extinction.

1.3.2 Capercaillie

The capercaillie (*Tetrao urogallus*) is a large and sexually dimorphic grouse species. It is suggested to be an ‘umbrella species’ of old-growth forest (Suter *et al.* 2004). In its continuous distribution range, the capercaillie inhabits boreal coniferous forests from Scandinavia to eastern Siberia. The distribution in western and central Europe is more scattered due to the naturally patchy distribution of montane conifer forests, but also due to severe habitat loss. The capercaillie still occupies most of its original range, although serious declines in western and central Europe have resulted in local extinctions (Status survey and conservation action plan 2002-2004). Currently the species is protected by law in several European countries and it is included in the respective national Red Books (Storch 2000). In the boreal distribution range, the population size of the capercaillie is

still relatively large, but has declined quickly over the last decades. Major threats include forest fragmentation and changes in the age composition of forests due to human land use (Helle *et al.* 1999, Rolstad 1989a).

The capercaillie is suggested to be susceptible to habitat changes due to its large spatial requirements. It also has a polygynous lek mating system, which has some characteristics that may increase the vulnerability of the populations in fragmented forest landscapes. Capercaillie mating occurs at leks that consist of small groups of displaying males visited by females (Rolstad 1989a). According to field observations, the distribution of mating is highly skewed and only a small proportion of the males reproduce (Hjorth 1970). This makes the effective population size compared with census size smaller than that of monogamous species. Lekking behaviour has also been suggested to lead to restricted dispersal. Males tend to be devoted to their leks and females usually prefer the same lek site and even the same male from year to year (Hjorth 1970; Wegge & Rolstad 1986). Moreover, it is commonly suggested that the dispersal of the capercaillie is very restricted and/or sex-biased with males being the philopatric sex (e.g. Lindén 2002, Segelbacher & Storch 2002). However, information is almost nonexistent concerning juvenile dispersal rates and dispersal distances, and their roles in population genetics, dynamics and persistence (Status survey and conservation action plan 2002-2004). Only a few estimates of dispersal of different sexes exist for the capercaillie (reviewed by Storch & Segelbacher 2000). These are based on marked individuals, and the sample sizes are typically small (one to 55 individuals). The results are somewhat conflicting; the mean dispersal distance of juvenile females is higher (5.2 km, N=18) than of males (1.2 km, N=6) in the Ural mountain whereas in Scandinavia the largest dispersal distances of juveniles are found in males (up to 75 km) (see Table 1 of Storch & Segelbacher 2000). Only a few genetic studies on the capercaillie are available. In addition to the high amount of gene flow detected among Finnish populations (mitochondrial DNA, Liukkonen-Anttila *et al.* 2004), extensive gene flow has been found even among highly fragmented populations in the Alps (nuclear DNA, Segelbacher & Storch 2002).

1.4 Aims of the study

In this thesis, I examine the effects of forest fragmentation on the genetic structure of wood ant and capercaillie populations with the aim of identifying and evaluating the different genetic and behavioural factors important for species conservation and management at different geographical scales. I examine the impact of effective population size and geographical isolation on the level of genetic diversity, population differentiation and inbreeding. I also aim to estimate how different behavioural factors are associated with the genetic vulnerability of the populations. Special characteristics of the study species, social behaviour and polygynous lek mating system, make it possible to study the effect of the behavioural factors on the vulnerability of the species or populations in fragmented forest landscape. Finally, I discuss the utility of the genetic methods in species conservation.

To examine the effect of sociality on the genetic vulnerability of fragmented populations, I evaluate how fragmentation, patch colonisation and changes of the population size affect the levels of genetic variation and differentiation in ants with different social organisation. To effectively direct conservation efforts and to understand spatial dynamics in general, it is important to obtain information on how the ant populations respond to local changes in forest landscapes and how clearly the genetic structure of populations reflects demographic changes (see e.g. Ingram & Gordon 2003). I also examine the social and genetic structure of a remnant population system of the hairy wood ant with the aim of identifying possible factors relevant to its persistence and conservation.

The capercaillie has a polygynous lekking system that may influence its response to habitat fragmentation. In this thesis, I examine the genetic structure and vulnerability of the capercaillie in Finland with the aim of identifying possible factors relevant to its conservation and management. I evaluate the effects of habitat fragmentation on the genetic variability, population structure and the level of inbreeding. The capercaillie is suggested to be susceptible to habitat changes due to its large spatial requirements and polygynous lek mating system. However, the effect of habitat fragmentation on the genetic structure and vulnerability of the species is largely unknown. I also examine the connection between dispersal and mating behaviour by estimating sex-specific gene flow between leks of capercaillie. The dispersal of the species is commonly cited to be female-biased and very restricted (e.g. Storch 1997; Segelbacher & Storch 2002) even though only a few estimates of dispersal of different sexes exist showing conflicting results across the studies (Storch & Segelbacher 2000).

2 Materials and methods

2.1 Sampling and populations studied

2.1.1 Ants (I, II)

Samples of wood ants were collected from populations in Russia (Paper I) and Ireland (Paper II). Populations of *Formica aquilonia* and *F. lugubris* in Russia represent recently fragmented populations in the continuous distribution ranges of the species, whereas populations of *F. lugubris* in Ireland were very small and geographically isolated.

The study area in Russia was located in Peshki in the Moscow region. It mainly consisted of mature spruce forest cut by the meandering Klyazma River. The area has belonged to the myrmecological reserve Verhnaya Klyazma (648 ha) since 1981. The populations of *F. aquilonia* and *F. lugubris* were observed for 33 years, from 1966 to 1998, by mapping the colonies annually. All main events, including changes in habitat, destruction of nests and formation of new nests, were recorded as part of the annual surveys. In years 1997-1999, samples for genetic analyses were collected from nine subpopulations (76 nests) of *F. aquilonia* and from four subpopulations (38 nests) of *F. lugubris* in Peshki. Individuals of *F. lugubris* were also collected from two subpopulations (9 nests) in another area, Shahovskaya, which is located about 100 km from Peshki.

Only two small populations of *F. lugubris* exist in Ireland. A larger population with 41 nests is located in Tipperary (18 km^2) in mid-western Ireland. The woods in Tipperary are afforested with introduced conifer species. A smaller population with 8 nests is located in Killarney National Park (within the area of 6 km^2) in south-western Ireland. The woods in Killarney valley are the largest remaining ancient native oak (*Quercus petraea*) forests in Ireland. In summer 2003, we mapped all known nests and collected samples from each nest found. Samples of *F. lugubris* were also collected from three nests in Scotland.

2.1.2 Capercaillie (III, IV)

The study of capercaillie was conducted in Finland which belongs to the continuous distribution range of the species. Samples collected for genetic analyses were faeces (papers III and IV), feathers and tissues (paper IV).

Faeces were collected from the lekking sites (N_{lek}=161) during springs 2002-2004. Leks sites were determined with the help of visual contact, sounds and tracks of the birds during the mating season of the capercaillie. The sex of the sample was inferred from the size difference of the faeces (Hjorth 1970), and was later confirmed by DNA-based sex identification according to Griffiths *et al.* (1998).

Most of the feather and tissue samples were from the birds hunted during the legal hunting season 1995-2003. Originally, birds were collected by hunters to be used for research at the Finnish Game and Fisheries Institute. Some of the samples were also collected from found carcasses or moulted feathers.

These non-invasive techniques provided a relatively large data set without disturbance of the birds. The sampling scene (described in paper III) was planned for separating different individuals in the field, and high variation of microsatellite loci made it possible to ensure that the final data set did not include any replication of individuals.

2.2 DNA isolation (I-V)

In ants, five workers from each nest (875 individuals in total) and all males sampled (N=314) were stored in 96% ethanol until genomic DNA was extracted by DNeasy tissue kit (Qiagen) following *DNeasy protocol for animal issues* (papers I and II). Before extraction, all the samples were examined using microscopy to confirm that they did not include pieces of other individuals.

In capercaillie, the faecal samples (N=489), most of which were fresh, were stored at -20C immediately after field collection. A small part of the samples consisted of dried droppings stored in -20C. DNA was extracted by QIAmp DNA stool Mini Kit (Qiagen) protocol for *Isolation of DNA from stool for Human DNA analysis* (papers III and IV).

Feathers and tissues of the capercaillie (N = 298) were either dried or frozen. The DNA from tissues was extracted by using the standard phenol-chloroform method (Sambrook *et al.* 1989) and from feather quills as described by Liukkonen-Anttila *et al.* (2004) (paper IV).

2.3 Molecular methods

I used microsatellite markers to reveal genetic variation in nuclear DNA in all papers (I-IV). Variation at mitochondrial DNA was examined by using single-stranded conformation polymorphism (SSCP) (papers II and III) and sequencing (papers II and IV).

2.3.1 Microsatellite fragment analysis

Microsatellite loci are segments of DNA with very short sequences repeated in tandem (Queller *et al.* 1993). The large number of microsatellite loci and their high variability makes them potentially important tools for examining the genetic structure of populations.

Variation at microsatellite loci in ants was investigated using primers originally designed for *F. exsecta* (Gyllenstrand *et al.* 2002): FE7, FE13, FE17, FE19, FE21, FE37, FE38, FE42, FE51, and for *F. paralugubris* (Chapuisat 1996): FL12, FL20, FL21 and FL29 (papers I and II). Genetic variation in capercaillie was assayed at nine microsatellite loci, TUD1-TUD6 and TUT1-TUT3 (Segelbacher *et al.* 2000) (papers III and IV). Genotypes were assayed by polymerase chain reaction (PCR) with fluorescent labelling, followed by electrophoresis (ABI Prism 377 system). To minimise the errors of genetic typing (Taberlet *et al.* 1999) most individuals (all individuals with rare genotype) were regenotyped to ensure consistent sizing of alleles.

2.3.2 SSCP fragment analysis

SSCP is based on 3-dimensional conformation, as determined by a single-stranded nucleotide sequence migrating in polyacrylamide gel (Sunnucks *et al.* 2000). Even a single nucleotide difference may change the conformation of the single strands enough to change their mobility in the gel. Thus SSCP is a useful method for detecting single nucleotide polymorphism without having to sequence the homologous DNA fragments isolated from a large number of individuals. The method has been applied to detect sequence diversity (Frankham *et al.* 2002).

In ants, we used PCR to amplify a 423 bp fragment by primers tRNA-ser/CytB3 (Jermiin & Crozier 1994) (paper II). Mitochondrial DNA control region variation in capercaillie was revealed by amplifying a 386 bp fragment from conserved control region I with primers LPPGLU (Liukkonen-Anttila *et al.* 2002) and 5'-TGA AGC TAG TCA TGA GGT ACA T-3' (paper III). The amplified PCR products were resolved on a nondenaturing polyacrylamide gel according to Sunnucks *et al.* (2000).

2.3.3 MtDNA sequence variation

The most direct way for examining genetic variability is to determine the nucleotide sequences in the DNA. Sequencing is relatively time-consuming and expensive, and has primarily been used for taxonomic purposes. However, new techniques have also made it usable for lager scale studies of population genetics (Frankham *et al.* 2002).

In ants, we sequenced 1509bp of the mtDNA, from the area including cytb, for both directions using the same primers as Goropashnaya *et al.* (2004) (paper II). For sequencing the mtDNA in capercaillie, we used the same primers as for SSCP (paper IV). Samples were purified and sequenced using BigDye termination (v3.1, Applied Biosystems) and resolved on an ABI prism 377 system.

2.4 Statistical methods

In paper III, microsatellite data were checked by Micro-Checker (Van Oosterhout *et al.* 2004) for detecting null alleles, stuttering and allele dropouts by comparing the consistency between the estimated allele and genotype frequencies. Microsatellite genotypes were tested for linkage disequilibrium and for deviation from Hardy-Weinberg at each locus using GENEPOP software (Raymond & Rousset 1995) in papers I, III and IV.

The mtDNA sequence alignment was done in Sequencher and Bioedit Sequence Alignment Editor (v. 7.0.0). In paper II, we used MEGA3 (Kumar *et al.* 2004) to analyse the sequence variation and substitution patterns of the mtDNA, and the program TCS (Clement *et al.* 2000) to construct the haplotype-network.

2.4.1 Genetic variability

Genetic variability in microsatellite data was mainly estimated as allelic richness and gene diversity by using software F_{STAT} 2.9.3 (Goudet 2001). Allelic richness (A) is the mean number of alleles per locus adjusted for differences in sample size, whereas gene diversity is the expected heterozygosity according to Hardy-Weinberg expectations (H_E).

In paper IV, the mtDNA variation was estimated as nucleotide diversity (π) and haplotype diversity ($\hat{h}$) was calculated by DNAsp (Rozas & Rozas 1999). Nucleotide diversity is defined as the average proportion of nucleotide differences between all pairs of DNA sequences in a population (Nei & Li 1979). Haplotype diversity is the probability of two haplotypes drawn at random being different (Nei &Tajima 1981).

2.4.2 Relatedness, inbreeding and inbreeding depression

Relatedness records the degree of shared genetic material between individuals with respect to random individuals from the same population. In ants, we estimated the genetic relatedness (*r)* among nestmate workers following Queller and Goodnight (1989). In the analysis, nests were weighted equally, and to obtain standard errors, the estimates were jackknifed over nests and loci. We used the same approach to estimate inbreeding coefficient *F* within populations (papers I and II).

The inbreeding coefficient, F_{IS} was used to estimate the level of inbreeding in populations of the capercaillie (paper IV). F_{IS} describes the divergence of observed heterozygosity from the expected heterozygosity within populations (See 2.4.3.1).

Inbreeding depression in ant populations was estimated as the proportion of diploid males (paper II). The frequency of diploid males is commonly used as an estimate of inbreeding depression in social insects because the fitness of the queens/nest is reduced when a large fraction of diploid offspring are males that use resources and cannot reproduce (See 1.2.2).

2.4.3 Genetic differentiation

2.4.3.1 Wright's F-statistics

Wright's F-statistics describe the level of differentiation among populations by portioning the overall variation into components within and among populations. F_{IS} is *the inbreeding coefficient* that measures the reduction in heterozygosity of an individual due to non-random mating within its population. F_{IT} is *the overall inbreeding coefficient* that measures the reduction in heterozygosity of an individual relative to the total population. F_{ST} is *the fixation index* that describes the reduction in heterozygosity within a population relative to the total population due to selection or drift. The F_{ST} value ranges between zero and one, although small sample sizes can lead to slightly negative values. Furthermore, the upper limit of F_{ST} is set by the average heterozygosity within a population (Hedrick 2005).

One of the most used F_{ST} estimates, that is equivalent to Wright's statistics, is the one described by Nei (1973). Other measures of population differentiation that are closely related to Wright's F_{ST}, have used the variance of the allele frequencies among populations divided by the product of the mean allele frequencies. One of the most widely used of these methods is that of Weir and Cockerham (1984). The approach of Weir and Cockerham differs philosophically from that of Nei (Lowe *et al.* 2004). Nei's measure describes how the total genetic variation is divided into within and between-population components, without any assumption about descendants. Weir and Cockerhamn in turn, assume that all the populations descend from a single ancestral population. Their method requires the assumptions that all populations are independent and constrained in size over time, having no selection, mutation or migration involved, conditions that are not found in natural populations. However, Cockerham and Weir's method is currently one of the most widely used because it enables sophisticated statistical analysis to assess the validity of the result (Lowe *et al.* 2004).

In all papers (I-IV), the F_{ST} estimate was based on Weir & Cockerham (1984) and was calculated by the standard frequentist method using software F_{STAT} 2.9.3 (Goudet 2001). In paper III, the levels of genetic differentiation (F_{ST}) were also estimated according to Nei (1973) by using the Bayesian model (Corander *et al.* 2003).

2.4.3.2 Isolation by distance (IBD)

Most organisms have a limited dispersal ability and thus, gene flow may be restricted by distance alone. This effect is known as isolation by distance (IBD) (Wright 1943). Isolation by distance is used to infer the extent of genetic connectivity within and between populations. It measures the regression of pairwise estimates of population structure (F_{ST}) on pairwise geographical distance (km).

In paper IV, IBD was calculated for both nuclear and mitochondrial DNA with the program ISOLDE in GENEPOP (Raymond & Rousset 1995), whereas Matlab was used in paper III. Significance of the spatial correlation was determined by a Mantel test (Mantel 1967) in both papers.

2.4.3.3 Bayesian statistics

Several Bayesian methods have been introduced in the literature for investigation of the genetic structure of a population. Such methods are recently viewed as a way of revolutionising statistical thinking in the field of genetics (Beaumont & Rannala 2004). Bayesian analysis is based on the notion of posterior probabilities, that is, probabilities estimated on prior expectations. The utility lies in offering a more direct approach to many questions, sometimes more straightforward interpretation of results, and the incorporation of prior knowledge into the analysis (Shoemaker *et al.* 1999). In papers I, III and IV, Bayesian analysis was used to discover genetic population structure.

In papers I and III, a Bayesian analysis of population structure was conducted using the program BAPS (Corander *et al.* 2003, 2004). This method uses prior knowledge of sampling location, and estimates posterior probabilities for all different combinations of populations. When the number of populations is less than 10, the program performs an exact Bayesian analysis by enumerative calculation, whereas the Markov Chain Monte Carlo algorithm is used for a larger number of populations.

In paper IV, we analysed the population structure at the level of the individual by using the spatial model in the software BAPS 4.1 (Corander *et al.* 2003, 2006a, 2006b), which is developed for the discovery of genetic discontinuities in populations from molecular marker data. This statistical tool provides an informative and illustrative way to reveal the population structure.

2.4.4 Sex-biased gene flow

In paper III, sex-bias in gene flow of the capercaillie was examined in two different ways. First, the two kinds of markers (nuclear and mtDNA) were expected to result in different population structure estimates when sex-biased dispersal occurs (Prugnolle & de Meeus 2002). For uni-parentally inherited markers (mtDNA) one sex does not contribute to the genome of its offspring, whereas for bi-parental markers (nuclear DNA), both sexes contribute to the genetic diversity of the progeny. In this respect, differences in the level of genetic structure between the two kinds of markers are expected when sex-biased dispersal occurs (Prugnolle & de Meeus 2002). However, it has been stressed that the results have to be interpreted carefully as a difference in the level of genetic structure between different markers may partly be due to their divergent effective population sizes (N_E) which in turn depends on breeding characteristics, such as polygamy of the species (Chesser & Baker 1996). Capercaillie males are highly polygamous as is typical for lekking species. To investigate the effect of polygamy on the ratio of F_{ST} estimates by different markers, we applied the approach of Ennos (1994) and Berg *et al.* (1998) (see more in paper III).

The population structure was also estimated separately for the sexes by bi-parentally inherited markers (microsatellites). As allele frequencies are equally randomized between males and females in the offspring, it is possible to detect contrasted population differentiation among sexes if individuals are sampled after dispersal. When utilising bi-parentally inherited markers, it is important to be aware of the following restriction: Since the genes of immigrants will be carried by both their daughters and sons, any difference between the sexes will disappear as soon as immigrants have reproduced. Therefore, the

male and female comparisons are quantifications of current, not past dispersal. In contrast, methods based on the comparison between different types of markers without reference to sex will detect historical differences in gene flow (Prugnolle & de Meeus 2002).

2.4.5 Effective population size

Effective population size (N_e) is the size of an idealized population that has the same genetic properties as observed for the actual population (Wright 1931). It depends on the number of individuals that reproduce and contribute to the gene pool of the next generation (Lowe *et al.* 2004). In ants, colonies are family units, and relatedness among the brood directly reflects the breeding structure within colonies. In paper II, we estimated the effective population sizes of wood ant populations. Genotypes of the individuals and estimates of relatedness based on microsatellite data were used to approximate the number of reproducing females in the population. The number of males was estimated from the effective queen mating frequency (Seppä *et al.* 2004). Finally, the effective population size was calculated according to Wright (1969).

Sudden reductions in effective population size are referred to as bottlenecks. Genetic bottlenecks increase the possibility of population extinction through increasing stochasticity and the deleterious effects of inbreeding (Lowe *et al.* 2004). Historical data on effective population sizes is not often available. However, the measures of genetic variability can be used to identify genetic bottlenecks. When bottlenecks occur, both allelic richness and heterozygosity decline with reduction of population size, but allelic richness declines faster due to loss of rare alleles. To detect possible population bottlenecks in paper I, we used the program BOTTLENECK 1.2.02 (Cornuet & Luikart 1996).

3 Results and Discussion

I examined the effects of forest fragmentation on genetic diversity and population structure with the aim of evaluating the importance of different behavioural and genetic factors in conservation and management of fragmented populations. The species studied were mound building red wood ants, *F. lugubris* and *F. aquilonia,* and the lekking bird the capercaillie, *Tetrao urogallus*. The effects of fragmentation in both wood ants and capercaillie were related to time since fragmentation and to the level of habitat loss and isolation from the other existing populations. The relationships were further dependent on species characteristics such as dispersal and mating behaviour. The present study provided important information on species conservation and management in terms of better understanding the species' biology and behaviour, as well as increased knowledge concerning genetic issues that should be taken into account when planning conservation actions.

3.1 The effect of fragmentation on genetic structure (I, II, IV)

Habitat loss and fragmentation had some effects on genetic structure in both groups of species. Even the effect of recent forest fragmentation (within 35 years) on population structure was seen in genetic relationships of the remaining subpopulations of the wood ant *F. aquilonia*. However, the potential effects of population fragmentation on the genetic population structure in the ant *F. lugubris* and capercaillie was possible to detect only when fragmentation was severe and/or long-lasting. The loss of genetic diversity was less observable than the differences in population structure. The severe loss of genetic diversity was observed only in very isolated and extremely small populations of the wood ant *F. lugubris* in Ireland. Similarly, the increased level of inbreeding due to small effective population size and/or strong isolation of populations were only detected in a remnant population of *F. lugubris*.

3.1.1 Fragmented populations of wood ants (I, II)

To examine the effect of recent forest fragmentation on population structure of wood ants, populations of *Formica aquilonia* and *F. lugubris* were monitored for 33 years, and the genetic structure was revealed at the end of the study period (paper I). Forest fragmentation led to a decline and spatial redistribution of populations. Changes in the spatial distribution of populations were seen in the genetic relationships of the remaining subpopulations, revealing the boundaries of the historical populations (high values of genetic differentiation, F_{ST}) and recolonisation histories (genetic affinities revealed by Bayesian analyses) (paper I). However, the effect of fragmentation on the level of genetic variability was not that obvious. Genetic analyses did not detect major differences in either the level of genetic variation or inbreeding between the subpopulations (Table 1), even though the populations differed in size. The most obvious sign of the loss of genetic variation was the significant distortion of allele frequencies indicating a recent bottleneck in one of the subpopulations of *F. aquilonia* (paper I).

The loss of genetic variation and increased inbreeding was more pronounced in the long-term isolated and small populations of *F. lugubris* in Ireland (paper II). The genetic variation measured as allelic richness and genetic diversity was lower than in any other studied population of the same or closely related species (Table 1). Additionally, no mtDNA variation was discovered in Ireland, whereas several haplotypes have been found, for example, in populations of *F. lugubris* in England (Gyllenstrand & Seppä 2003). We also detected signs of inbreeding and inbreeding depression in the Tipperary population (Table 1). The inbreeding coefficient (F_{IS}) was significantly positive and higher than the inbreeding coefficient in populations of *F. lugubris* in Russia or any other studied population (Table 1). To estimate the level of inbreeding depression, we calculated the frequency of diploid males in nests. Diploid males were found in 22% of nests producing diploid sexuals in Tipperary. Similar and lower levels (0-17%) have been detected earlier in isolated island populations of five *Formica* species with monogynous/weakly polygynous nests (Pamilo *et al.* 1994). The proportion of matched mating i.e. mating of parents sharing a sex allele in common, was at least 11.6% in the Tipperary population. The probability of matched mating (p) is related to the effective number of alleles (K) as $p = 2/ K$ (Adams 1977), suggesting 17 alleles in *F. lugubris* in Tipperary. The estimate in other hymenopteran populations usually ranges between nine and 20 (reviewed by Cook & Crozier 1995). The theoretically expected number of alleles at this strongly selected locus depends on the effective population size (Yokoyama and Nei 1979). The estimated N_E in Tipperary (<100) would predict only 3-5 alleles, depending on the mortality effects. Even though our estimate of the number of alleles is an overestimate, there is a discrepancy between the estimates of the current population size and the allelic diversity of the sex locus. This may reflect a recent reduction of the population size that has not yet influenced the genetic variation at the sex locus to the same extent as at the putatively neutral microsatellites. The unexpectedly high genetic diversity found at the sex-determining locus may be a consequence of the timing of diploid male production (Pamilo *et al.* 1994) and of selection on queens (Ross & Fletcher 1986). Genotypic (microsatellite) diversity was significantly lower in nests that produced diploid males (paper II), indicating a connection between inbreeding and diploid male production.

Table 1. The results of the genetic studies on wood ant (F. aquilonia and F. lugubris) and capercaillie shown with their references. The number of studied individuals (N) for nuclear (nuc) and mitochondrial (mtDNA) data, nests (N_{NEST}), subpopulations (N_{SP}) and leks (N_L). Genetic diversity (H_E) is the average value over studied units. Relatedness (r) and inbreeding coefficients are based on Queller and Goodnight (F) and F-statistics (F_{IS}). Genetic differentiation is the average F_{ST} over studied units for nuclear (nuc) and mitochondrial DNA (m). Many estimates are reported with standards errors (± se). The size of the study area is approximately the size of the sampling area (km).

Species / population	N	$N_{NEST/SP}$	H_E	r ± se	F ± se	F_{ST} / nuc ± se	F_{ST} / m	Study area (km)	Paper
Wood ants									
F. aquilonia									
Russia	380	76/9	0.48	0,03 ± 0,04	0.00*	0.09		2 x 2	I
F. lugubris									
Russia (Peshki)	190	38/4	0.48	0,19 ± 0,04	0.04	0.04 *		2 x 2	I
Russia (Shahovskaya)	45	9/2	0.49	-0,02 ± 0,02	-0,11	0.12 *		0.1 x 2	I
Ireland (Tipperary)	205	41	0.37	0.59 ± 0.03	0.10 ± 0.04	0.45 ± 0.13**		2.5 x 2.5	II
Ireland (Killarney)	40	8	0.30	0.47 ± 0.08	-0.02 ± 0.09			3 x 6	II
England	370	74/5	0.61	0.05*	0.03 ± 0.01	0.03 ± 0.00	0.53	4 x 4	Gyllenstrand & Seppä 2003
Finland	74	16	0.63	0.70 ± 0.06					Gyllenstrand & Seppä 2003
Switzerland	144	18	0.47	0.50 ± 0.02	0.08 ± 0.02	0.16 ± 0.01**		0.1 x 0.8	Bernasconi *et al.* 2005
Switzerland	328	41		0.12 ± 0.01	0.04 ± 0.03			0.1 x 0.8	Bernasconi *et al.* 2005
Capercaillie	$N_{nuc}/_{mtDNA}$	$N_{L/SP}$			F_{IS}				
N-Finland / lek	268/187	102	0.69			0.098 ± 0.01	0.093	250 x 400	III
Males / lek	89	51	0.68			0.208 ± 0.01[B]		250 x 400	III
Females / lek	77	51	0.69			0.226 ± 0.01[B]		250 x 400	III
Finland / subpopulation	787/508	16	0.76		0.22	0.018 ± 0.003	0.012	400 x 1000	IV
Russia / subpopulation	42	2	0.71		0.14	0.047*		?x1500	Rutkowski *et al.* 2005
Poland / subpopulation	90	4	0.59		0.24	0.114		500 x 700	Rutkowski *et al.* 2005
Alps / subpopulation	292	18	0.59		-0.08	0.046*		150 x 300	Segelbacher & Storch 2002
Jura / lek, subpopulation	238	50/15	0.55	0.22 (lek)	0.02	0.033		30 x 50	Regnaut *et al.* 2006

* The mean of pair-wise estimates. ** The estimates are between Irish and Swiss populations, respectively. [B] The estimates are based on Bayesian analyses

The loss of genetic variation and increased inbreeding in *F. lugubris* populations in Ireland is a consequence of very small effective population sizes and of the restricted gene flow due to strong and long-term isolation of populations. The estimated effective population size of *F. lugubris* in Ireland is very small (<100 breeding individuals) compared to, for example, population in Peshki (paper I). The total population size of *F. lugubris* in Peshki could be estimated as a few hundred reproductive females, whereas the *F. aquilonia* population consisted of tens or hundreds of thousands of reproductive queens. Decimation of such a population size does not lead to a quick reduction of gene diversity, unless the populations go through severe bottlenecks. Such bottlenecks could take place during foundation of new nests and new subpopulations (paper I). The low level of genetic variation in Ireland also reflects the strong isolation of populations. In Russia, the gene flow among subpopulations may have maintained the high level of genetic diversity as subpopulations were located very close to each other and also to other populations in close proximity to the study area. Irish populations instead were highly isolated, being located more than 100km away from each other. The reduced variability of the Irish *F. lugubris* populations resembles the findings in oak populations (*Quercus* sp.), in which the haplotype diversity was lower in Ireland than in Great Britain and Southern-Europe (Kelleher *et al.* 2004). The reason for the loss of variation was suggested to be isolation and a limited founder population.

3.1.2 Genetic diversity and population structure of the capercaillie (IV)

High effective population size and short-term fragmentation may explain the high level of genetic variation and weak genetic differentiation of capercaillie subpopulations in Finland (paper IV). The level of genetic variability, population differentiation and inbreeding coefficient were compared between subpopulations that were clustered into source and sink populations according to Linden *et al.* (2000). The basis of source and sink dynamics is in the intensity of forest habitat fragmentation that was expected to be more severe in southern and western Finland than the northeastern part of the country; the subpopulations in northeastern Finland formed source populations, whereas subpopulations in further west and south were recorded as sink populations. No clear trend between genetic variability and the level of habitat fragmentation was found (see table 2 in paper IV). Additionally, the level of genetic differentiation did not increase from source to sink populations, with the exception that the genetic differentiation was highest in the southernmost sink population.

Forest fragmentation in Finland may not have been severe and long-lasting enough to lead to changes in the genetic composition of the capercaillie. Moreover, the species may tolerate fragmentation better than earlier thought. In contrast to views that the species is highly philopatric and/or vulnerable on the fragmentation of old growth forests, results from new studies imply that (i) the capercaillie is not strictly adapted to mature forests (e.g. Rolstad & Wegge 1989, Helle *et al.* 1994, Rolstad *et al.* 2005), (ii) potential dispersal ability of the species seems relatively good (e.g. Wegge & Larsen 1987, Mäkinen *et al.* 1997) and (iii) the effective population size of the species is relatively high in Finland (see more in paper IV).

In contrast to Finland, the severe and long-term habitat fragmentation, together with the low effective population size in Central and Western Europe, may have decreased the genetic variability and increased the level of genetic differentiation of capercaillie populations. The genetic diversity (H_E) found in Finnish populations is at a similar level as has been estimated in populations in Russia (Rutkowski *et al.* 2005) (Table 1), but is higher than that obtained in populations in Poland (Rutkowski *et al.* 2005) and further west, in the Alps (Segelbacher & Storch 2002) and in the Jura mountains (Regnaut *et al.* 2006). The populations in Central and Western Europe are also more differentiated from each other than those in Finland (Table 1), but whether the differences are significant cannot be calculated from the available data. The lower genetic variability and higher differentiation found in populations in the Central and Western Europe can be an artefact from different sampling schemes, study scale or different loci used, but it may also reflect the differences in their landscape history. Forest fragmentation is much more severe and longer-lasting in Central and Western Europe than in Finland. In addition to the differences in landscape, the population size differs between the areas. The effective population size of capercaillie is much lower in isolated populations in the Alps and Jura mountains than in the species' main range in Finland. Similar results have been found in a closely related species, the black grouse *Tetrao tetrix* (Caizergues *et al.* 2003). The level of genetic variation in more differentiated populations of the black grouse in the Alps (θ=0.06, H_E=0.74, A=5.4) is lower than in Finland (θ=0.02, H_E=0.78, A=6.4). In addition to habitat fragmentation, the reason for the loss of genetic variation in black grouse in the Alps is suggested to be the low effective population size and strong isolation from the species' main range (Caizergues *et al.* 2003).

3.2 Behaviour and conservation genetics (I-IV)

Even if the study species of different taxa (insects and avian) had similar responses to habitat fragmentation, the response was greatly dependent on their behaviour. Furthermore, the response differed even inside the group (wood ants, paper I). Behavioural factors influence the effective population size, and dispersal ability of the species, which in turn were connected to the level of genetic diversity, inbreeding and interpopulation gene flow. Overall, the effect of habitat fragmentation was more pronounced in wood ants than in capercaillie. This could mainly be due to the great differences in population sizes and dispersal ability between the groups, shown also as different geographical scales of the studies.

3.2.1 Sociality and genetic vulnerability of fragmented populations (I, II)

Sociality affected the genetic vulnerability of wood ant populations by decreasing the level of gene diversity (paper II), increasing the level of inbreeding depression (II) and restricting the gene flow between subpopulations (paper I). The polygynous social form is often connected to restricted dispersal (e.g. Pamilo 1983, Sundström 1993, Chapuisat *et*

al. 1997). However, the interaction between sociality and dispersal ability seemed to be more complicated than that. For example, highly polygynous *F. aquilonia* colonised new habitat patches within small distances during and after habitat fragmentation, whereas *F. lugubris* was not successful in colonising any, even though the lower level of differentiation of subpopulations implied a better dispersal ability of this weakly polygynous species (paper I). The large population size of *F. aquilonia* in connection with the social form of the species as well as the short distances between habitat patches may have created a competitive advantage for the species within the study area. Additionally, the high inbreeding values found in monogynous populations of *F. lugubris* suggest a partly restricted dispersal of the species (paper II).

3.2.1.1 Spatial structure and genetic differentiation of populations (I)

In paper I, I examined whether species with different social organisation respond differently to habitat fragmentation. The two species studied, *F. aquilonia* and *F. lugubris*, are closely related and ecologically similar, so the difference in the level of polygyny may be assumed to be the major determinant of any differences in dispersal and population changes associated with habitat fragmentation. Studied nests of *F. lugubris* in Peshki were weakly polygynous, whereas nests of *F. aquilonia* were highly polygynous. The number of queens was roughly estimated from the relatedness of worker nest mates. The relatedness of 0.19 in *F. lugubris* implies that the nests would have on average 3.8 queens (paper I), assuming that the nests recruit their own daughters as new queens (Gyllenstrand & Seppä 2003) and that the relatedness within a brood of a single female is 0.6 as is commonly observed in *Formica* ants (Pamilo 1993, Sundström 1993). The low worker relatedness in *F. aquilonia* suggests a high level of polygyny, with some tens or hundreds of queens per nest.

The effect of habitat fragmentation on spatial population structure differed between the species. The nest aggregations of *F. lugubris* were already patchily distributed prior to fragmentation, and degradation of the area did not have a large impact on the overall spatial distribution of its subpopulations. In contrast, highly polygynous populations of *F. aquilonia* were widely distributed and consisted of large numbers of nests, and the spatial distribution changed due to extinction or range expansions of some subpopulations, splitting of nest aggregations and colonisation of new patches of forest. The different response of species to fragmentation was also seen in genetic population structure. When genetic structure of subpopulations was estimated by F_{ST} and Bayesian clustering, the genetic differentiation and clustering of *F. lugubris* subpopulations was weaker than in *F. aquilonia* (Table 1). The lack of genetically differentiated subpopulations of *F. lugubris* in Peshki agrees with the hypothesised link between social organisation and gene flow (Pamilo and Crozier 1997). Species with monogynous (or only weakly polygynous) nests generally show weak or no genetic structure, whereas species with highly polygynous nests can show strong structure (Pamilo 1983, Sundström 1993, Chapuisat *et al.* 1997, Liautard & Keller 2001, Gyllenstrand 2002).

Even if the spatial structure in the *F. aquilonia* population was more apparent than in *F. lugubris*, the major differences among the *F. aquilonia* subpopulations reflected the initial subdivision of the population rather than changes that would have taken place

during the study period. These differences remained through the study period even though there clearly was gene flow between other subpopulations within similar geographical distances. This indicates the role of social structure in restricting gene flow. It has been shown that polygynous colonies generally produce highly male-biased sex ratios, and gene flow is largely carried by males (Doums *et al.* 2002, Gyllenstrand 2002, Gyllenstrand & Seppä 2003). However, polygynous ants have been observed mating in the nests (Chapuisat *et al.* 1997, Chapuisat & Keller 1999), which suggests that dispersal of males might also be restricted.

Habitat fragmentation led to establishment of new subpopulations in *F. aquilonia* but not in *F. lugubris*. The colonisation of new patches was also influenced by interspecific competition. *Formica aquilonia* has been shown to exclude other wood ant species in large habitat areas with many nests (Punttila *et al.* 1994). In contrast, in small habitats with a small number of individuals, they cannot compete with larger individuals of monogynous species which can successfully monopolise and defend their territories. In addition, fragmentation increases the edge areas which often favour less polygynous or monogynous species such as *F. lugubris* (Punttila *et al.* 1994). In contrast to our expectations, *F. aquilonia* managed to expand its range and to colonise new patches more efficiently than *F. lugubris*. The large size of the *F. aquilonia* population due to high number of nests and queens per nest, and the short distances must have given it a competitive advantage within the study area.

3.2.1.2 The loss of genetic diversity and increased inbreeding (II)

Habitat fragmentation had no clear effect on the level of genetic diversity and inbreeding in either of the studied wood ant species *F. aquilonia* or *F. lugubris* in Russia (paper I). However, the small effective population size connected to the monogynous / weakly polygynous form may have led to the decreased level of genetic diversity in *F. lugubris* populations in Ireland (paper II).

The studied populations of *F. lugubris* in Ireland were mainly monogynous having only one, or occasionally two, queens per nest. The effective population sizes were estimated as 71 and 19 in Tipperary and Killarney, respectively. The genetic variation estimated for Irish populations was lower than found in polygynous populations of the species (paper I, Gyllenstrand & Seppä 2003, Bernasconi *et al.* 2005) (Table 1). For example, Gyllenstrand and Seppä (2003) found an unexpectedly high amount of genetic variation in fragmented populations of the hairy wood ant in England. They suggested that the high genetic diversity was due to the high level of polygyny in nests increasing the effective population size (average N_e=719). The genetic diversity calculated from the same set of loci was much lower in Ireland (H_E = 0.37) than in England (H_E =0.61). We also compared allelic richness by randomly sampling one individual per nest from Ireland (Tipperary) and England (data from Gyllenstrand & Seppä 2003). In every comparison, the number of alleles was much higher in England (38.1 on average) than in Ireland (22.9 on average) although the number of nests in Tipperary was about twice that of any English population.

Even though the monogynous populations are expected to have good dispersal ability, the high inbreeding values found in monogynous populations suggest a partly restricted

dispersal. When the level of inbreeding found in Irish populations is compared with populations of *F. lugubris* with different social structure, the average inbreeding coefficient is higher than in any studied populations of *F. lugubris* (Table 1). A high level of inbreeding is also found in monogynous populations of *F. lugubris* in Switzerland (Bernasconi *et al.* 2005), and *F. exsecta* in Finland (Sundström *et al.* 2003), which indicates that dispersal within their local populations may be spatially restricted. A low population density can lead to mating between relatives when the area of the studied population exceeds dispersal distances. Even though dispersal is often believed to be less restricted in monogynous than in polygynous species / populations, the findings in *F. exsecta* (Sundström *et al.* 2003) indicate that monogynous queens tend to establish new nests close to their parental nest, leading to spatial genetic structure and the possibility of inbreeding.

3.2.2 Lekking behaviour and restricted gene flow (III, IV)

Even though the dispersal of capercaillie seems effective enough to maintain a high level of genetic diversity in Finland, lekking behaviour may restrict the dispersal of individuals. The dispersal of capercaillie is often cited to be female-biased (e.g. Lindén *et al.* 2000, Segelbacher & Storch 2002, Regnaut *et al.* 2006), even if the results from field studies show conflicting results across the studies (reviewed by Storch & Segelbacher 2000). The results obtained in this thesis suggest equal effective dispersal of sexes, where the dispersal of both sexes, not only males, is partly restricted.

3.2.2.1 Equal effective dispersal of sexes (III)

Contrary to the traditional view that the males of lekking grouses are highly philopatric and females are the dispersing sex, we found roughly equivalent dispersal of the sexes in capercaillie (paper III). The level of polygamy has a strong influence on the effective population size and on the effective dispersal of the sexes. Overall, the importance of male dispersal in the capercaillie and other lekking grouse species may have been underestimated earlier, by neglecting the effects of demographic factors like polygamy on the patterns of genetic differentiations and the rare long-distance natal dispersal of males.

Sex-biased dispersal is expected to lead to disparity in the pattern of genetic differentiation obtained for both sexes or for differently inherited markers (Prugnolle & de Meeus 2002). The genetic differentiation among leks was slightly stronger in females than in males (Table 1), but the difference between sexes was not statistically significant ($p > 0.1$). When genetic differentiation was compared between differently inherited markers, slightly more structure was found in nuclear than in mtDNA (Table 1). As the effective population size differs between the markers used, we compared the genetic structure by inserting the effect of polygamy (p) on the interpretation of the estimate of sex-biased dispersal (d = relative male dispersal rate). In general, the level of polygyny strongly influenced the expected patterns of genetic differentiation and the inference of sex-specific dispersal rates. Our estimates of genetic differentiation suggest an equal effective dispersal by females and males if the level of polygyny (p) is 8.5. There is no

good estimate of the level of polygyny in capercaillie but in light of existing results (Lumsden 1961, Hjorth 1970, Moss 1980, Rolstad 1989b, Rolstad & Wegge 1989, reviewed in paper III) the parameter p may well be close to 8.5 suggesting more or less equal dispersal of the sexes. It should be noted hat the details of breeding characteristics and the number of surviving progeny further affect the effective population size and the inference of sex-biased dispersal (Prugnolle & de Meeus 2002).

Our results suggest that the dispersal of the capercaillie is roughly equal in both sexes. The often stated expectation of capercaillie males being highly philopatric is mainly based on few ecological field studies, even though it has been observed that long-distance male dispersal up to 75km can occur (Storch & Segelbacher 2000). Young males have been observed to migrate towards regions with lower densities of birds (Borchtchevski 1993). In addition, juvenile and subadult males can have large home ranges and visit several leks (Wegge & Larsen 1987). Thus, the present result is compatible with ecological studies, and questions the often cited expectation of male residence. Especially, the equal or nearly equal effective dispersal does not imply that males and females exhibit similar patterns of dispersal; females can be more prone to disperse but generally disperse short distances, whereas males only sometimes disperse but do so over great distances. If the dispersal of both sexes is partly restricted (see 3.2.2.2), the rare long distance dispersal events are especially important and have a marked effect on the genetic structure of the species.

There are only a few earlier studies that examine the contrasting patterns in population structure of different markers (greater prairie chicken *Tympanuchus cupido pinnatus*, Johnson *et al.* 2003) or sexes (black grouse, Höglund *et al.* 1999, Caizerques *et al. 2003*) in lekking grouse species. None of these studies have evaluated the effects of demographic factors such as polygamy of the species on the interpretation of the genetic results, or have found any difference in genetic population structure between the sexes. The available studies on lekking grouse species therefore leave it open regarding whether any sex-biased gene flow exists.

The conception of female-biased dispersal in lekking grouse species is partly based on the resource defence hypothesis of Greenwood (1980), who, however, questions whether it applies to lekking species. Moreover, several studies have proposed that lekking species in different taxa (e.g. marine iguana *Amblyrhynchus cristatus* Rassmann *et al.* 1997) including birds (great bustard *Otis tarda* Martin *et al.* 2001) illustrate male-biased dispersal. Currently, the existing literature strongly suggests that for many species it is inappropriate to consider sex bias in dispersal to be a species constant. Many of the studies reviewed by Clarke *et al.* (1997) demonstrated that sex biases were only found within some populations, sites or years. This may also be the case with the capercaillie. It is very likely that dispersal behaviour is influenced by environmental factors such as habitat availability. Juvenile dispersal is not given by a fixed probability but is made conditional based on the presence of free territories in a patch, and that individuals born in full patches will more easily disperse.

3.2.2.2 Restricted dispersal and inbreeding (III, IV)

Although dispersal of the capercaillie may be effective enough to prevent genetic differentiation and loss of genetic diversity in Finland, the isolation by distance in both nuclear and mtDNA and high values of inbreeding coefficient found at the subpopulation level (paper IV), suggest restricted dispersal of both sexes. Moreover, strong structuring at the lek level (paper III) proposes a restricting effect of the lekking system on the dispersal behaviour of the species.

According to the results in papers III and IV, the genetic differentiation is five-fold weaker at the subpopulation level than at the lek level ($p<0.001$, t-test) (Table 1). High inbreeding coefficients were found in every studied subpopulation (Table 1) and an excess of homozygote genotypes was found at each locus (paper IV). The high inbreeding estimates could result from errors in study methods such as existence of null alleles or allelic drop out. However, we did not find any evidence for that because all loci showed similar patterns with continuous distribution of allele sizes. The similar level of inbreeding coefficient (F_{IS}) has been also found in other populations of the capercaillie in Poland and in Russia (Rutkowski *et al.* 2005) (Table 1), and also in other lekking grouse species (0.19-0.31 in the lesser prairie chicken *Tympanuchus pallidicinctus* (Bouzat & Johnson 2004). However, the inbreeding coefficient in populations of capercaillie in the Alps showed huge variation between populations (-0.41-0.10), and was negative on average (Segelbacher & Storch 2002) (Table 1).

The high inbreeding values found in capercaillie and other lekking grouse species imply that females and males are closely related due to restricted dispersal of both sexes. It has been suggested that the restricted dispersal of males in lekking species may evolve via resource competition (Greenwood 1980) or other advantages to males obtained through kin selection (Kokko and Lindström 1996). Kin selection theory predicts that by joining a lek where a relative is likely to reproduce, the attractiveness of the lek is raised and males unsuccessful in obtaining copulations may gain via inclusive fitness. It has been shown that leks of the capercaillie in the Swiss and French Jura Mountains contain a mixture of close kin and unrelated individuals (Regnaut *et al.* 2006). High relatedness of males may lead to overall reduction in genetic variation causing inbreeding. However, this seems not to be the case in the capercaillie, as genetic variation is relatively high in Finland. Additionally, the level of inbreeding seems to be much weaker in populations with lower level of genetic variability in the Alps (Segelbacher and Storch 2002). Thus, the most probable explanation for increased inbreeding is that dispersal of females is also restricted. In capercaillie and many other lekking species, females provide the parental care and may also increase their fitness by remaining near to their natal site. Moreover, if the female offspring copy their mothers in mate choice (Höglund *et al.* 1990, Gibson *et al.* 1991), the chance for inbreeding greatly increases.

The high variance in inbreeding coefficients between areas in the Alps could reflect dispersal behaviour due to high variation in geographical isolation and size of the habitat patches. When a habitat patch is small and cannot recruit many individuals, birds have to disperse but when it is possible, they stay close to their birth area. When population density is high and/or part of the habitat is destroyed, staying at the same lek with relatives may not be a good option, especially for the cocks. When all available territories

at the lek are occupied, the cocks can fight until the death of the other. This does not increase the breeding success of related cocks but may favour male dispersal.

3.3 Critical interpretation of the genetic results

Several problems may arise when interpreting the possible effects of habitat fragmentation on the basis of genetic results. Some of these problems are pronounced when comparing results obtained from different species, from different genetic markers within a species or from studies with different geographical scales. One of the most difficult methodical "problems" is the importance of statistical significance in interpretation of the results; (i) is a significant difference always biologically relevant, and (ii) if no difference is found does it mean that there is no difference in reality or is it just a lack of power in the methods used. Additionally, (iii) in many studies in existing literature, a difference between two populations is erroneously concluded from one population departing from a null hypothesis and the other which does not, without comparing the populations against each other.

The effects of fragmentation on genetic structure were in presented studies mainly examined by estimating the level of genetic diversity, population differentiation and inbreeding. These estimates greatly depend on the geographical scale of the study. The differences in results obtained from studies at different scales greatly affect the interpretation of the result giving important information but also easily leading to false conclusions. The results and interpretations depend on the unit used in the analysis, whether the units are populations or social groups (leks or ant colonies). For example, when the genetic structure of the capercaillie was examined, isolation by distance was detected at the subpopulation but not at the lek level. The difference can be explained by strong differentiation at the lek level. If leks consist of some individuals that are closely related, as suggested by Regnaut *et al.* (2006), even leks close to each other can be highly differentiated. The results indicate that the dispersal of the capercaillie may be rather bimodal; moderate dispersal at the geographical scale (at the subpopulation level), and less dispersal at the landscape scale (at the lek level).

One of the most difficult problems in interpretation of molecular genetic data relies on the association of statistical significance and biological importance. It is important to consider the biological relevance of statistically significant results as discussed by Hedrick (2001) and Shoemaker *et al.* (1999). For example, one of the most commonly used estimates in population genetics is F_{ST} that describes the genetic differentiation of populations. If the estimate is significantly positive ($p<0.05$), the gene flow between populations is restricted. Yet, there might be statistical significance even if there is no meaningful biological difference. This may become a major concern in conservation biology as a large number of highly variable markers become available in many species. The statistical power for determining differentiation between groups is closely related to the number of independent alleles, so that there can be extremely high statistical power even for a few highly variable microsatellite loci. With such a power, extremely small genetic differences can become statistically significant (Hedrick 1999).

The question of the statistical power and its biological meaning is also important when the results do not reject the null-hypothesis. For example, when genetic structure

was compared between the sexes of the capercaillie, microsatellites showed similar differentiation (based on F_{ST} and the number of clusters made by BAPS) in both females and males suggesting no strong sex bias in dispersal (paper III). More structure was found in females than in males, but the difference between sexes was not significant. Given the result of a non-significant difference, the question arises whether the performed analyses had sufficient statistical power to detect significant differences if such differences would have existed. As the number of analysed leks was quite high, the result is not expected to be sensitive to the number of samples. In particular, there is no statistical reason for expecting that this would lead to large systematic changes in the structure estimates of both sexes with respect to each other.

Statistical significance can also be used misleadingly. For example, if the population structure is significantly greater than zero in one population, but not in another, the misleading conclusion could be that the dispersal ability of the species differs between populations. There is no evidence that the population structure differs between compared groups that would indicate differences in dispersal. Yet, this type of conclusion can be found in literature. Such conclusions are especially dangerous when comparing results obtained from different methods, as molecular markers differ in their variability making great differences in statistical sensitivity.

3.4 Management of fragmented populations (I-IV)

The results of this thesis emphasise the importance of preserving effective population size and the connectivity of habitat patches when planning management strategies for species. There were great differences in conservation needs among the species, which should be taken into account especially in local management actions. Moreover, conservation actions should be planned on different geographical scales as species have different spatial needs and dispersal abilities.

3.4.1 Sociality and wood ants

When planning management actions for social insects, the knowledge of the social structure of the species and / or populations concerned is very important. Demographic and genetic results presented here suggest that habitat fragmentation forms a clear threat at a local scale with large negative effects on ant population viability (papers I and II). As species differing in their social structure have different response to fragmentation, the management and conservation needs of their populations differ consequently. To avoid harmful effects of habitat fragmentation, patch size is important. This is especially important for a polygynous species which requires a large enough population size to compete with other species. Weakly polygynous or monogynous species also colonise distant patches and protect them against other species even if the patches are small. However, if the populations of monogynous species are too small, they may not survive in competition with polygynous ant species (paper I). Additionally, occupation of small and isolated patches may not be enough for long-term survival as the genetic diversity

can greatly decrease when the nests have only one or few breeding individuals (paper II). On the scale of landscape, forest management should pay special attention to the connectedness of threatened ant populations. It should also be noted that even though the ant populations concerned would not be under threat of complete extinction, changes in the population size of such a dominating component of the boreal forest ecosystem can have drastic consequences.

The conservation status of the species determines the need of conservation and management. Management is always greatly dependent on local needs and conditions. The results presented here point to important population characteristics of wood ants, which need to be taken into consideration when formulating future management strategies for the species in Ireland (paper II). According to our results, the hairy wood ant can be considered as a native species to Ireland and populations are subject to a high risk of extinction in the near future. The greatest threat for both populations is a small N_E and the main action needed for conservation is preservation of habitat. Preserving part of the forest in the vicinity of the existing population would allow the population to expand. Harmful effects of introduced species on natural habitat must be minimized, and in Tipperary it is urgently necessary to review the management policy of forests which hold the small remaining wood ant population. Unfortunately, even in the recent past (after our sample collection), part of nests and nest habitat have been destroyed by clear-felling. The nests located in the clear-cut area had many unique alleles. The conservation of habitat between these and other nests is especially important for the preservation of gene flow and maintenance of genetic diversity in the population. One management option could be to introduce gene flow between the Tipperary and Killarney populations. The introduction of *F. lugubris* from other non-Irish populations to supplement the diminishing Irish populations should not be the first option as the social structure differs drastically between the populations, and social structure may be at least partly an adaptation to the local environment (paper II).

3.4.2 The importance of lek conservation in Capercaillie management

The results presented in this thesis support the importance of connectivity of forest cover in the management of the capercaillie populations, which should be taken into account in forest planning. In addition to the conservation of the capercaillie in its continuous distribution range, knowledge of the genetic structure of the capercaillie aids in the management of declined and threatened populations elsewhere in terms of a better understanding of the genetic factors associated with the species' biology.

Genetic population structure of the capercaillie seems relatively stable in Finland. However, even if high levels of genetic variability are found in all subpopulations in Finland, it does not necessarily imply that the capercaillie is not genetically vulnerable in the long term. Changes in allelic distributions and especially reduction of genetic diversity may take a long time unless the population goes through a severe bottleneck. Forest fragmentation in Finland may not have been severe and long-lasting enough to lead to changes in the genetic composition of the capercaillie. Additionally, although the potential dispersal ability of the capercaillie seems very good and genetically effective,

the high level of inbreeding and partly restricted gene flow between leks indicates that restricted dispersal caused by forest fragmentation may have severe long-term consequences on population viability.

When planning conservation strategies for lekking grouse species, the preservation of leks of different sizes is essential and should be taken into account in management actions. The study of effective dispersal behaviour of the capercaillie showed the importance of the level of polygyny in the distribution of genetic variation among the populations. It has been observed that larger leks with many displaying males attract more females than smaller leks (Alatalo *et al.* 1992), implying that the size of available leks may influence the level of polygamy of the species. This in turn affects relative dispersal levels of the sexes. Changes in landscape structure due to human land use tend to decrease the size of the leks, which in turn can decrease the level of polygamy of the species. Thus, the size of the leks may also have an important influence on maintaining genetic diversity in capercaillie populations.

In forestry planning, it is especially important to maintain a sufficient network of well functioning lek sites but also of suitable unoccupied sites to sustain efficient natal dispersal of the capercaillie. As the genetic population structure seems to be relatively panmictic and genetic diversity consistently geographically distributed, it is important to try to preserve connectivity between all areas with no special division of populations.

3.5 Concluding remarks and future aspects

The studies of this thesis show that genetic methods can be used to evaluate issues important to species conservation and to determine management options for a species or for local populations. By examining the genetic structure of the species it was possible to clarify their conservation status including the effective population size, the question of origin, and genetic vulnerability (genetic diversity, inbreeding and inbreeding depression) of the populations and/or species. The information obtained from genetic structure could also be used to dissect the recent historical changes underlying the present population structure, and to understand the species biology, for example of dispersal and breeding behaviour of the species.

Even if the results of this thesis gave new information on species conservation, it also showed that there is a lot more to be learnt. In social insects, the avoidance of the effect of inbreeding depression is very important. However, only a small amount of data on diploid male production is available. A long-term genetic study combined with field studies in small and isolated wood ant populations, such as those in Ireland, could help to understand the actual mechanisms (e.g. selection) that prevent inbreeding depression in nature. Additionally, the wood ant species seems a very good study species for habitat fragmentation, and could be used as indicator species of forest fragmentation.

Most of the earlier studies on capercaillie are based on small data sets obtained in field studies. When reviewing the literature, it was surprising that many strong expectations concerning the behaviour of the capercaillie were actually paradigms based on opinions and hypotheses, and not empirical results. Much research is needed for example to obtain better estimates of dispersal rates especially of young individuals (natal dispersal). In

addition to dispersal behaviour, little is still known about the lekking behaviour of the species, for example there is no estimate of the level of polygamy in this species. The genetic methods could be further used to estimate the relatedness between individuals in leks and to examine the movement of individuals between leks. This kind of genetic study at the landscape level, connected with ecological studies, could provide valuable information important for conservation and management of the species. Genetic results could be further connected to landscape information to obtain information on how behavioural factors such as lekking and dispersal behaviour depend on the environmental factors at the landscape scale.

Conservation and management in practise is usually a local action. Thus, it would be important to make the information obtained from scientific studies available for local management and decision markers. To get more specific information in a “usable” form, analyses that would include local information, for example landscape data, would be greatly needed. Unfortunately, producing this kind of local information is not always scientifically productive and thus, not supported financially. Luckily, there is an increasing willingness among scientists and decision makers to co-operate to fill this gap.

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I Mäki-Petäys H, Zakharov A, Viljakainen L, Corander J & Pamilo P (2005) Genetic changes associated to declining populations of *Formica* ants in fragmented forest landscape. Mol Ecol 14: 733-742.

II Mäki-Petäys H & Breen J (2006) Genetic vulnerability of a remnant ant population (Conserv Genet, DOI10.1007/s10592-006-9182-1).

III Mäki-Petäys H, Corander J, Aalto J, Liukkonen T, Helle P & Orell M (2007) No genetic evidence of sex-biased dispersal in a lekking bird, the capercaillie (*Tetrao urogallus*) (J Evol Biol, Accepted).

IV Mäki-Petäys H, Liukkonen T, & Orell M (2007) Genetic structure and vulnerability of the lekking bird, the Capercaillie (*Tetrao urogallus*), in fragmented forest landscape (Manuscript).

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