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Kaisa Rikalainen

Genetic Diversity in the Wild

Cyclic Population Dynamics and Population Isolation

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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2013

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ABSTRACT

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Genetic diversity within natural populations is essential for populations' vitality, adaptation and evolution. Small population size, population isolation, and fragmentation are potential risks of losing genetic variability. Also cyclic population dynamics can cause the loss of genetic diversity because of reductions in population size. Lowered genetic diversity often translates to decreased fitness of individuals. In this thesis I aimed to study the maintenance of genetic diversity in the contexts of population dynamics and fragmentation. More specifically, I examined if frequent population crashes and fragmentation affect genetic diversity, how it is maintained (especially the role of density-dependent selection), and how it is translated to individual fitness. I assembled a panel of genetic markers for a cyclic rodent, the bank vole (*Myodes glareolus*). The panel consisted of anonymous, expressed sequence tag-linked and social behavior-associated (vasopressin and oxytocin-linked) microsatellite loci. I used long-term data to see if genetic diversity was maintained within a population and if density-dependent selection was affecting on social behavior-associated loci. I also contrasted mainland and island populations to examine if fragmentation had reduced their genetic diversity. Finally, I studied whether lowered genetic variability translated to decreased fitness of individuals. I found that genetic variability was maintained within populations despite the frequent crashes in population size. Genetic diversity was presumably promoted by high effective population size and migration, and density-dependent selection did not substantially act on those social behavior-associated loci. Furthermore, my research showed that population fragmentation was associated with reduced genetic diversity of females, and, surprisingly, the females with lower genetic diversity gained fitness benefit. Taken together, this thesis hopes to bring new insights into the associations between population size, population isolation and genetic diversity. It shows that the genetic diversity might bear both positive and negative effects on individual fitness. Moreover, it suggests that cyclic population dynamics do not provoke density-dependent selection on social behavior-associated loci.

Keywords: Bank vole; fitness; genetic diversity; population cycle; population fragmentation; population isolation; social behavior.

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FOREWORD

Genetic diversity is the premise of evolutionary potential within wildlife populations. It has an impact both on population's vitality today and its adaptability for the challenges encountered in the future. Genetically variable, or heterozygous, individuals often show superiority over others in terms of viability and breeding. These reasons support the maintenance of genetic diversity of populations in the wild. The evolutionary theory, however, states that the amount of genetic variation within a population should be reduced due to the fitness benefit of the most fitted genotype. In theory, natural selection should drive the population towards the fixation of the best allelic state and the fittest phenotype. The controversy between the existence of genetic variation and theory has been a fundamental question in evolutionary biology for decades.

In this thesis I examined the possible reasons for the existence and maintenance of genetic diversity within wild populations. More specifically, my aim was to study how genetic diversity is affected by the risks of population size reduction and population fragmentation or isolation. I also aimed to explain how genetic diversity is maintained and how it is turned to fitness of the individuals. More broadly, my attempt was to elucidate how genetic diversity, population demography, isolation and fitness are intertwined in the wild.

Jyväskylä 14.2.2013
Kaisa Rikalainen

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by the Roman numerals I-V. I am the only author in one paper (I) and the first author in three papers (II, III, V), where I carried out the most of the study planning, molecular and statistical analyses and writing. In paper IV, I participated the study planning, analyses and writing.

I Rikalainen, K. Fast isolation by AFLP of sequences containing repeats (FIASCO). *Methods in Molecular Biology*, in press.

II Rikalainen, K., Grapputo, A., Knott, E., Koskela, E. & Mappes, T. 2008. A large panel of novel microsatellite markers for the bank vole (*Myodes glareolus*). *Molecular Ecology Resources* 8: 1164-1168.

III Rikalainen, K., Aspi, J., Galaza, J.A., Koskela, E. & Mappes, T. 2012. Maintenance of genetic diversity in cyclic populations - a longitudinal analysis in *Myodes glareolus*. *Ecology and Evolution* 2: 1491-1502.

IV Watts, P., Aspi, J., Galarza, J.A., Koskela, E., Kyröläinen, S., Lönn, E., Mappes, T. & Rikalainen, K. No evidence for selection at the vasopressin 1a and oxytocin receptors as a response to cyclical changes in mammalian population density. Manuscript.

V Rikalainen, K., Galarza, J.A., Koskela, E., Mappes, T., Parri S. & Grapputo, A. Genetic variability and benefits of inbreeding within vole populations in fragmented and continuous habitats. Manuscript.

CONTENTS

1 INTRODUCTION

1.1 Maintenance of genetic variation in the wild

Genetic variation within wild populations is a topic of continuous interest in ecology and evolutionary biology. A paradox originates from evolutionary theory stating that selection and genetic drift should reduce genetic variation (Fisher 1930). However, empirical evidence shows that there is substantial amount of genetic variation within natural populations (e.g. Merilä & Sheldon 2000, Sheldon et al. 2002).

The main mechanisms suggested to maintain genetic variation in wild populations are mutation-selection balance and the balancing selection, the latter of which includes heterosis (or heterozygote advantage), antagonistic pleitotropy, negative frequency-dependent selection and environmental heterogeneity (categorized e.g. by Roff 1992, and Whitlock 2004). Mutation is the ultimate source of genetic variation and the only process that really generates new genetic material. In *mutation-selection balance* mutations are expected to compensate for the homogenizing actions of selection by bringing new genetic variation into the population (Lande 1975). *Heterosis* (or *heterozygote advantage*) refers to the boosted vigour of heterozygote individuals compared to the lower performance of homozygous individuals (Ewens & Thomson 1970). Genetic diversity is maintained through the fitness benefit of the heterozygous ones. *Antagonistic pleiotropy* refers to a situation where one allele/gene has a positive effect on one component of fitness but negative on another (see e.g. Rose 1982). This leads to a status where neither of the two alleles/genes is selected for but are both maintained within the population. The phenomenon is the genetic basis of trade-offs in life history theory, which is evidenced by negative genetic correlations within wild populations (Roff 1992). In *negative frequency-dependent selection* the selection on a phenotype depends on its frequency within the population (Ayala & Campbell 1974, Gromko 1977). The selection is assumed to favor the rarest phenotype, leading to the maintenance of both types. *Environmental heterogeneity* refers to the ability of both environmental and temporal variation to maintain genetic variation (Levene 1953, Hedrick et al. 1976, Hedrick 1986). The phenomenon is based on an

assumption that different phenotypes and genotypes have different fitness in different environments or at different times. The system can maintain genetic variation in situations where a population experiences heterogeneous environments.

In theory, especially the traits that are closely associated with individual fitness should show reduced genetic variation (Fisher 1930, DeRose & Roff 1999). These traits, commonly known as life-history traits, include e.g. infant survival, fecundity and litter size. As these traits are strongly connected with reproduction and survival, they should be under strong selection and hence show lower genetic diversity than other (e.g. morphological) traits (as shown e.g. by Gustafsson 1986).

1.2 Cyclic population dynamics

1.2.1 Vole population cycles

Cyclic population dynamics refers to a phenomenon where the size of a population changes regularly with high amplitude in time. Regular population size fluctuation is fairly common in the nature and it has been recorded in mammals (Elton 1924, Korpimäki et al. 2005), birds (Moss & Watson 1991), fishes (Sanderson et al. 1999), and insects (Berryman 1996). Probably the most popular example of mammalian population cycles is the periodical fluctuation in the Norwegian lemming population (*Lemmus lemmus*, Elton 1924). Other northern Scandinavian small rodents also exhibit regular population cycles (Krebs 1996). An example of those, the bank vole (*Myodes glareolus*), which is the species studied in this thesis, has a population cycle of 3-4 years in Finland (Korpimäki et al. 2005, Kallio et al. 2009). Typically the cycle consists of two years of increasing population size followed by a peak year during which the population size reaches its maximum. After the peak the population size crashes, almost as a rule during the winter, and the cycle starts over with increasing population size next spring.

The population cycle of bank vole is in synchrony with other vole species at the same area (Sundell et al. 2004, Korpimäki et al. 2005). This further intensifies the influence of vole cycles on the ecosystems: e.g. the abundance of voles has profound effects on their predator numbers (Sundell et al. 2004), the prevalence of Puumala hantavirus (Kallio et al. 2009), and the amount of forest damage with its economic consequences (Gill 1995, Baxter & Hansson 2001, Huitu et al. 2009). Fennoscandian vole cycles are characterized by a latitudinal gradient, from non-cyclic populations in the south to high-amplitude cycles in the north (Hansson & Henttonen 1985, 1988).

Various explanations have been suggested for the formation and maintenance of rodent population cycles. The explanative factors fall into two categories: extrinsic and intrinsic. *Extrinsic factors* refer to environmental variables such as food resources and predator pressure. Experimental

restriction in food abundance has shown to be a potent factor in reducing vole numbers during the crash phase (Huitu et al. 2003, 2007). Poor physical condition caused by malnutrition is suggested to induce mortality through starvation and susceptibility to predators and pathogens. Reduction in predator numbers has also shown to be associated to an increase in vole numbers (Korpimäki & Norrdahl 1998, Sundell et al. 2004). Predator abundance has been suggested to underlie the interspecific synchrony of vole species (Sundell et al. 2004, Korpimäki et al. 2005). *Intrinsic factors* refer to phenotypic and/or genotypic variables and may involve behavioral and physiological parameters. The original intrinsic hypothesis, although later widely challenged, was stated by Chitty (1967) who suggested that individuals' social behavior is heritable and different states of aggressiveness (reflecting individuals' ability to compete for resources) are selected for at opposing cycle phases. At low density phases nonaggressive types with high reproductive effort are selected for, whereas aggressive types with low reproductive effort are selected for during high density phases. The cyclic population dynamics forms as a result from the varying reproductive effort. Charnov and Finerty (1980) proposed another behavioral hypothesis that was based on kin selection. They hypothesized that the individuals breed in kin groups during low density phases, and high reproductive effort during these phases is due to the reduced aggressiveness towards the kin. At high density phases the increased dispersal disrupts the kin groups, which results in increased aggressiveness and reduced reproductive effort. The changes in reproductive performance, dispersal and kin presence drive the population cycle. In addition, also changes in physiological parameters such as in body size (see Boonstra & Krebs 1979) and the included references) and in population's age structure (Boonstra 1994) have been recorded and suggested to lie behind the population cycles.

1.2.2 Genetic diversity within cyclic vole populations

The substantial reductions in population size during the crash phases predispose the population to genetic *bottlenecks* (Nei et al. 1975), i.e. temporary or permanent declines in effective population size (Beebee & Rowe 2004). During bottlenecks, the population is more prone to genetic drift, which can further reduce the population's genetic diversity. Nevertheless, despite the strong oscillation in population size of cyclic vole populations and the possible threat of repetitive bottlenecks, high heterozygosity has been frequently observed among several vole populations (see e.g. Plante et al. 1989, Berthier et al. 2005, 2006, Ehrich & Jorde 2005, Redeker et al. 2006, Ehrich et al. 2009).

Processes that can maintain the high genetic diversity within these populations include e.g. differences in the migration pattern of individuals, inbreeding avoidance (via spacing behavior or kin recognition), and environmental heterogeneity (density-dependency). The high genetic variation in cyclic vole populations can be maintained if the loss of genetic diversity during the crash phases is outweighed by the intensive *migration* between subpopulations (Berthier et al. 2006). When the population size is small the

arrival of just a few immigrants that successfully reproduce can overcome genetic drift and balance out bottleneck effects (Keller et al. 2001). In other terms, gene flow impedes the degenerative effects of genetic drift. The emergence of new allelic combinations within the population can refer to gene flow from distant populations.

Inbreeding avoidance (i.e. prevention of mating between relatives) can be achieved by different mechanisms. At least two of them, dispersal and kin recognition, have been recorded in microtine rodents (Bollinger et al. 1993, Kruczek 2007). Dispersal, referring to individual *spacing behavior*, might associate with population size or density. If dispersal in a cyclic population increases with the population size/density, the genetic variation within the population would increase during the peaks, as shown by Berthier et al. (2006). If, in contrast, dispersal increases during the crash phases, as evidenced by Ims & Andreassen (2005), the accelerated gene flow would compensate for genetic drift. In both cases the genetic diversity would be maintained within the population as genetic drift is cancelled out by gene flow. Kin recognition in the bank vole serves probably both for inbreeding avoidance, as females recognize related males (Kruczek & Golas 2003, Kruczek 2007), and for *kin tolerance*, as breeding and territorial females accept close relatives at nearby locations (Mappes et al. 1995). Kin recognition is important in inbreeding avoidance, whereas kin tolerance becomes relevant in social situations such as territory occupancy, as I will discuss below (see sections 1.3.2, 3.3.2 and Conclusions).

One possible mechanism that can maintain genetic diversity within cyclic populations is *environmental heterogeneity*. The numbers of conspecifics (Korpimäki et al. 2005), together with food resources (Potapov et al. 2004, Huitu et al. 2007), predation and pathogen pressure (Sundell et al. 2004, Huitu et al. 2007) and intraspecific competition (Hansen et al. 1999) can vary according to the cycle phase, which challenges the individuals and may set different selection pressures on them. The differential selection pressures (i.e. density-dependent selection) in the bank vole have been evidenced by the changes in reproductive effort (Helle et al. unpublished manuscript) and reproductive tactics (Mappes et al. 2008b) that vary according to population density. As I discussed previously (see section 1.2.1), Chitty (1967) suggested that it is possible that the traits (e.g. associated with social behavior) also show different responses according to the cycle phase. This mechanism could maintain genetic variation if different traits are selected for at opposing cycle phases.

1.3 Population isolation

1.3.1 Population isolation and inbreeding

Population isolation is known to risk a population's genetic diversity. Isolated populations may suffer from *founder effects* (i.e. the establishment of a population by only a few individuals, Hamilton 2009), and they are often small

in individual numbers which promotes mating between relatives and, furthermore, population inbreeding. Small populations are also susceptible to the random actions of *genetic drift* (i.e. random fluctuation of allele frequencies due to finite population size, Whitlock 2004). The action of inbreeding and genetic drift can lead to reproductive isolation of populations to such a degree that, in extreme cases, the events of speciation or extinction might take place (Lande 1980, Saccheri et al. 1998, Frankham 1995, 1998). Also populations living in fragmented habitats share many of the features of isolated populations, such as the increased risk of mating between relatives and the increased risk of losing their genetic diversity (see e.g. Couvet 2002, Aguilar et al. 2008).

Both inbreeding and genetic drift can lead to increased *homozygosity* within populations. Homozygosity may have harmful effects on individuals' fitness due to the expression of recessive deleterious alleles (the dominance hypothesis) and due to the loss of heterozygosity at loci with heterozygote advantage (the overdominance hypothesis) (Charlesworth & Willis 2009). Recessive deleterious alleles are alleles present in a population in recessive state, i.e. suppressed by the healthful allele at the locus. As a consequence of inbreeding these recessive alleles may be homozygous within an inbred individual and the deleterious allele is expressed. In the case of overdominance, heterozygous individuals have superior fitness compared to homozygous individuals. Because of inbreeding increases homozygosity it also reduces the proportion of superior heterozygotes.

When the population size is small, the importance of genetic drift in determining allele frequencies can outweigh the effects of selection (Frankham et al. 2002). However, as homozygosity increases within the population, natural selection has a better opportunity to act against recessive deleterious alleles (*purging*, Glémin 2003). Therefore, it is not simple to predict how the allele frequencies change under these counteracting forces.

1.3.2 Features of island vole populations

Island populations of terrestrial species are good examples of habitat fragmentation and population isolation: the water barrier usually impedes or prevents migration among island populations and also between mainland and island populations. Island vole populations possess a few exceptional features that deserve a short discussion here.

First, it is important to note, that the bank vole is an energetic disperser with dispersal distances extending up to 8.5 kilometers in continuous landscapes (Gliwicz & Ims 2000). Hence, it is very likely that the species is capable of migrating in patchy insular landscapes, especially over the ice cover during winter (see also Crone et al. 2001 for the migration abilities of the field vole, *Microtus agrestis*).

Second, metapopulation processes have been proposed to prevail in some small mammalian populations, including some insular populations of the field vole (*Microtus agrestis* in a Fennoscandian archipelago (Crone et al. 2001) and water vole (*Arvicola terrestris* in a Scottish river basin (Telfer et al. 2001)).The

term *metapopulation* refers to a situation where a variety of local discrete populations (i.e. the metapopulation) interact via migration and gene flow (Hanski & Gaggiotti 2004). The processes of local extinctions and recolonizations characterize metapopulation dynamics (Slatkin 1977), and thus *migration* among the local populations plays a central role in the metapopulation framework. Frequent extinctions and recolonizations affect the genetic composition of the populations: the genetic differentiation should be high within newly established populations due to metapopulation dynamics (Whitlock & MacCauley 1990, Whitlock 1992, Ingvarsson et al. 1997).

Third, if water barriers restrict individual dispersal effectively, overexploitation of food resources is often the main reason for vole populations' extinctions from islands (Pokki 1981). This overgrazing is generally referred as *the fence effect* (Krebs et al. 1969), which is sometimes observed in predator-free ecosystems, such as on islands (Fey et al. 2008).

Finally, individuals on isolated islands show differences in traits, and the phenomenon is sometimes termed *the island syndrome* **(**Adler & Levins 1994; but see also Meiri et al. 2008). These differences include e.g. larger body size, higher population density, and reduced aggressiveness. Islands, especially the small ones, often lack predators (Adler & Levins 1994, Fey et al. 2008), which, together with restricted dispersal, contributes to higher population density. Isolated islands are also limited with resources (e.g. food and/or territories), which increases inter- and intraspecific competition (Adler & Levins 1994, Koskela et al. 1997). In these settings, tolerance towards others may play a critical role in survival and fecundity. In several vole species (see e.g. Lambin & Krebs 1993, Kawata 1987, Pusenius et al. 1998), including the bank vole (Mappes et al. 1995) the relatedness of females enhances their reproductive success, which is in accordance with the kin selection theory (Hamilton 1963). Therefore, in isolated vole populations *kin tolerance* may be critical in determining the fitness of the individuals and, furthermore, the frequencies of alleles passed to next generations.

1.3.3 Maintenance of genetic diversity within subdivided populations

If we consider restricted migration among isolated or fragmented populations, the situation can be referred to as *population subdivision*. The processes that maintain genetic diversity within natural populations, as I discussed above (section 1.1), include mutation-selection balance and balancing selection, and these mechanisms can also preserve variation within subdivided populations. However, I want to make a few general comments concerning the special quality of these populations.

Mutation *per se* is not considerably affected by population subdivision, but the intensity of selection can change with population structure (Whitlock 2004). As population subdivision increases, the proportion of homozygous individuals increases, and selection can act against recessive deleterious alleles (Whitlock 2002). In consequence, genetic variation can be reduced in subdivided populations as homozygous individuals are restricted in numbers

by selection. The ability of heterozygote advantage to maintain genetic diversity within subdivided populations might be reduced if either of the homozygous states has better fitness than the other one; in this case the population might be fixed for the allele with the most fit genotype (Whitlock 2004). In frequency-dependent selection different alleles can be maintained in different subpopulations, which turns out as an increase in the total diversity of the species. Finally, environmental heterogeneity can maintain genetic variability if there are selectively and epistatically different alleles in different local populations (Whitlock 2004).

1.4 Fitness effects of genetic diversity

Fitness, described as an individual's ability to both survive and reproduce, in relation to the other individuals in the population, can be linked to genetic diversity in various ways. First, reduced genetic diversity, i.e. increased homozygosity, can result in *inbreeding depression* of individuals, which is expressed as reduced survival and fertility of offspring of related individuals due to the higher likelihood of having deleterious recessive alleles (Charlesworth & Charlesworth 1999, Charlesworth & Willis 2009). The phenomenon was first documented in plants (Darwin 1876), but inbreeding lowers fitness-related traits also in many animal species (e.g. Charlesworth & Charlesworth 1987, Saccheri et al. 1996, Crnokrak & Roff 1999, Hedrick & Kalinowski 2000, Nielsen et al. 2012), and can also cause heritable diseases in humans (see e.g. Peltonen et al. 1999 for Finnish disease heritage).

Second, quite the opposite of inbreeding depression is the phenomenon termed *heterosis*, (or hybrid vigour) which I already discussed as one mechanism potentially maintaining genetic diversity within wild populations (see section 1.1). Heterosis is, indeed, a beneficial fitness effect of genetic diversity. Typically recorded within the first filial generation (F1) crossed from individuals of inbred lineages or different populations (Shull 1948), heterosis has been detected in wild mammalian species, where heterozygous individuals have higher fitness compared to homozygous ones (Coltman et al. 1999, Slate et al. 2000). Heterosis is suggested to result from the overall increased heterozygosity within hybrid individuals, since in the heterozygous state recessive deleterious alleles are masked and the heterozygosity of overdominant alleles is restored (Lynch 1991, Charlesworth & Willis 2009).

Third, increased heterozygosity does not always translate to positive fitness effects. *Outbreeding depression* is encountered when the hybrid offspring show reduced fitness compared to their parent (Lynch 1991). Outbreeding depression can result from the disruption of local adaptations, if parental populations have adapted to different environmental conditions and their progeny is adapted to neither of the parental environments (Montalvo & Ellstrand 2001). The other explanation for outbreeding depression is the Dobzhansky-Muller incompatibility (Dobzhansky 1936, Orr & Turelli 2001)

where hybrid offspring show reduced fitness due to incompatible combination of alleles. These incompatibilities can arise even in the absence of either beneficial or detrimental effects of parental alleles. Yet another process, the segregation of co-adapted gene complexes, can cause outbreeding depression (Lynch 1991). Co-adapted gene complexes are formed by beneficial alleles at multiple loci, which are passed to offspring as a whole and the destruction of these associations will have detrimental fitness effects.

A vast amount of research has been conducted to find linkage between genetic heterozygosity and individual fitness. These studies, commonly known as *heterozygosity-fitness correlations*, or HFC's, have been used to study the effects of inbreeding in non-pedigreed wild populations (reviewed e.g. by Chapman et al. 2009, but see also Szulkin et al. 2010). In HFC an individual's variation at genetic marker loci is correlated with variation in fitness or fitness-related traits. Three hypotheses have been proposed to explain these correlations (Hansson & Westerberg 2002). In *the direct effect hypothesis* markers used to estimate genetic variation are themselves affecting an individual's fitness (David 1998). This situation can take place when using e.g. allozyme markers which often have strong linkage to enzyme function and hence straight association with fitness. In *the local effect hypothesis* associative overdominance is proposed to create the correlation between genetic diversity and fitness. The fitness increase is due to linkage disequilibrium between fitness loci and marker loci (David 1998). In *the general effect hypothesis* fitness loci and marker loci are suggested to be in identity disequilibrium, referring to the nonrandom association of diploid genotypes in zygotes and therefore more multiheterozygotes and multihomozygotes occur than expected from single-locus heterozygosities (David 1998, Chapman et al. 2009).

1.5 Genetic markers

1.5.1 Microsatellites

Genetic markers are essential in the research of genetic variation and the genetic consequences of population size changes and population isolation. Molecular markers are sections of the genome, which are presumed to represent much larger segments of DNA. Useful genetic markers have alternative sequences, termed *alleles*, present in the population, i.e. they are polymorphic. The polymorphism of the markers allows the discrimination of specimens, to the level of individuals or groups of individuals, which is often required in population genetic analyses. Many genetic markers are considered to be selectively neutral, which permits the assumption that the variability across marker loci reflects the total genetic variability of an individual or population. However, not all microsatellites are neutral in terms of selection, and probably a more appropriate term for these would be anonymous microsatellite loci.

One type of genetic markers are *microsatellites*, which are tandem repeats of a sequence of 2-6 base pairs of nuclear DNA, located in both coding and non-coding regions of the genome. The length of the repeat is highly variable because of high mutation rate within these regions (Tautz 1989), most likely due to strand slippage during DNA replication (Schlötterer et al. 1991). The high variability together with codominance (i.e. neither of the alleles is dominant over the other) makes microsatellites ideal tools for studying variation at population and individual levels (Tautz 1989). Moreover, since some microsatellites are associated with functional genes (i.e. located at the transcription regulation site or within introns) those loci may affect the transformation or activity of corresponding genes, and can be used in e.g. candidate gene studies. Microsatellite loci have become also fairly easy and cheap to obtain (Zane et al. 2002), and their usage in genetic analyses is fast because of the polymerase chain reaction (PCR) technique. The within-species-conserved sequences flanking the repeat allow locus-specific PCR primers to be designed.

Genetic databases, such as GenBank (http://www.ncbi.nlm.nih.gov/genbank/), are filled with various sequences, including genetic markers. Sequences for non-model species, however, are often lacking and therefore they have to be isolated *de novo*. Different strategies have been used for microsatellite isolation (reviewed by Zane et al. 2002). Traditionally, microsatellites were isolated from genomic libraries by screening hundreds or thousands of colonies with hybridization of repeat-containing probes (Rassmann et al. 1991). Since then, several methodologies have been proposed and applied to the isolation of microsatellite loci, such as modifications of randomly amplified polymorphic DNA (RAPD, Williams et al. 1990, Wu et al. 1994) and primer-extension (Ostrander et al. 1992, Paetkau 1999). Today the most widely used protocols utilize *selective hybridization*, originally proposed by Karagyozov et al. (1993), Armour et al. (1994) and Kijas et al. (1994). Most recently, next generation sequencing techniques have been started to use in microsatellite development (see e.g. Guichoux et al. 2011).

The protocol of *Fast Isolation by AFLP of Sequences Containing Repeats*, i.e. the FIASCO protocol, originally proposed by Zane et al. (2002), utilizes the method of amplified fragment length polymorphism (AFLP, Vos et al. 1995). In short, the genomic DNA is simultaneously fragmented by a restriction endonuclease enzyme and ligated to specific oligonucleotide adaptors. The adaptor sequences bear annealing sites for PCR primers, which allows the amplification of DNA-adaptor complex by PCR. The amplified fragments are hybridized at specific conditions with a biotinylated probe consisting of the desired repeat sequence. Hybridized fragments are selectively captured by streptavidin-coated magnetic beads, after which the DNA is separated from the complex. This is followed by the precipitation and amplification of the DNA, and as a result, the isolated microsatellite loci are ready to be characterized.

1.5.2 Social behavior-associated microsatellite loci

In addition to selectively neutral (or anonymous) loci, microsatellites can also be associated with functional genes. Examples of this are microsatellite loci situated at the upstream 5′ regulatory region of neuro-hormone receptors for vasopressin (*avpr1a*) and oxytocin (*oxyr*) in mammals (Hammock & Young 2005). In rodent models vasopressin and oxytocin play important roles in adjusting social and reproductive behavior, such as parental care, monogamy and maternal attachment (reviewed by Donaldson & Young 2008). It has been shown that vasopressin associates with social behavior through its interaction with *avpr1a* (*e.g*. Winslow et al. 1993, Insel et al. 1994; reviewed by Donaldson & Young 2008), and the same apparently holds true with oxytocin and its receptor (Insel & Shapiro 1992, Gimpl & Fahrenholz 2001).

A fascinating quality of this system is that the prevalence of *avpr1a* in the brain is determined by allelic variation at the adjacent microsatellite locus in such a way that longer alleles are associated with higher densities of *avpr1a* (Lim et al. 2004, Hammock & Young 2004, 2005, but see Hammock et al. 2005). Consequently, individual genotype on the *avpr1a*-associated microsatellite locus should determine the density of the receptor and also behavioral response of the individual.

1.6 Aims of the thesis

The focus of my thesis was to explore the genetic diversity in wild bank vole (*Myodes glareolus*) populations. More specifically, I aimed to

1. Design genetic tools for studying wild bank vole populations
2. Investigate how populations' genetic diversity was affected by and maintained within cyclic population dynamics
3. Study whether cyclic population dynamics create density-dependent selection on behavior-associated genetic loci within the population
4. Study if population isolation/fragmentation reduced populations' genetic diversity
5. Examine the fitness consequences of genetic variability

Being a non-model organism, genetic markers for the bank vole were not abundant before this study (Gockel et al. 1997, Gerlach & Musolf 2001; II). Therefore it was essential to isolate and characterize usable tools for studying genetic diversity and its consequences within wild bank vole populations (*aim 1*). As the wild bank vole populations exhibit oscillating population cycles

(Korpimäki et al. 2005, Kallio et al. 2009) they provide natural settings for studying the effects of population size changes on population's genetic diversity (*aim 2*) and density-dependent selection (*aim 3*). During the crash phases the population might face erosion in its genetic composition, presumably up to the brink of a genetic bottleneck (Nei et al. 1975, Saccheri & Hanski 2006). Moreover, at different cycle phases the individuals may face different selection pressures (Mappes et al. 2008b, Helle et al. unpublished manuscript) which provides an opportunity to study the contribution of environmental heterogeneity on population's genetic diversity (*aim 2*) and density-dependent selection (*aim 3*). The bank vole, being probably the most common small rodent in Finland, is able to energetically inhabit insular habitats creating natural framework for studying the genetic effects of population isolation and fragmentation (*aim 4*). Furthermore, the bank vole has a good trappability (Koskela et al. 1997, Koivula et al. 2003), it can be successfully reared in laboratory facilities (Oksanen et al. 2003, Schroderus et al. 2010) and its life history is well characterized (e.g. Koivula et al. 2003, Mappes & Koskela 2004, Mappes et al. 2008a,b), which make the species suitable for fitness research (*aim 5*).

The first aim of this thesis was to develop genetic tools for further studies in the bank vole. I revised, modified (I) and applied (II) a method to enrich microsatellite-containing fragments from bank vole genome and designed a panel of informative genetic anonymous microsatellite markers for use in my study populations. We also developed three expressed sequence tag (EST) - linked microsatellite loci (IV) that were not assigned to any biological function. These EST-loci were developed to see if gene-linked microsatellites *per se* respond to natural selection.

With the genetic tools in hand I proceeded to studying the effects of cyclic population dynamics on population's genetic diversity (III). I contrasted population cycle peaks and crashes to see whether population size reduction lead to reduction in genetic diversity.

Moreover, I evaluated possible mechanisms, including environmental heterogeneity that may contribute to genetic diversity in the population (III). I also examined more specifically if environmental heterogeneity created temporally varying density-dependent selection within the population, or, more specifically, social behavior-associated genetic loci (IV).

Then I studied the effects of population isolation/fragmentation on genetic diversity (V). I used genetic data collected from both fragmented island habitats and from a continuous mainland habitat to see if fragmentation reduced genetic diversity within the island populations. Moreover, I analyzed how individual fitness was affected by inbreeding.

2 MATERIALS AND METHODS

2.1 Study species

The bank vole *Myodes glareolus* (Fig. 1) is one of the most common wild mammals in boreal coniferous and deciduous forests and fields in northern Europe (Stenseth 1985, Bujalska & Hansson 2000). It mainly feeds on plants, seeds and fungi (Hansson 1985), and sometimes on animal material (Hansson & Larsson 1978, Gebczynska 1983). Small predators, such as the least weasel (*Mustela nivalis nivalis*) and the stoat (*Mustela ermine*), together with several avian predators (e.g. Eurasian kestrel (*Falco tinnunculus*), the short-eared owl (*Asio flammeus*) and Tengmalm's owl (*Aegolius funerus*) prey on wild bank vole populations in Fennoscandia (Korpimäki & Norrdahl 1989, Korpimäki et al. 1991, Norrdahl & Korpimäki 1995).

The species is characterized by a short life span, young age of maturation, high fecundity and promiscuous mating system (Tkadlec & Zedja 1998, Mills et al. 2006), and there is both phenotypic and genotypic variation in these life-history traits (Koivula et al. 2003, Mappes & Koskela 2004, Mappes et al. 2008b, Schroderus et al. 2012).

The breeding season of bank voles in our study area in Central Finland lasts from late April to early September during which the females give birth to several litters with one to ten pups (Koivula et al. 2003). The females exhibit *post partum* oestrus (i.e. are able to become gravid within a few hours after parturition), which enables them to be gravid while lactating, i.e. for most of the breeding season. Environmental conditions in terms of food and territorial abundance are crucial for successful breeding of the females (Koskela et al. 1998).

In northern Fennoscandia the bank vole populations show dynamic population cycles, i.e. fluctuations in individual numbers (Korpimäki et al. 2005). The cycles are characterized by a length of three to five years and high amplitude (i.e. high difference in population size between the crash and the peak phases) (Kallio et al. 2009).

FIGURE 1 The study species, the bank vole (*Myodes glareolus*). Photograph by Heikki Helle.

2.2 Study populations

The concept *population* usually refers to a group of individuals capable of mating with each other. Hence, population boundaries can consist of either space or time. My thesis was concerned with changes in both of these dimensions: in space (population fragmentation) and in time (the phases of population cycle). In this thesis I have used the term population in distinguishing the groups of individuals sampled both at spatially different locations (V) and during a certain year (II-IV).

The bank vole populations used in this thesis were situated in Konnevesi (62°37′N, 26°20′E), Central Finland (Fig. 2). The bank vole density in the study site oscillated with regular three-year population cycles (Fig. 3). For monitoring the population cycle, bank vole individuals were trapped using live traps (Ugglan multi-capture live traps, Grahnab, Hillerstorp, Sweden) four times per year: in May (early breeding season), July (middle of the breeding season), August (late breeding season) and late October-early November (after the breeding season). 20 trapping sites were distributed over an area of approximately 100 km^2 and situated in coniferous, deciduous or mixed forests (Fig. 2).

The size of bank vole populations was estimated as the trapping index (Fig. 3). The trapping index is the number of captured individuals per 100 trap

nights where monthly trapping data are interpolated from the trappings carried out four times per year. The population cycle phase categorization (III and IV, Fig. 3) was established based on an autocorrelation analysis according to Kallio et al. (2009).

The 20 trapping points on mainland localities (III-V; Fig. 2) were essentially the same used for monitoring the population cycle (see above). The 25 island populations (Fig. 2) were located on the islands of the lake Konnevesi and their trapping points were situated at the places the voles frequently use.

The samples used in characterizing the microsatellite markers (II) were collected at the crash year 2000 (N=20) and at the following peak year 2002 (N=20). The samples for studying the effects of population dynamics (III) and density-dependent selection (IV) were collected during the years 1999-2006, a period of three consecutive population cycles. The samples used in studying population isolation/fragmentation and its fitness effects (V) were collected in the year 1999. The bank voles were trapped during the reproductive season. Morphometric features, such as weight and head width, and reproductive status of the animals were recorded and tissue samples were taken for genetic analyses (II-V). Morphometric characteristics and body mass was used to determine the maturity state of the individuals (II-V).

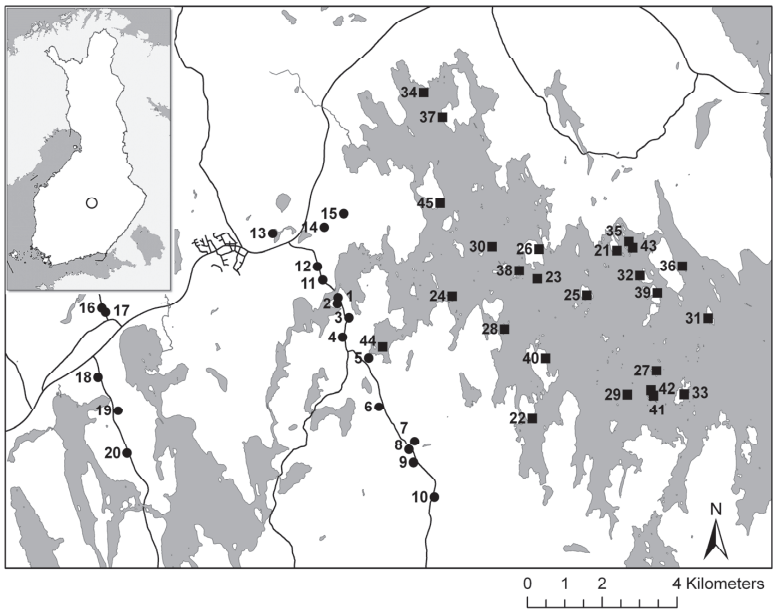

FIGURE 2 The trapping sites. The small figure shows the location of the study site (open circle) at Konnevesi, central Finland. The large figure shows the locations of 20 mainland trapping sites (closed circles) and 25 island trapping sites (closed rectangles).

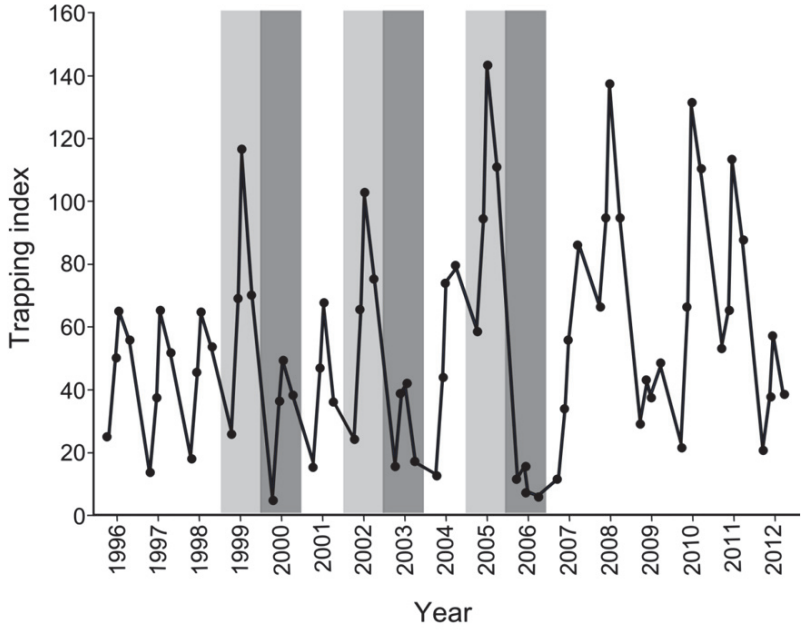

FIGURE 3 The population cycle of bank voles in the study area. The number of voles is presented as the trapping index (i.e. captured individuals/100 trap nights, monthly data are interpolated from the trappings carried out four times per year), the trappings are indicated with circles. The analyzed years (in studies III and IV) are indicated with light (peak phases) and dark (crash phases) bars.

2.3 Laboratory procedures

2.3.1 Animal housing (V)

All adult females (based on their outer characteristics and weight) were brought into the laboratory for fitness measurements. The animals were housed in Eurostandard type III cages (425×266×155 mm) with wood chips and hay and maintained in 16:8 light/dark photoperiod. Food and water was provided *ad libitum*.

2.3.2 Measuring individual fitness (V)

The fitness of adult female individuals was studied in terms of breeding probability and litter size. Because of the *post partum* oestrus the breeding bank vole females are gravid regularly throughout the whole breeding season. This allowed us to determine the breeding probability as the gravidity status of wild-captured individuals. The gravid females were checked daily for

parturition. The pups were immediately counted and weighted and the litter size was determined as the number of pups delivered by the female.

The correlation between individual fitness and individual inbreeding status (see section 2.4 for details) of the females was calculated and compared between the fragmented and continuous populations (Sas version 9.1; Sas Institute Inc., U.S.).

2.3.3 Genetic analyses (I-V)

In this study, three kinds of microsatellite markers were used: anonymous microsatellites (II-V), EST-linked microsatellites (IV) and social behavior - associated microsatellites (IV). Total genomic DNA was extracted from tissue samples using either a solution of 5% chelex resin (V; Sigma-Aldrich, U.S.) or Kingfisher magnetic particle processor (Thermo Fisher Scientific, U.S.) with QIAGEN chemistry (II-V; Qiagen, Germany). For enriching the (anonymous) microsatellite-containing repeats (I), a hybridization-based isolation protocol was revised, modified and applied (Zane et al. 2002). The oligonucleotide probes $(CA)_{22}$, $(CAG)_{11}$, $(GA)_{12}$ and $(CATA)_8$ were used in hybridization process. The insert-containing clones of the size 500 base pairs and larger were sequenced with BIGDYE chemistry and ABI PRISM (Applied Biosystems, U.S.). The primers flanking feasible microsatellites were designed with the software PRIMER 3 (Rozen & Skaletsky 2000). In addition to the anonymous microsatellite loci characterized in the study I, I used another six anonymous microsatellite loci described by Gockel et al. (1997) and Gerlach and Muslof (2001).

We also developed three EST-linked microsatellite loci (IV). These loci were derived from a bank vole heart trancriptome (Babik et al. 2012) and could not be assigned to any biological process (BLASTx, Altschul et al. 1990). The microsatellites from the transcriptome sequence were detected and primers were designed using QDD (Meglécz et al. 2010) and PRIMER 3 (Rozen & Skaletsky 2000). Moreover, I used three social behavior-associated (*avpr1a*- or *oxyr*-associated) microsatellite loci (IV, Watts et al. unpublished data). Two of these were associated to *avrpr1a* (one located at the transcription regulation site and the other within the intron), and the third was situated at the 5′ regulation region of *oxyr*.

PCR amplifications were carried out in 10 µl reactions consisting one of the three mixes (Table 1). PCR conditions differed slightly according to the primers used (Table 2). The amplified fragments were either (V) run in a LI-COR GENE READIR 4200 automatic sequencer (Li-Cor Inc., U.S.) or (II-IV) detected with an ABI PRISM 3100xl (Applied Biosystems, U.S.). The alleles were (V) scored by eye and using LI-COR GENE PROFILER (Li-Cor Inc.) or (II-IV) using GENEMAPPER version 3.7 software (Applied Biosystems). The sizes of alleles were determined by (V) running the sequence on the plasmid pUC18 (Thermo Fisher Scientific, U.S.) or (II-IV) by GENEMAPPER version 3.7.

TABLE 1 Polymerase chain reaction mixes.

	Experiment		
	II, III, IV	IV	V
DNA	70 ng	75 ng	20-50 ng
Taq Polymerase	0.25 U (Biotools)	0.05 U (Fermentas)	0.5 U (Gibco)
PCR Buffer	1×$MgCl_2$-free PCR Buffer (Biotools)	1×DreamTaq Buffer (Fermentas)	75 mM Tris-HCl 20 mM $(NH_4)SO_4$ 0.01% Tween
dNTPs	2 mM each	200 nM each	2 mM each
unlabelled forward primer	4.5 µM	-	4.5 pM
labelled forward primer	0.5 µM	0.3 µM	0.5 pM
reverse primer	5 µM	0.3 µM	5 pM
MgCl2	locus-optimized conc.	-	1.25 mM

TABLE 2 Polymerase chain reaction thermocycling conditions.

	Microsatellites described by		
	Rikalainen et al. 2008 (II)	Watts et al. (IV)	Gockel et al. (1997) and Gerlach and Muslof (2001) (V)
Initial denaturation	2 min, 94 °C	3 min, 95°C	5 min, 94°C
Thermocycling denaturation annealing extension	30 cycles of 45 s, 94 °C 45 s, 50 °C or55 °C 1 min, 72 °C	35 cycles of 30 s, 95°C 40 s, 50°C 30 s, 72°C	30 cycles of 30 s, 94°C 30 s, 55°C 45 s, 72°C
Terminal elongation	-	-	5 min, 72°C

2.4 Population genetics (II-V)

The initial analyses of the genetic data included scoring for null alleles, large allele drop-out, stuttering (MICRO-CHECKER; Van Oosterhout et al. 2004) and

linkage disequilibrium (GENEPOP on the web; Raymond & Rousset 1995). Highly susceptible loci were omitted (III, IV). The deviations from Hardy-Weinberg expectations were estimated (FSTAT version 2.9.3.2, Goudet 1995) and Bonferroni procedures (II, IV, V; Rice 1989) or false discovery rate approach (III; Benjamini & Hochberg 1995) was used whenever multiple testing could have resulted in possible type I errors.

Genetic diversity was measured as observed heterozygosity, expected heterozygosity and allelic richness (i.e. the number of alleles independent of sample size). These measures were compared between the crash and peak phases of the population cycle (III, IV) and between fragmented and continuous populations (V). Changes in effective population size and possible encountered population bottlenecks (III) were also estimated (DIYABC, Cornuet et al. 2008; BOTTLENECK version 1.2, Cornuet & Luikart 1996). The number of private alleles (i.e. alleles discovered only at one temporal population, III) was determined (ARLEQUIN version 3.0; Excoffier et al. 2005) to see if new allelic material was frequently or cyclically introduced to the population and would thus refer to individual migration.

Population genetic structure was estimated as *Fst* according to Weir and Cockerham (Weir & Cockerham 1984). High *Fst* values refer to population sub-structure via e.g. isolation. Pairwise *Fst* values were calculated for each temporal population (III) and for fragmented and continuous populations (V) and these values were compared between crash and peak phases (III) and between island and mainland populations (V). The correlation between *Fst* and temporal distance matrices (III) was calculated (TFPGA version 1.3, Miller 1997). Spatial genetic structure (III) was analyzed (STRUCTURE, Pritchard et al. 2000; SPAGEDI, Hardy & Vekemans 2002) to see e.g. differences in migration pattern of the individuals.

Environmental heterogeneity (III) was studied in terms of temporal changes in the genetic composition of the population with the analysis of molecular variance (AMOVA, ARLEQUIN version 3.1, Excoffier et al. 2005). Evidence for density-dependent selection (IV) was examined by searching for outlier loci based on *Fst* (LOSITAN, Antao et al. 2008, Beaumont & Nichols 1996) and reduced levels of genetic diversity (lnRH test, Kauer et al. 2003).

Inbreeding within populations was estimated as *Fis* (Weir & Cockerham 1984) and it was contrasted between the population phases (III) and between the fragmented and the continuous populations (IV). Moreover, individual inbreeding coefficients (multilocus heterozygosity, [the proportion of loci for which an individual is heterozygous], internal relatedness (Amos et al. 2001), homozygosity by loci (Aparacio et al. 2006), and standardized heterozygosity (Coltman et al. 1999)) were calculated (IV; the last three by using IR MACRON3, available at
http://www.zoo.cam.ac.uk/zoostaff/meg/amos.htm#ComputerPrograms)
and the values were contrasted between island and mainland populations.

3 RESULTS AND DISCUSSION

3.1 Development of genetic tools (I, II, IV)

3.1.1 Anonymous microsatellite loci (I, II)

I revised, modified and applied a protocol of *Fast Isolation by AFLP of Sequences Containing Repeats* (FIASCO) for anonymous microsatellite loci isolation (Zane et al. 2002). The protocol was originally developed and tested for one avian and three marine species, but the usage for mammalian species has been infinitesimal.

In total, 987 clones containing inserts were sequenced, and primers flanking the repeats were designed for 221 of these. As a result, 66 microsatellite loci from the bank vole proved to be stable and highly polymorphic and therefore usable for further studies. Some deviations from Hardy-Weinberg expectations were detected, possibly due to the presence of null alleles at those loci. Though some linkage was detected between some of the loci, it was not present in both of the sampled populations. The sequences of the characterized loci are placed at public disposal at GenBank.

3.1.2 EST-linked microsatellite loci (IV)

In addition to anonymous microsatellite loci (section 3.1.1), also EST-linked microsatellite loci were developed (IV). Raw data from the bank vole heart transcriptome (provided kindly by W. Babik) was screened for microsatellite sequences, and those were searched for protein annotations. The three EST-linked loci used in this study could not be annotated with any behavioral function; however, these loci were included in study IV in order to determine the possible selection response to gene-associated loci *per se* (i.e. to distinguish their selective response from social behavior-associated loci).

3.2 Genetic diversity in relation to cyclic population dynamics (III, IV)

3.2.1 Genetic diversity throughout the population cycle (III)

Cyclic population dynamics can predispose populations to genetic erosion, i.e. reduction in genetic variation, because of low individual numbers during the crash phases. This is most obviously seen in populations that have encountered genetic bottleneck (Nei et al. 1975, Saccheri & Hanski 2006). The population I studied went through substantial reductions in population size during the study period (see Fig 3). During the three crashes the population size was lowered from 54 to 90 per cent per crash. Reductions of this magnitude might significantly reduce the effective population size and cause genetic bottlenecks.

Genetic *bottlenecks* can be detected from a single temporal sample by using heterozygosity excess as evidence. During a bottleneck, rare alleles are lost due to drift more rapidly that the common ones (Nei et al. 1975). Thus, during a bottleneck allelic diversity is reduced faster than heterozygosity. As a consequence, there is a difference between the observed heterozygosity and the heterozygosity expected from the number of alleles (Cornuet & Luikart 1996). This heterozygosity excess can be taken as a sign for recent bottleneck. In this study, I did not find any signs of genetic bottlenecks within the populations even during the crash phases. Despite the drastic changes in population size as measured by the trapping index, the *effective population size* remained fairly stable and high even during the crash phases.

I also found that population's *genetic diversity* (observed or expected heterozygosity) was not affected by the cyclic population dynamics. Allelic richness was the only parameter that showed marginal, but presumably not biologically significant difference between the crash and peak phases. Other studies on the genetic diversity of cyclic rodent populations have also showed high heterozygosity values in different phases of the population cycle (Plante et al. 1989, Berthier et al. 2005, 2006, Ehrich & Jorde 2005, Redeker et al. 2006, Ehrich et al. 2009). Compared to heterozygosity, however, allelic richness is suggested to be a more sensitive estimator of short and severe genetic crashes, because of its ability to detect the loss of rare alleles (Nei et al. 1975, Spencer et al. 2000). Also my results support this, since allelic richness was slightly lowered during the crash phases. Nevertheless, this state was shortly cancelled out during the next peak phase.

3.2.2 Processes maintaining genetic diversity (III)

According to my results, during the peaks new alleles (i.e. private alleles) accumulated in the population and the spatial genetic pattern was more homogenous compared to the crash phases. During the crash phases the population was characterized by isolation-by-distance. Both uniform population structure and the emergence of new alleles refer to accelerated gene

flow during the peak phases. This suggests accelerated *migration* rates during the peaks, as proposed by Berthier et al. (2006).

On the other hand, my analyses did not indicate distinctive breeding units during the population crashes. During the crash phases the population might be expected to display more patchy population structure if discrete breeding clusters form within the population (Berthier et al. 2006). In this system, however, the bank vole population apparently never reaches very low individual numbers and therefore discrete breeding units do not form during the crash phases. Alternatively, discrete breeding units may not form if migration is effective enough even during the crash phases (see Ims & Andreassen 2005).

In this study the population structure (isolation-by-distance) during the crash phases was obvious, whereas during the peak phases it was absent. These differences in *spacing behavior* of individuals refer to positive density-dependence (Berthier et al. 2006), where large population size promotes migration. On the contrary, other empirical evidence suggests negative density-dependence within vole species (Ims & Andreassen 2005, Andreassen & Ims 2001). In addition to this I detected one more peculiarity about spacing behavior: I discovered that male individuals were responsible for the isolation-by-distance that predominated during the crash phases whereas the females comprised more homogenous population structure. This is ambiguous since the bank vole females are known to be territorial and form kin structures during breeding seasons (Mappes et al. 1995, Lambin & Yoccoz 1998), while the males usually disperse (Gliwicz & Ims 2000). All these controversies imply that the dispersal and migration in arvicoline rodents are dynamic and complex processes which are also affected by factors other than population density and inbreeding avoidance (e.g. mate searching, competition and habitat quality; Le Galliard et al. 2012).

I did not find evidence for *environmental heterogeneity* to be affecting the genetic diversity of bank vole populations (see also section 3.2.3). According to my results none of the analyzed anonymous loci were consistently found at a certain cycle phase that would refer to possible linkage between the genetics and the environment. Moreover, most of the genetic variation was found within cycle phases, not among them, indicating that there are no major (anonymous marker –based) genetic differences between the cycle phases. Instead of environmental heterogeneity, the new migration-originated alleles together with constantly high effective population size are a plausible explanation for the high genetic diversity found in the cyclic bank vole population.

3.2.3 Density-dependent selection on social behavior-associated loci (IV)

As I stated previously (see section 3.2.2), there was no evidence of environmental heterogeneity when considering neutral (anonymous) genetic markers (III). However, selective sweeps affect specifically those genes that are under selection (Kauer et al. 2003), and can be therefore distinguished from selectively neutral loci. Social behavior, such as aggression, maternal

attachment and pair bonding, is strongly linked with fitness since pro-social individuals (individuals with stronger pairbonding and maternal affiliation) often gain benefit in mating and produce larger litters (see Castelli et al. 2011). The vole individuals face quite different social environment at the opposing phases of the population cycle. For example, during population peaks intraspecific competition for resources as well as predator and pathogen pressure are more vigorous compared to the situation at low population density (Korpimäki et al. 2005, Huitu et al. 2003, 2007, Soveri 2000). This can create temporally varying environments - and an opportunity for natural selection to erode individuals with the less fit behaviors.

Contrary to these expectations, I did not find evidence for density-dependent selection on different allele lengths during the population cycle. There were no signs that behavior-associated loci, *avpr1a* and *oxyr*, were selected either for or against at the opposing cycle phases. Instead, genetic diversity at these loci was consistently high throughout the population cycle, and the loci did not appear as outliers in terms of genetic differentiation. The only exceptions to this were one anonymous microsatellite locus and another locus situated in the intron of vasopressin receptor gene that were identified as outliers.

Being a rather complex trait, social behavior is likely to be affected by several loci instead of one. This seems a compelling and plausible explanation for my results about *avpr1a* and *oxyr*. In addition to this, the bank vole reproductive success is determined by several factors (e.g. male testosterone level, Mills et al. 2009; immunocompetence, Mills et al. 2010), and the joint effect of these factors might not be easily distinguished from the allelic variation at a single genetic locus. It is also possible that selection acting on pro-social traits in my study population has not been strong enough to give detectable signs specifically on these microsatellite loci. Even though previous research has shown the association between *avpr1a* microsatellite variation, pro-social behavior and fitness (Donaldson & Young 2008), my results do not give any support for temporally varying density-dependent selection at these social behavior-associated loci.

3.3 Genetic diversity in relation to population isolation / fragmentation (V)

3.3.1 Genetic diversity in fragmented and continuous populations

Population isolation can be of great importance in determining the genetic variability within a population (Frankham 1998, Saccheri et al. 1998). Mating between relatives, founder effects and random genetic drift are major causes of an increase in inbreeding and hence a reduction in heterozygosity (Nei et al. 1975, Whitlock 2004). These same risks apply also to fragmented populations

(Couvet 2002, Aguilar et al. 2008). Consistent with this, my results showed that the bank vole populations in fragmented island habitats exhibited lower *genetic diversity* compared to the population in continuous habitat. Moreover, the fragmented habitat populations showed higher genetic structure (*Fst*) than the continuous habitat population. This finding is in concordance with both the metapopulation theorem (Whitlock & McCauley 1990, Hanski & Gaggiotti 2004) and empirical evidence, which show that newly established populations exhibit higher population differentiation due to extinction-recolonization dynamics (Whitlock 1992, Ingvarsson et al. 1997). These results suggest that the reduced genetic diversity detected within island populations can be due to e.g. founder effects or genetic drift and that metapopulation dynamics may also characterize bank vole populations in these settings.

The vole population size peaked during the studied summer and after that crashed to very low numbers in the study area (see Fig. 3). During the crash many of the island populations went to extinction (Mappes et al. unpublished data). Also this demography of extinctions and putative recolonizations refers to *metapopulation* structure. Inbreeding within an isolated population has been advocated as a reason for extinction (Frankham 1995, 1998), and there is empirical evidence for increased extinction risk with decreasing heterozygosity within the metapopulation framework (Saccheri et al. 1998). However, also stochastic environmental factors, such as resource availability and predation, may have an important effect on extinctions of isolated populations (Fey et al. 2008).

Given the dispersal ability of bank voles (Gliwicz & Ims 2000), colonization and/or immigration might be frequent among island patches especially during winter due to ice cover. Even low numbers of immigrants might rescue the population's genetic diversity (Mills & Allendorf 1996). Therefore the detected decrease in genetic diversity might be only a temporal state if migration among islands or between island and mainland localities is frequent.

3.3.2 Fitness effects of genetic diversity

In this study I focused specifically on females' fitness in relation to inbreeding status. My results showed that the individuals were more inbred within fragmented habitats than within the continuous one, which agrees with the theoretical predictions of isolated localities stated above (Frankham 1998). Individual inbreeding often results in harmful effects on fitness, commonly known as *inbreeding depression* (Charlesworth & Charlesworth 1999, Charlesworth & Willis 2009). Contrary to the assumption that inbreeding is disadvantageous for individual fitness, I found that inbreeding enhanced the probability of the females to breed. The females seemed to gain fitness benefit of shared relatedness.

According to the kin selection theory, the presence of kin enhances females' reproductive performance (Hamilton 1963), which has also shown to hold true with several vole species (see e.g. Kawata 1987, Lambin & Krebs 1993,

Pusenius et al. 1998), including the bank vole (Mappes et al. 1995). Living near kin can lower the levels of aggression and reduce the competition with conspecifics as evidenced by the tolerance towards related individuals (Mappes et al. 1995). Especially isolated island populations are characterized by reduced aggression because of the increased relatedness of the individuals (Adler & Levins 1994). My results showed that the individuals within fragmented habitats had overall higher probability to breed than the mainland individuals. It is possible that the individuals on the islands were more tolerant towards each other because of the shared relatedness, which results in beneficial effects on individual fitness.

4 CONCLUSIONS

Genetic diversity within a population is a premise for population viability, adaptive potential and evolution. I discussed above (see sections 1.1, 1.3.3) several mechanisms that are hypothesized and identified to maintain genetic diversity in the wild. However, reductions in population size and population isolation/fragmentation are among the acknowledged risks for decreasing the genetic variability in natural populations (Nei et al. 1975, Saccheri et al. 1998, Frankham 1998, Couvet 2002, Whitlock 2004). In this thesis I examined whether these factors reduce the genetic variation within wild bank vole populations, whether one specific selection mechanism (density-dependent selection) could be working in these circumstances, and whether the reduced genetic variability results in fitness loss of individuals.

My results showed that genetic variation in the mainland bank vole population was high throughout the population cycle (III, IV). Even the drastic reductions in population size did not show on genetic level, neither on anonymous (III) nor on behavior-associated loci (IV). This suggests that changes in population density do not impose density-dependent selection on these social behavior-associated loci in this population and, therefore, there has to be other mechanisms preserving high genetic variation in these settings. Based on the results of my thesis and previous research I have attributed the constantly high population size (even during the crash phases) and effective migration to be the main factors in genetic diversity maintenance in cyclic vole populations. However, as the linkage between social behavior-associated locus (*avpr1a*) and fitness has been established, it would be compelling to study these questions in other vole populations that face even more severe selection pressures.

The results of my thesis showed that the effects of population size reduction and fragmentation were not similar. The drastic and repetitive reductions in population size did not erode its genetic diversity (III, IV) whereas population fragmentation seemed to be associated with reduced genetic diversity and increased population sub-division (V). These results are deducible given the notable migration abilities of the bank vole (Gliwicz & Ims 2000). The mainland population I studied seemed to be comprised of one global

unit where the individuals were able to move at their will. Moreover, the landscape was most likely rich in suitable resources (food, territories, mates) throughout the population cycle, which contributed to the maintenance of high effective population size and, furthermore, high genetic diversity. On the islands the settings are quite the opposite, since the habitat bears many limitations. Especially the restricted space use is likely to constrain either the number of breeding females (Bujalska & Saitoh 2000, Koskela et al. 1997) or their quality in terms of social tolerance (Mappes et al. 1995). Also other resource limitations (such as food and mates) might affect the breeding success, reproductive effort and, furthermore, population inbreeding and structure. It would be interesting to examine the local extinctions and recolonizations on the islands to see whether the metapopulation processes are valid for the bank vole and how the genetic diversity is maintained in these settings in the long run.

The results of my research revealed an intriguing and quite unusual association between individual inbreeding and fitness, since the more inbred females had higher probability to breed and, in this sense, gained a fitness benefit. As I stated above, living on an isolated island, more or less crowded, might bring about atypical social situations among the conspecifics. In these settings tolerance towards others might become significant in determining resource allocation: if less energy is spent on aggressiveness or competition towards others more resources can be allocated to e.g. reproduction (as demonstrated e.g. by Boulay et al. 2010). This is a captivating mechanism but whether these predictions actually lie behind the present results remain to be studied in the future. Also other mechanisms, such as the occurrence of local adaptations on the islands, still wait their turn to be addressed in the forthcoming research.

To sum up, the pathway from population size reduction or isolation via inbreeding to impaired fitness is neither a straightforward one nor should be taken for granted. Genetic diversity, its maintenance and its fitness consequences within wild populations are likely to be formed by the complex interplay of selective forces, individual characteristics, social interactions, and more or less stochastic events within individuals (genetics) and environment. In this thesis I have addressed the question how some of these factors operate within the wild. However, my results also bring about new questions and perspectives to the field: e.g. what are the roles of other selective forces (heterozygote advantage, antagonistic pleitotropy, frequency-dependent selection) in the maintenance of genetic variability in cyclic populations; what are the explicit or prevailing processes maintaining genetic diversity on the islands and do they differ from those of continuous populations; what is the actual intensity of migration within and among bank vole populations, both in mainland and island landscapes; to what extent are individual characteristics, including social behavior, relevant to the maintenance of population genetic diversity? Among many others, these questions might serve as stepping-stones as the research on the maintenance of genetic variation in the wild continues.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Populaation syklisyyden ja eristyneisyyden vaikutuksista geneettiseen monimuotoisuuteen

Geneettinen monimuotoisuus on luonnonpopulaatioiden elinehto: se on populaatioiden sopeutumiskyvyn perusta mahdollistaen lajien evoluution. Geneettisesti monimuotoiset yksilöt ovat usein myös elinvoimaisimpia ja tuottavat eniten jälkeläisiä populaatioon. Pieni populaatiokoko on usein riski populaation geneettisen monimuotoisuuden säilymiselle. Pienessä, eristyneessä tai pirstoutuneessa populaatiossa sisäsiitoksen määrä kasvaa ja geneettisen satunnaisajautumisen vaikutukset voimistuvat, mistä usein aiheutuu yksilöiden homotsygotiatason lisääntyminen ja edelleen kelpoisuuden lasku. Kelpoisuuden lasku puolestaan pienentää populaatiokokoa entisestään. Populaatiokoon lasku saattaa aiheuttaa geneettisen pullonkaulailmiön, jossa efektiivinen populaatiokoko (lisääntyvien yksilöiden lukumäärä populaatiossa) äkillisesti laskee. Pullonkaulailmiö lisää edelleen sisäsiitoksen ja geneettisen satunnaisajautumisen mahdollisuutta. Homotsygotian lisääntyminen laskee yksilön kelpoisuutta resessiivisten haitallisten alleelien ilmentymisen vuoksi ja/tai heterotsygotiaedun hävitessä.

Sisäsiitokseen liittyvä kelpoisuuden väheneminen, sisäsiitosheikkous, ilmenee sisäsiittoisten yksilöiden heikkona lisääntymis- ja elinkykynä ulkosiittoisiin yksilöihin verrattuna. Tälle vastakkainen ilmiö, heteroosi, on seurausta haitallisten resessiivisten alleelien peittymisestä terveiden alleelien ilmiasun alle. Toisaalta on havaittu myös tilanteita, joissa ulkosiittoisuus on haitaksi esimerkiksi paikallisten sopeumien tai yhteensopivien geenikompleksien hajoamisen seurauksena.

Väitöskirjatyössäni tutkin geneettisen monimuotoisuuden esiintymistä ja säilymistä luonnonpopulaatioissa. Tavoitteeni oli selvittää syklisen populaatiodynamiikan ja populaation eristyneisyyden tai pirstoutuneisuuden vaikutuksia geneettiseen monimuotoisuuteen ja edelleen monimuotoisuuden vaikutusta yksilöiden kelpoisuuteen. Tutkimukseni mallilaji oli metsämyyrä (*Myodes glareolus*), hyvin yleinen pikkunisäkäs Suomen ja koko Pohjois-Euroopan metsissä ja peltoalueilla. Suomen leveysasteilla metsämyyräpopulaatioiden koko vaihtelee syklisesti 3-4 vuoden jaksossa. Tyypillisesti populaatiosyklissä on kaksi kasvuvuotta, joiden aikana populaatiokoko kasvaa saavuttaen huippunsa, minkä jälkeen kolmannen vuoden aikana se romahtaa ja sykli alkaa uudelleen alusta. Populaation koko voi huippuvuonna olla useita kymmeniä kertoja suurempi romahdusvuoteen verrattuna, joten populaatiokoon romahdus saattaa olla riski populaation geneettisen monimuotoisuuden säilymiselle. Metsämyyrä levittäytyy voimakkaasti ja kansoittaa tehokkaasti elämiseen soveltuvat alueet. Se asuttaa luontaisesti myös pirstoutuneita ja eristyneitä elinympäristöjä, kuten saaria. Maalla eläville lajeille, kuten metsämyyrälle, saaria ympäröivä vesi on varsin

tehokas liikkumisen ehkäisijä, mistä johtuen erilliset saaret saattavat yhdessä muodostaa metapopulaation. Metapopulaatiossa erilliset populaatioyksiköt ovat toisiinsa yhteydessä yksilöiden liikkumisen ja sen aikaansaaman geenivirran kautta.

Väitöskirjatyöni alkoi geneettisten merkkialueiden kartoittamisella. Tätä tarkoitusta varten etsin metsämyyrän genomista toistoalueita, mikrosatelliitteja, joiden avulla metsämyyräpopulaatioiden geneettisen monimuotoisuuden tutkiminen olisi mahdollista. Mikrosatelliitit ovat geenisekvenssejä, joissa sama 2-6 emäsparin mittainen jakso toistuu useita kertoja peräkkäin. Nämä toistojaksot muuntuvat helposti mutaation kautta, mistä johtuen niistä on populaatiossa edustettuna useita eri muotoja, alleeleja. Alleelien osuuksien avulla on mahdollista populaatiogenetiikan keinoin määrittää muun muassa populaatioiden geneettistä rakennetta ja monimuotoisuutta. Anonyymien, eli geenien toimintaan liittymättömien mikrosatelliittien eristämisessä käytin valikoivan hybridisaation menetelmää, jossa biotinyloidun koettimen avulla genomista poimitaan halutut toistojaksot. Tämän jälkeen sekvensoin toistojaksoja ympäröivät DNA-juosteet, ja suunnittelin näiden sekvenssien avulla toistojaksokohtaiset alukkeet, joita saatoin käyttää tutkittavien metsämyyräyksilöiden genotyyppien selvittämisessä. Anonyymien mikrosatelliittialukkeiden lisäksi kehitimme tutkimusryhmässämme myös DNA:sta transkription kautta ilmentyneihin alueisiin liittyviä merkkialueita monistavia alukkeita (nk. expressed sequence tag- eli EST-markkerit) sekä kahteen neuropeptidiin, vasopressiiniin ja oksitosiiniin liittyviä geneettisiä markkereita. Vasopressiini ja oksitosiini liittyvät kiinteästi yksilöiden sosiaaliseen käyttäytymiseen, kuten parinmuodostukseen ja kiintymykseen. Näitä kahta viimeistä markkerityyppiä (EST- ja vasopressiini/oksitosiinimarkkerit) saatoin käyttää selvittäessäni tarkemmin tiheydestä riippuvan valinnan vaikutusta syklisessä metsämyyräpopulaatiossa.

Syklisen populaatiodynamiikkansa vuoksi metsämyyrä soveltuu hyvin populaatiokoon ja geneettisen monimuotoisuuden välisiä yhteyksiä selvittävään tutkimukseen, sillä samalla alueella elävät yksilöt muodostavat säännöllisesti ja toistuvasti pienen ja suuremman populaation. Populaatiokokoon liittyvän tutkimukseni aineistona käytin pitkäaikaisseurantaa keskisuomalaisesta metsämyyräpopulaatiosta, jota analysoin kolmen populaatiosyklin ajalta. Määritin populaation geneettisen monimuotoisuuden kussakin huippu- ja romahduspisteessä ja vertasin vastakkaisia syklin vaiheita toisiinsa. Tutkin myös populaatiorakennetta syklin eri vaiheissa ja arvioin mahdollisia populaation geneettiseen monimuotoisuuteen vaikuttavia tekijöitä, eritoten tiheydestä riippuvan valinnan vaikutusta.

Tutkimukseni tulokset osoittivat, että syklinen populaatiodynamiikka ei vaikuttanut merkittävästi populaation geneettiseen monimuotoisuuteen. Havaitsin heikkoja merkkejä geneettisen monimuotoisuuden tason vaihtelusta populaatiosyklin vaiheiden mukaan, mutta toistuvista populaatiokoon romahduksista huolimatta efektiivinen populaatiokoko säilyi riittävän korkeana, jotta pullonkaulailmiötä ei syntynyt eikä geneettinen monimuotoisuus laskenut merkittävästi. Tutkimukseni tulokset eivät antaneet viitteitä tiheydestä riippu-

vasta valinnasta kyseisessä populaatiossa. Vasopressiiniin ja oksitosiiniin, ja niiden kautta edelleen yksilöiden sosiaaliseen käyttäytymiseen vaikuttaviin geenialueisiin ei kohdistunut erilaisia valintapaineita populaatiosyklin eri vaiheissa. Tämän vuoksi myyräpopulaatiossa onkin oltava muita mekanismeja, jotka ylläpitävät korkeaa geneettistä monimuotoisuutta. Tutkimukseni tulosten mukaan korkean efektiivisen populaatiokoon lisäksi myös yksilöiden tehokas liikkuminen ja sitä seuraava pariutuminen etäälläkin olevien yksilöiden kanssa, etenkin populaatiosyklin huippuvuosina, piti yllä korkeaa geneettisen monimuotoisuuden tasoa.

Populaation eristyneisyyteen ja pirstoutuneisuuteen liittyvässä osiossa työni aineisto koostui 25 saaresta ja 20 manneralueesta, joiden metsämyyräyksilöitä analysoin. Määritin populaatioiden geneettisen monimuotoisuuden ja tutkin, oliko se alhaisempi eristyneessä/pirstoutuneessa elinympäristössä. Lisäksi vertasin populaation geneettistä rakennetta pirstoutuneessa ja yhtenäisessä elinympäristössä sekä tutkin yksilön kelpoisuuden ja sen sisäsiitosasteen välistä yhteyttä. Työni tulokset osoittivat, että eristyneissä/pirstoutuneissa elinympäristöissä populaatioiden geneettinen monimuotoisuus oli alhaisempi kuin yhtenäisellä manneralueella. Metapopulaatiodynamiikkaan viittasi havaintoni siitä, että saaripopulaatiot olivat geneettisesti eriytyneempiä kuin manneralueiden yksilöt. Yllättävää oli, että vaikka yksilöt olivat sisäsiittoisempia saarilla mantereeseen verrattuna, sisäsiittoisuus ei laskenut naaraiden kelpoisuutta, vaan sisäsiittoisempien naaraiden kelpoisuus oli korkeampi ulkosiittoisten kelpoisuuteen verrattuna. Saamani tulokset kertovat siitä, että eristyneissä tai pirstoutuneissa elinympäristöissä myyräyksilöt pariutuvat useammin läheistä sukua olevan yksilön kanssa, mikä laskee geneettistä monimuotoisuutta ja nostaa sisäsiitosastetta. Kelpoisuuden lisääntyminen yhdessä sisäsiitosasteen kasvun kanssa saattaa viitata sukulaisvalintaan, jonka mukaan sukulaisyksilöiden läsnäolo lisää naaraan lisääntymismenestystä. Sukulaisryhmissä eläminen saattaa vähentää yksilöiden välistä kilpailua, jolloin energiaa voidaan käyttää enemmän lisääntymiseen liittyviin toimintoihin.

Väitöskirjatyöni tulokset osoittavat, että syklinen populaatiodynamiikka ja populaation pirstoutuneisuus eivät vaikuta samansuuntaisesti pikkunisäkäspopulaatioiden geneettiseen monimuotoisuuteen. Rajutkaan populaatiokoon laskut eivät välttämättä vähennä geneettistä monimuotoisuutta, jos populaation koko palautuu nopeasti ennalleen ja yksilöiden liikkuminen mahdollistaa tehokkaan geenivirran. Tiheydestä riippuva valinta ei vaikuta merkittävästi kyseenä oleviin sosiaaliseen käyttäytymiseen liittyviin geenialueisiin. Populaation eristyneisyydestä tai pirstoutuneisuudesta aiheutuva yksilöiden homotsygotiatason kasvu puolestaan ei suoraviivaisesti johda kelpoisuuden vähenemiseen, vaan sisäsiittoisuus saattaa joissain tilanteissa tai elinympäristöissä olla jopa eduksi.

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