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Postglacial Population History of the Common Shrew (*Sorex araneus*) in Fennoscandia

Molecular Studies of Recolonisation, Sex-Biased Gene Flow and the Formation of Chromosome Races

BY

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Abstract
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The common shrew, *Sorex araneus*, has one of the most variable karyotypes among mammals, displaying numerous chromosomes races throughout its distribution, which can be categorized into different karyotypic groups. The objective of this thesis was to examine the postglacial population history of Fennoscandian common shrews using autosomal microsatellites, mitochondrial DNA (mtDNA) and a Y chromosome specific microsatellite (L8Y).

Autosomal microsatellites and mtDNA revealed weak genetic structure over a hybrid zone between the karyotypically divergent Northern and Western karyotypic groups. However, the genetic structure displayed by the Y chromosome microsatellite was orders of magnitude higher. Hence, considerable chromosomal differences between the groups do not prevent female gene flow, while male gene flow is reduced (cf. Haldane's rule). Further, the results suggest that the Haldane effect may be caused by the chromosomal differences between the karyotypic groups.

No mtDNA differentiation was observed either between chromosome races or between the Northern and Western karyotypic groups in Fennoscandia. The combined pattern of karyotypic and mtDNA variation of Fennoscandian common shrews, suggest bi-directional postglacial recolonisation from a single refugium in Europe. The variation of the Y-linked microsatellite supported this conclusion. In contrast, significant mtDNA structure, discordant with the karyotypic variation, revealed that common shrews in southern Finland belong to a different lineage than remaining Fennoscandian regions, implying postglacial recolonisation from a different source.

MtDNA variation of the chromosome races in Sweden supports the hypothesis that three races of the Western karyotypic group have been formed through whole arm reciprocal translocations (WARTs), as suggested by their mutual karyotypic variation. The variation of the molecular markers supports the theory of rapid karyotypic evolution in the common shrew.

Keywords: Sorex araneus, chromosome race, postglacial recolonisation, hybrid zone, sex-biased gene flow, chromosomal evolution

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In memory of Håkan Tegelström

List of Papers

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

I **Andersson A-C, Narain Y, Tegelström H, Fredga K. 2004.** No apparent reduction of gene flow in a hybrid zone between the West and North European karyotypic groups of the common shrew, *Sorex araneus*. *Molecular Ecology,* 13, 1205-1215.

II **Andersson A-C**. Lack of mitochondrial DNA structure between chromosome races of the common shrew, *Sorex araneus*, in Sweden. Implications for chromosomal evolution. (Manuscript).

III **Andersson A-C, Alström-Rapaport C, Tegelström H**. Fennoscandian phylogeography of the common shrew (*Sorex araneus*). Postglacial recolonisation – combining information from chromosomal variation with mitochondrial DNA data. (Manuscript).

IV **Andersson A-C, Alström-Rapaport C, Tegelström H**. Reduced levels of male gene flow in a hybrid zone between the North and West European karyotypic groups of the common shrew, *Sorex araneus*. Chromosomally based explanation for Haldane's rule? (Manuscript).

V **Andersson A-C, Utter M, Alström-Rapaport C, Tegelström H**. Y-chromosome microsatellite variation among common shrews (*Sorex araneus*) in northern Europe. (Manuscript).

Contents

INTRODUCTION

Chromosomal variation between individuals of the same species is not uncommon in mammals and has intrigued researchers since the phenomenon first was discovered. It has been argued that chromosome rearrangements promote speciation (White 1978; King 1993) but this opinion is controversial and has been criticised by researchers favouring genic causes of speciation (e.g. Coyne 1993; Coyne & Orr 1998). Irrespective of the role chromosomes might play in the formation of new species, karyotypic variation within species is a remarkable characteristic and some species show high levels of chromosomal polymorphism. One of the most well described species is the common shrew, *Sorex araneus*; a small insectivore that has been the subject of numerous studies since karyotypic variation first was observed in this species (Sharman 1956; Ford *et al.* 1957; see Searle & Wójcik 1998). The karyotypic variation in the common shrew is of Robertsonian origin and chromosome number can vary both within and between populations. Most genetic studies of this species examined chromosome morphology to unravel various evolutionary aspects of chromosomal polymorphism, but since 1980 a diverse range of genetic studies have accumulated using allozymes, sequence data and microsatellites, continuously adding molecular information to the vast chromosomal knowledge (see Ruedi 1998; Searle & Wójcik 1998, Hausser *et al*. 1998).

Six chromosome races of the common shrew have been observed in Sweden (Fredga 1996). Despite distinct chromosomal differences they are not considered to be reproductively isolated from each other (Narain & Fredga 1997; 1998) and are therefore particularly interesting in the context of karyotypic evolution. During the last glacial maximum Sweden was completely covered with ice and consequently had to be recolonised by representatives of animal and plant taxa surviving in refugia outside Fennoscandia. Further, studies of the karyotypic variation suggest that Sweden was recolonised from two directions by common shrews exhibiting considerable chromosomal differences (Fredga 1996). Moreover, the distribution and karyotypes of chromosome races in southern Sweden indicate that these races may have been formed by a specific type of Robertsonian rearrangement, whole arm reciprocal translocations (WARTs) (Fredga 1996; Narain & Fredga 1996). However, the importance of this mechanism in karyotype evolution of the common shrew has been under some debate (see Searle and Wójcik 1998).

In summary, these characteristics of the chromosome races of the common shrew in Sweden have been thoroughly studied predominantly from a chromosomal aspect (e.g. Fredga 1973; 1982; 1987; 1996; Narain & Fredga 1996; 1997; 1998; Fredga & Narain 2000) but allozyme studies have been conducted as well (Frykman *et al*. 1983; Frykman & Bengtsson 1984). Mo-

lecular studies of the chromosome races of the common shrew in Sweden could add new insights into the details of the intricate problem of karyotype evolution. In this thesis I address various questions of postglacial population history of the karyotypically diverse common shrew populations in Fennoscandia using molecular markers as microsatellites, mitochondrial DNA and a Y chromosome specific microsatellite.

The common shrew

Biology

Insectivores are among the most ancient of mammals, first appearing shortly after the extinction of the dinosaurs (Churchfield 1990). The common shrew, *Sorex araneus*, is a small Palaearctic Insectivore, abundantly distributed as far north as the Arctic coast and eastwards to lake Baikal. In Europe it is absent from most of France, the Mediterranean zone (Spain, Italy and Greece) as well as on several islands, for example Ireland, the Outer Hebrides and the Isle of Gotland in the Baltic (Mitchell-Jones *et al.* 1999).

The common shrew feeds on almost any kind of invertebrates that it encounters: beetles, earthworms, spiders and snails. Having poor vision the common shrew predominantly uses the olfactory tactile and acoustic senses in locating prey. Due to the high metabolic rate, a common shrew must eat between 80 and 90 % of its body weight each day, which means that an average sized shrew every day has to consume about 100 prey items (of 10 mm size) (Churchfield 1990). Consequently, common shrews reach high densities only in habitats where invertebrates are abundant, e.g. moist habitat with dense vegetation cover (Mitchell-Jones *et al.* 1999).

Common shrews have a life span of 12-13 months. They overwinter as immatures and breed in the following spring. *Soricine* shrews seem to be unable to build up energy reserves in the wild and as a consequence they do not hibernate and thus have to be active during winter. However, during the cold months the rest periods tend to be longer and the activity periods shorter compared to the rest of the year, which may be a way to conserve energy (Churchfield 1982).

In the northern regions, the breeding period starts in April or early May, with males reaching maturity about three weeks earlier than females. The females are in oestrous only for a few hours every three weeks and will permit the closeness of a male only during this brief period (Churchfield 1990). Gestation lasts for 24-25 days and thereafter 5-7 young are born in each litter. Often the female get pregnant again immediately after the birth of the first litter. After 22-25 days the young are fully weaned, and completely independent. The young instinctively start to catch food, and the female has never been observed to catch food for her young. Females produce one to two litters per breeding season, which ends in late July (Churchfield 1990).

Common shrews of both sexes disperse during the immature stages (Hanski *et al*. 1991), and reach maturity after 4-6 months.

Common shrews are territorial and both females and males maintain equal sized territories as immatures (Croin Michielsen 1966). When mature, females extend their territories as they have an increased need for food. Mature males expand their territory to a much greater extent than females (Stockley 1992). Male ranges can overlap with that of two or more females but they always share their territory with several other males. Because there is no exclusive access to females, males have a promiscuous breeding pattern spending time in search for receptive females instead of maintaining an exclusive territory, and thus there is no paternal investment by common shrews (Stockley & Searle 1998). Males maturing early in the season stay in areas with high population density, whereas males that mature late often have to abandon the home rage and disperse for longer distances to find receptive females (e.g. Shillito 1963). Female shrews are also highly promiscuous; evidence from DNA fingerprinting of wild common shrews showed that eight of nine litters were the result of multiple matings. On average, each litter had 3.3 different fathers with a maximum of six (Searle 1990; Tegelström *et al*. 1991; Stockley *et al*. 1993). Because females mate with many males it has been suggested that sperm competition is important in the common shrew. Common shrew males also have larger testes in relation to their body size compared to mammals with other reproductive strategies (Kenagy & Trombulak 1986). As the reproductive success of male common shrews is not significantly correlated to body mass (Stockley 1992), sperm competition may be more important than direct competition (Stockley & Searle 1998).

Chromosomal polymorphism

The chromosomal polymorphism observed in the common shrew has arisen through Robertsonian rearrangements. The ancestral karyotype of the common shrew most likely consisted of telocentric (single armed) chromosomes (see review in Wójcik *et al*. 2002) and most of the karyotypic variation is thought to have arisen through Robertsonian fusions, where two telocentrics are combined to form a metacentric (bi-armed) chromosome (Fig. 1a). The reversed process, Robertsonian fissions, is believed to be rare in the common shrew (Searle & Wójcik 1998). An additional process, whole arm reciprocal translocations (WARTs), has also been suggested to produce novel metacentrics in a procedure where one metacentric chromosome interchange arms with either a non-homologous metacentric or a telocentric chromosome (Fig. 1b) (see Wójcik *et al* 2002; Fredga 1996). The WART model of chromosomal evolution has been put forward as a plausible explanation for the origin of races in Finland (Halkka *et al*. 1987), Siberia (Polyakov *et al*. 2001) and southern Sweden (Fredga 1996; Narain & Fredga 1996).

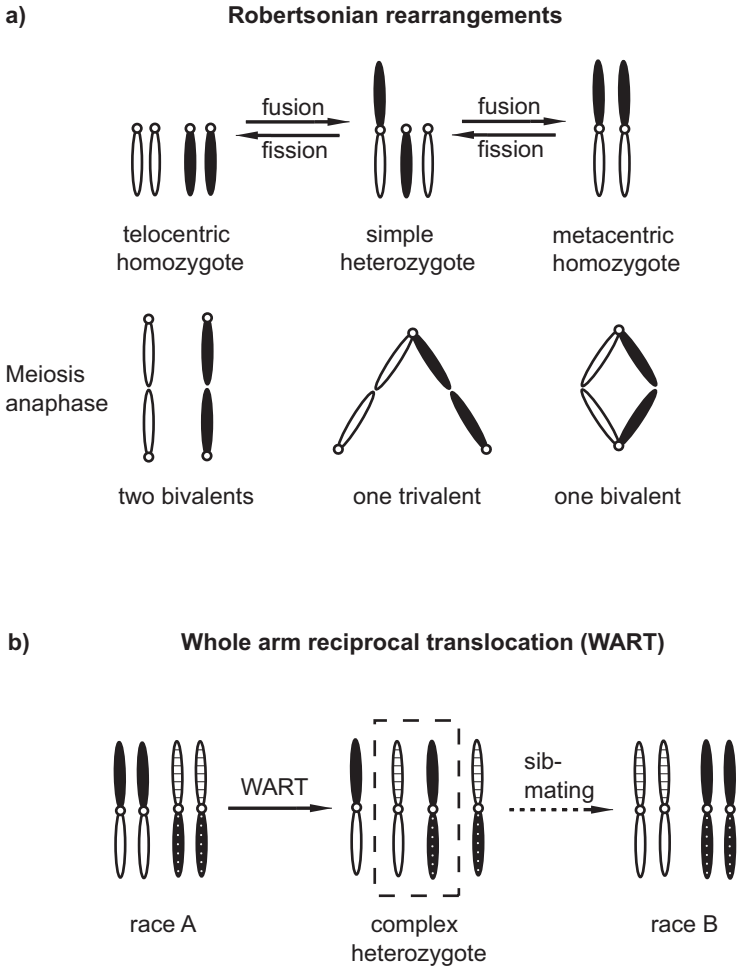

Figure 1. Chromosomal rearrangements, giving rise to simple (a) and complex (b) heterozygotes. (a) Robertsonian rearrangements. Formation of a new metacentric chromosome by fusion of two telocentrics, or fission of one metacentric, the latter process believed to be rare in the common shrew. Meiotic configurations of the different chromosome types are shown for comparison. (b) Formation of two novel metacentrics (in the hatched box) by a whole arm reciprocal translocation (WART) between two non-homologous metacentric chromosomes. This process produces an individual with a "complex" heterozygous karyotype. Under specific circumstances (e.g. sib-matings) individuals homozygous for the novel metacentric may be produced, which then is the first step in the formation of a new race (race B). Note that the complex heterozygote also can be an F1 hybrid between races A and B. Meiotic configurations of complex heterozygotes are shown in Figure 2.

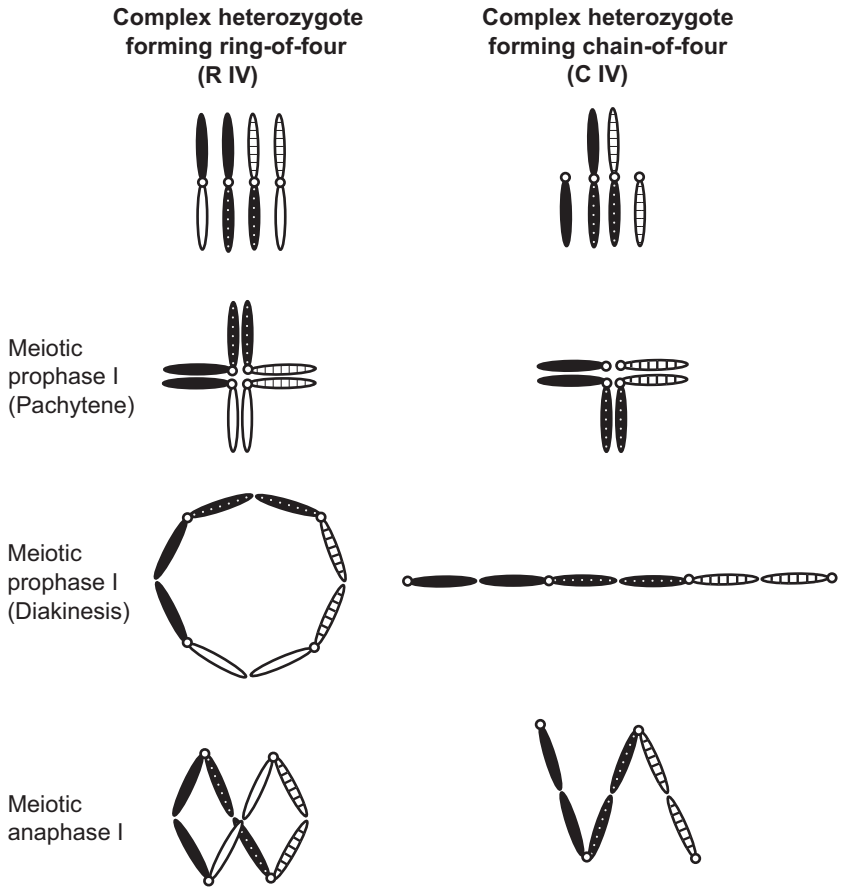

Figure 2. Meiotic configurations of complex heterozygotes. Complex heterozygotes forming ring quadrivalents (R IV) are more stable during meiosis than those forming chain quadrivalents (C IV).

Twelve telocentric pairs are involved in the polymorphism, and thus the chromosome number for the common shrew varies between 2n=20 and 2n=33 (males have XY_1Y_2 sex chromosomes, females XX). The number of chromosome arms is always constant (nombre fondametal, NF = 40). Karyotypes are described according to the combination of autosomal arms, where each arm is given an italicised lower-case letter of the alphabet, the largest arm denoted *a* (Searle *et al.* 1991). All shrew karyotypes have identical sex chromosomes and share three pairs of metacentric autosomes (*af*, *bc* and *tu*). Remaining arms (*g*-*r*) can exist either as telocentric or metacentric chromosomes. Geographically proximate populations, sharing the same set of meta-

centric and telocentric chromosomes by descent, are defined as chromosome races (for definition see Hausser *et al.* 1994). In addition, chromosomal polymorphism can be observed within a race. In races showing Robertsonian polymorphism one or several metacentric chromosomes also exist in the telocentric form. As a result, an individual shrew of a pure race can be a Robertsonian heterozygote, with one metacentric and two telocentric chromosomes (simple heterozygotes) or a homozygote, either with two metacentrics or four telocentrics (Searle *et al.* 1990).

In spite of this considerable chromosomal polymorphism, no variation in external morphology coinciding with the different chromosome races has been recorded (e.g. Sulkava *et al*. 1985). Based on specific chromosome arm combinations, the different races can be grouped into larger evolutionary units named karyotypic groups. Worldwide, 68 races have been described, most of which can be classified into four karyotypic groups (Wójcik *et al.* 2002; Wójcik *et al.* 2003).

Fertility of Robertsonian heterozygotes

Searle *et al*. (1990) made the distinction between simple heterozygotes and complex heterozygotes. Simple heterozygotes form trivalents during meiosis because at least one pair of homologous chromosomes is present both in the metacentric and telocentric form (Fig. 1a). During meiosis of a complex heterozygote, longer chain or ring elements are formed due to the presence of at least two metacentric chromosomes having only one arm in common (Fig. 2). In mice and other mammals, which display chromosomal polymorphism, individuals showing either multiple simple heterozygosity or complex heterozygosity almost always are infertile or sterile (Searle 1993). However, data for the common shrew suggest that Robertsonian heterozygotes do not suffer from infertility as substantially as other taxa (Searle 1993; Narain & Fredga 1997; 1998). Nevertheless, for the common shrew complex heterozygotes are assumed to be less fertile compared to simple heterozygotes, and furthermore complex heterozygotes forming chain configurations are less fertile than those forming ring configurations of equal length (Searle 1993; reviewed in Searle & Wójcik 1998).

Formation of chromosome races

Establishment of a novel chromosomal race requires two different processes to occur, first mutation resulting in a new chromosomal variant and second, either local fixation or an increase of the frequency of the recently formed chromosome.

An uncomplicated fixation model for new chromosomal variants involves genetic drift. A high mutation rate and low heterozygous disadvantage for simple Robertsonian heterozygotes (forming meiotic trivalents) in the common shrew can result in local fixation of a new chromosomal variant by genetic drift (Searle & Wójcik 1998). However, the offspring of an individ-

ual, which has undergone a WART event, will show a complex heterozygous karyotype, carrying at least one newly created chromosome together with the metacentric chromosome(s) present in the ancestral karyotype. This individual will probably suffer from reduced fertility due to pairing difficulties during meiosis (as it must form at least a four element meiotic complex). Thus the recently formed metacentric chromosome probably requires a bottleneck event in order to be fixed in the population (Searle & Wójcik 1998). However, both ecological (Croin Michielsen 1966) and molecular studies (Bengtsson & Frykman 1990, Wyttenbach & Hausser 1996) found no evidence of subdivision of present populations of the common shrew. On the other hand the ecological conditions during the time of race formation may have been different from the present day conditions and genetic drift in small populations may have been responsible for creating new chromosome races (Wójcik *et al*. 2002; see Narain & Fredga 1996).

Increased frequency or local fixation of a chromosome variant could also occur through selection. A global advantage for metacentric chromosomes at the expense of the telocentric homologues is unlikely. Several extant chromosome races of the common shrew show predominately telocentric karyotypes, adjacent to races with metacentrics (Searle & Wójcik 1998). However, Wyttenbach *et al*. (1998) showed in an elegant breeding experiment meiotic drive for some metacentric chromosomes in common shrew males. Although meiotic drive could explain local fixation of metacentrics this does not explain the existence of intraracial Robertsonian heterozygosity. Wyttenbach *et al*. (1998) proposed that when the metacentric chromosome reaches high frequencies it loses the advantage of preferential transmission because most karyotypes are homozygous for the metacentric condition. The weak selection against simple Robertsonian heterozygotes starts to play a more important role, resulting in a stabilised frequency of metacentrics and telocentrics in a local population.

The large distributions of many current chromosome races suggest that local fixation of a metacentric chromosome is followed by an expansion of the newly formed chromosome race. The most straightforward way in which chromosome races could have increased their ranges is by colonisation of new areas. After the last ice age, when the common shrew continuously increased its territory north- and westwards by recolonising formerly inhabitable areas, many races originated and enlarged their range.

A specific evolutionary model for a group of chromosome races present in Poland was suggested by Wójcik (1993) when he observed that the distribution of the chromosome races in Poland fit White's chain process variant of the stasipatric model of chromosome evolution remarkably well (White 1978). According to White's theory, various metacentric chromosomes have spread different distances into an ancestral race of telocentric homologues. In a recent study, Ratkiewicz *et al.* (2002) also found support for White's theory, in a study of common shrews representing different chromosome

races in Poland as variation of the mitochondrial cytochrome *b* gene showed no evidence of a recent bottleneck event. However, Ratkiewicz *et al.* (2002) suggest that the cytochrome *b* gene might not be appropriate for investigating molecular differences between closely related chromosome races as low levels of variation were found both among the Polish shrews (Ratkiewicz *et al.* 2002) and among shrews in western Europe (Taberlet *et al.* 1994).

Chromosome races in Sweden

Chromosome number of common shrews in Sweden varies from 2n=20 to 2n=27. Abisko is the most northern of the chromosome races in Sweden and belongs the North European karyotypic group (NEKG, referred to as the Northern group) (Fredga 1996). The Northern group is characterised by the arm combination *ip* and consists of a group of northern and eastern races believed to share common ancestry (Wójcik *et al.* 2002). The Abisko race shows considerable intraracial Robertsonian polymorphism; the frequency of metacentrics of the different arm combinations varies between localities (Fredga 1996). Remaining Swedish chromosome races belong to the West European karyotypic group (WEKG, referred to as the Western group), a group characterised by arm combination *hi* (Searle & Wójcik 1998) (Fig. 3).

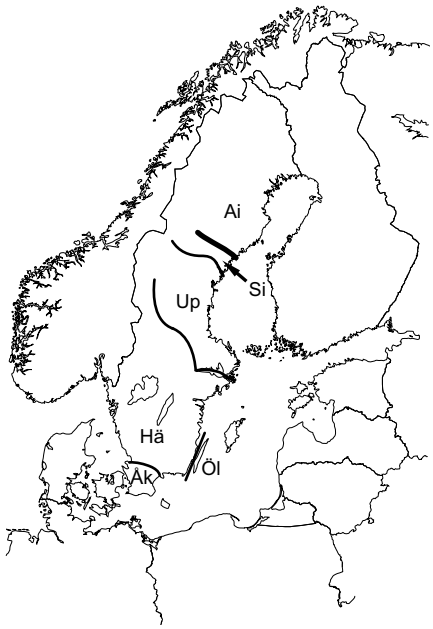

Figure 3. The distribution of Swedish chromosome races of the common shrew. The Abisko race (Ai) belongs to the Northern karyotypic group. The Sidensjö (Si), Uppsala (Ua), Hällefors (Hä), Åkarp (Åk), and Öland (Öl) races all belong to the Western karyotypic group (from Fredga 1996).

Table 1. Chromosome arm combination of the Swedish chromosome races. The Danish race Sjaelland is also included. Only the variable arm combinations are shown. Bold letters signify arm combinations proposed to be involved in consecutive whole arm reciprocal translocations (WARTs) (as shown by arrows). Slashes indicate that the specific arm combination can be found either in the metacentric or the telocentric state, i.e. the race shows intraracial Robertsonian polymorphism. The Sidensjö race may be of hybrid origin, resulting from a cross between the two neighbouring races, because it shares metacentric chromosomes with both the Abisko and Uppsala races. (Modified from Fredga 1996.)

Chromosome race (karyotypic group)	Chromosome arms metacentrics						telocentrics			
Abisko (Northern)	*g/m*	*h/n*	*j/l*	*i/p*	*k/q*	*o/r*				
Sidensjö (Western)	*gm*	*hi*	*j/l*		*k/q*	*n/r*	*o*	*p*		
Uppsala (Western)	*gm*	*hi*	*jl*		***k/p***	*n/r*	***o/q***			
Hällefors (Western)	*gm*	*hi*	*jl*		***ko***	***nr***	***pq***			
Åkarp (Western)	*gm*	*hi*	*jl*		***ko***	***nq***	***pr***			
Sjaelland (Western)	*gm*	*hi*	*jl*		***kq***	***no***	*pr*			
Öland (Western)	*gm*	*hi*	*jl*		*k/o*		*n*	*p*	*q*	*r*

The northernmost representative of the Western group, the Sidensjö race is localized between the Abisko and Uppsala races. The Sidensjö race may be of hybrid origin, resulting from a cross between the two adjacent races having *kq* from the Abisko race and *hi* and *nr* from the Uppsala race (Fredga 1996). The Sidensjö race shows intraracial Robertsonian variation and is characterised by a high number of telocentrics. Arms *o* and *p* are always unfused and *nr* is often found in the telocentric form. The Uppsala race, further to the south, has a wide distribution and the characteristic karyotype has all autosomes in the metacentric state except *oq*, which shows Robertsonian polymorphism. In the north, *o* and *q* are mostly unfused, and occasionally metacentrics *kp* and *nr* can be found in the telocentric state. In the Uppsala area, the frequency of the metacentric *oq* is 0.55 but towards the hybrid zone with the Hällefors race the frequency is 0.99 (Fredga 1996). The Hällefors race also has a wide distribution, occupying central and southern Sweden, but this race show no Robertsonian polymorphism. All autosomes are in the metacentric state. The southernmost race, Åkarp, has a restricted distribution and only occurs in the province of Skåne. All chromosome arms are in the

metacentric state and no Robertsonian polymorphism exists. An additional chromosome race occurs on the Isle of Öland in the Baltic, only 4 km from the mainland of southeast Sweden. The Öland race is characterised by 4-6 pairs of telocentric chromosomes, in contrast to the karyotypes of races found on the mainland in southern Sweden. Furthermore, two pairs of telocentrics (*k* and *o*) show Robertsonian polymorphism (Fredga 1996).

Fredga (1996) also observed that a succession of whole arm reciprocal translocations (WARTs) could have formed three races of the Western group on mainland Sweden (Table 1), which fits the geographic distribution of these races well (Fredga 1996; Fredga & Narain 1996). Furthermore, Fredga (1996) suggested that common shrews recolonised Sweden from two different directions, from the northeast by representatives of the Northern group, and from the south by representatives of the Western group. Because of their chromosomal differences, these two different karyotypic groups were considered to have survived the Last Glacial Maximum (LGM) in separate refugia (Fredga 1996; Fredga & Narain 2000).

Hybrid zones: reproductive characteristics and gene flow

Numerous hybrid zones between different chromosome races have been studied both between chromosomally similar races and between races exhibiting great chromosomal differences (reviewed in Wójcik *et al*. 2002). Depending on the karyotypes of the two encountering races hybrids of different levels of complexity are produced.

Hybrids between two chromosome races in Great Britain, the Oxford (*kq*, *no*) and Hermitage (*k*/*o, n, q*) races are simple heterozygotes and form one to three trivalents during meiosis (Searle &Wójcik 1998). Fertility studies of male hybrids in this zone revealed no significant differences in reproductive characteristics (e.g. germ cell death) between simple heterozygotes and Robertsonian homozygotes (Searle 1986; Garagna *et al*. 1989; Mercer *et al*. 1991). In contrast, significantly higher germ cell death was observed in female simple heterozygotes, but this did not affect fertility in practise (Wallace & Searle 1990).

Two hybrid zones in Sweden have been thoroughly studied, both regarding chromosomal characteristics of the hybrid zone and the fertility of the hybrids, namely the Uppsala-Hällefors hybrid zone in central Sweden and the Abisko-Sidensjö hybrid zone situated further to the north. The chromosomal differences between the Abisko race (Northern group) and the Sidensjö race (Western group) are large and in addition both races show intraracial chromosomal polymorphism (Fredga 1996). Hybrids between these two races are often complex heterozygotes, which form chain- or ring-of-four elements (CIV, RIV) during meiosis (Fig. 2)(Narain & Fredga 1998; Fredga & Narain 2000). Hence, the chromosomal differences between these two races are of a magnitude that theoretically should lead to reduced fertility of hybrids (Fredga & Narain 2000). Significant variation of reproductive char-

acteristics was also found in this hybrid zone, with complex heterozygotes displaying lower testis weight and higher germ cell death than simple heterozygotes and homozygotes (Narain & Fredga 1998). In a study of allozyme variation, Frykman and Bengtsson (1984) found evidence for gene flow between the races in the Abisko-Sidensjö-Uppsala hybrid zones, although this gene exchange appeared to be restricted. However, this clinal pattern was visible only in one locus, which raises the question of the magnitude of gene flow.

In a study of reproductive characteristics in the Uppsala-Hällefors hybrid zone, no difference between hybrids (complex heterozygotes, forming ring-of-four elements only) and individuals of the pure races (with homozygous karyotypes) was detected (Narain & Fredga 1997). Wyttenbach *et al*. (1999b) used microsatellites in an investigation of gene flow in this hybrid zone between the Uppsala and Hällefors chromosome races. They found weak structuring both within and between the chromosome races, indicating unrestricted gene flow between the races. The chromosomal cline in the hybrid zone was narrower than expected (Narain & Fredga 1996).

Using mitochondrial DNA (mtDNA), autosomal microsatellites and one microsatellite situated on the Y-chromosome Balloux *et al*. (2000a) studied two divergent common shrew races in the western Alps, now considered to be two different species (Brünner *et al*. 2002a). Balloux *et al*. (2000a) obtained different estimations of genetic structure for the three different markers. Autosomal microsatellites and mtDNA indicated restricted genetic exchange, whereas the Y-chromosome microsatellite showed complete absence of male gene flow. The absence of male gene flow is concluded to be caused by male sterility and hence is interpreted as a classical example of Haldane's rule (Balloux *et al*. 2000a). Haldane formulated (1922) a theory, which states that when one sex is absent, rare or sterile among hybrids between two races (species) it is the heterogametic sex (Haldane 1922). Balloux's *et al*. (2000a) investigation of the hybrid zone in western Alps pinpoints the importance of using multiple molecular markers when examining the interactions between individuals in, for example, a hybrid zone.

Phylogeography in Fennoscandia

In the field of phylogeography, the biogeographical study of a single species, Fennoscandia represents one of the most suitable as well as thoroughly studied areas (Jaarola *et al.* 1999). During the Last Glacial Maximum (LGM), 21 000-17 000 ^{14}C years before present (BP), the entire area was covered with perennial ice (Andersen & Borns 1997), and when the ice retreated, virgin land appeared which later was recolonised by flora and fauna residing in areas outside Fennoscandia. Two major colonisation routes existed, from the south via land bridges and from the northeast via pre-historic Finland (Björck 1995; Ignatius *et al.* 1980).

The first land bridge between the Scandinavian Peninsula and the European continent appeared 11 200 BP and lasted until 10 800 BP (Björck 1995). During the end of this period the climate deteriorated which probably resulted in extinction of many of the early colonisers (see Jaarola *et al.* 1999). The main colonisation period via the southern route thus probably took place on the second land bridge, which emerged 10 300 BP and lasted until 9 200 BP (Björck 1995; Jaarola & Tegelström 1996). For some additional time, Sweden was connected to the Danish island Zealand until 8 200 BP, when the Öresund strait opened up and finalised the continental connection (Björck 1995).

Colonisation from north-east was possible from around 10 000 BP with the beginning of deglaciation of south-west Finland (Ignatius *et al.* 1980), and about 9 000 BP northern parts of Sweden were connected with the southern parts by an ice free corridor, which permitted north colonisers to come in secondary contact with colonisers from the south (Björck 1995; see Jaarola *et al*. 1999).

Several studies, mainly based on mtDNA have revealed different Fennoscandian recolonisation patterns for various vertebrate taxa. The wood lemming (*Myopus schisticolor*) is an example of a coloniser using the northeast route exclusively (Fedorov *et al*. 1996), whereas colonisation solely from the south can be exemplified by a species introduced via human agricultural activity, the house mouse (*Mus musculus*) (Prager *et al*. 1993). Many taxa used both colonisation paths, resulting in a phylogeographic pattern seen for example in small mammals (reviewed in Jaarola *et al*. 1999), adders (Carlsson & Tegelström 2002), bears (Taberlet *et al*. 1995) and plants (e.g. Nordal & Jonsell 1998). This bi-directional recolonisation often resulted in secondary contact between, sometimes diverse, mitochondrial lineages of one species somewhere in Fennoscandia (see Jaarola *et al*. 1999; Carlsson & Tegelström 2002). The field vole (*Microtus agrestis*), the bank vole (*Clethrionomys glareolus*) and the brown bear (*Ursus arctos*) exhibit similar phylogeographical patterns and the secondary contact zones of these species coincide in the north of Sweden (Jaarola & Tegelström 1995; Tegelström & Jaarola 1998; Taberlet *et al*. 1995). These contact zones together constitute one of the major suture zones in Europe (Jaarola & Tegelström 1995; Taberlet *et al*. 1998) as defined by Remington (1968). The location of the suture zone may be a consequence of the possibility that the glacial ice remained longer in this particular geographic region, preventing the two colonising fronts to unite until an ice free corridor was established around 9 000 BP (Björck 1995; Jaarola & Tegelström 1995). In the common shrew the location of this suture zone corresponds exactly to the position of the contact zone between the Northern and Western karyotypic groups (Fredga 1996).

Postglacial recolonisation of the common shrew

Patterns of post-glacial recolonisation based on the karyotypes and the distribution of different chromosomal races, have been reconstructed for the western (Brünner *et al.* 2002b) and eastern parts of Europe (Polyakov *et al.* 2000, 2001) as well as Fennoscandia (Fredga 1996; Halkka *et al*. 1987; 1994). Brünner *et al.* (2002b) argue that immigrants from a possible refugium situated near the Black Sea coast used a colonisation path along the northern slopes of the Carpathian arc, via the north European lowlands and Denmark to recolonise Scandinavia. Descendants of these colonisers all belong to the Western group (Searle & Wójcik 1998). The chromosome races belonging to the Northern group are suggested to have survived LGM in a common refugia further to the east (Halkka *et al.* 1994; Fredga 1996), possibly situated somewhere in the Ural mountains (Halkka 1994; Polyakov *et al.* 2000, 2001).

Traditionally many mammals are considered to have survived the LGM in Mediterranean refugia situated in Iberia, Italy and the Balkans (reviewed in Hewitt 1999). However, there is increasing evidence that small mammals survived in refugia located further north, in central or eastern Europe (Bilton *et al.* 1998; see Jaarola & Searle 2002). In the common shrew, there is no indication of an Iberian LGM refugium, as the haplotype variation of cytochrome *b* of Andorra and southern France does not appear to be diverged from other European populations. Instead, haplotypes appear to be similar among individuals distributed over a vast area, from Andorra and England to east Siberia (Bilton *et al*. 1998; Haynes *et al.* 2000). Neither the Italian peninsula is currently considered as a recent refugium for the common shrew. Italy is presently inhabited by *Sorex samniticus* and a close relative of the common shrew, the Valais shrew (*Sorex antinorii*) which until recently was considered to be a race variant of the common shrew. The distinctness of the Valais shrew has led researchers to elevate it to species status; and many of its characteristics probably arose in isolation during the repeated glaciations of the Pleistocene (Brünner *et al*. 2002a).

OBJECTIVES

The main objective of this thesis was to investigate molecular differences of Fennoscandian common shrews using autosomal microsatellites, mitochondrial DNA and a Y chromosome specific microsatellite to examine their postglacial population history. First, I examined the level of gene flow between the two karyotypically most divergent groups in Sweden (Paper I), and also assessed if gene flow was equal in the two sexes (Paper IV). Further I investigated the mtDNA differences between the different chromosome races in Sweden to evaluate if molecular markers can reveal possible evolutionary scenarios for these races (Paper II). By combining the existing chromosomal knowledge with mtDNA variation I also investigated the postglacial recolonisation pattern of common shrews in Fennoscandia (Paper III). Finally I examined if Y chromosome microsatellite variation of common shrews in northern Europe corroborated the phylogeographic patterns observed with other markers (Paper V).

MOLECULAR MARKERS

Mitochondrial DNA

The mitochondrion is a maternally inherited organelle participating in cell respiration. The mitochondrial genome consists of a circular molecule with an average size of 16 kb in mammals, encoding for two rRNA, 22 tRNA and 13 proteins. It also contains a stretch of DNA termed the control region (1-2 kb), which carries the replication origin for the heavy strand. Mitochondrial DNA has been widely used in molecular studies especially of geographic structure of populations due to several reasons. In each animal cell there are about 100 to 100 000 mitochondria depending on cell type and each mitochondrion in turn have 2-10 mtDNA molecules, resulting in a relatively easy DNA isolation procedures even from degraded tissues (Hartl & Clark 1989, see Savolainen 1999). The mtDNA molecule does not seem to recombine which results in more simple branching structure of its gene trees than nuclear genes. This feature also has drawbacks as it results in each mtDNA molecule is inherited as a single entity only representing the evolutionary history of a single gene, and as a result reveal the evolutionary history of the gene rather than the population history of the species under survey (e.g. Tajima 1983; Pamilo & Nei 1988)

Because several mitochondria are present in a single cell there is a possibility that mutations can lead to more than one mtDNA haplotype in a single individual, a phenomena called heteroplasmy. In practise, this does not affect phylogeographic studies to a great extent because genotypic sorting takes place in a few generations resulting in that most individuals are homoplasmic for a single mtDNA haplotype (Avise 2000).

An additional useful property of the mtDNA molecule is the high rate of sequence evolution. The substitution rate is 5 to 10 times greater than nuclear genes, average divergence rate of 2 % per million years (Myr) in mammals (10^{-8} per lineage per nucleotide site per year). The evolutionary rate might be correlated to body size, being faster in for example rodents (3-11% per Myr) (Martin & Palumbi 1993). The high substitution rate of mtDNA is thought to be either a high rate of nucleotide misincorporation or a consequence of the lack of proofreading of the mtDNA polymerase. The drawback of the high evolutionary rate is that there is a large variation of the mutation rate between sites in mtDNA resulting in the occurrence of multiple substitutions of some nucleotide sites (Hartl & Clark 1989). The fastest evolving part of the mtDNA genome is the control region, the divergence rate in humans have been estimated to 7-22% per Myr (e.g. Hasegawa *et al*. 1993).

MtDNA variation in shrews

The presence of tandem repeats in the control region has been reported for many different vertebrate taxa (see Fumagalli *et al*. 1996). In the common shrew the left variable domain of the control region has a stretch of sequence of 78 or 79 bp repeated in tandem several times. In addition an imperfect repeat of equal size is present in all individuals (Stewart & Baker 1994; Fumagalli *et al*. 1996). Stewart & Baker (1994) investigated the divergence rate of the different repeats and their flanking regions between two species of the genus *Sorex*, and observed that the estimates differed both among the different repeats and the flanking regions. The highest estimate of sequence divergence was calculated for the unique sequence region flanking the stretches of repeated sequence, and was estimated to 15-20% per Myr.

Independence between chromosomal and mitochondrial evolution in the common shrew has been suggested between chromosome races in Europe (Taberlet *et al.* 1994; Fumagalli *et al.* 1999) and in Poland (Ratkiewicz *et al.* 2002). Several molecular studies indicate that the formation of chromosomal races is a fairly recent phenomenon (Taberlet *et al.* 1994; Fumagalli *et al.* 1999). However, it has also been proposed that the cytochrome *b* gene used in the studies in continental Europe, might not be appropriate for investigating molecular differences between chromosome races (Ratkiewicz *et al.* 2002).

In this thesis I sequenced a fragment of the mitochondrial genome including a part of the hypervariable left domain of the control region. The fragment consisted of part of the cytochrome *b* gene (105 bp), tRNAthr (66 bp), tRNApro (67 bp) and part of the non-coding control region (207 bp).

Autosomal microsatellites

A microsatellite is composed of a short nucleotide sequence of one to six base pairs that are repeated in tandem. The number of repeats normally varies from one, to a maximum of about 60. Every microsatellite has a fixed position in the genome, and thus each individual has a specific microsatellite locus, with two alleles that may differ in repeat numbers (Goldstein & Pollock 1997).

Microsatellites can be separated into three groups according to their composition: pure, compound and interrupted. Pure microsatellites are composed of one single type of tandemly repeated nucleotide sequence, whereas a compound microsatellite consists of two or more types of repetitive units. An interrupted microsatellite has one or several short sequences of other nucleotide composition integrated in the repetitive sequence (Jarne & Lagoda 1996). The mutation rate of microsatellite alleles is inversely related to the size of the repeat unit and increases with the number of repeats. The lowest levels of polymorphism have been found in interrupted microsatel-

lites, probably because of the stabilising effect the interruption exercises on the repeated sequence (reviewed in Jarne & Lagoda 1996).

The mutation rates of microsatellite loci are usually orders of magnitude higher than mutation rates at other loci within the same genome, and therefore they show a high degree of polymorphism (Hughes & Queller 1993). Levinson and Gutman (1987) suggested that events of slipped-strand mispairing together with unequal crossing-over, could explain the expansion and mutation processes of microsatellites. Slipped-strand mispairing is described as a mechanism in which the complementary bases at the site of an existing microsatellite, is mispaired due to denaturation and displacement of the two strands in a DNA molecule during replication (Levinson & Gutman 1987). This type of mutation process generates mutations, which consist of addition and deletion of a few numbers of whole repeat units (Goldstein & Pollock 1997).

The stepwise mutation model (SMM), was first presented by Ohta and Kimura (1973) and was later reintroduced and remade to agree with the requirements of microsatellites by Edwards *et al*. (1992). In this model, new microsatellite alleles arise by addition and deletion of one single repeat unit. However, the allele patterns at many other loci do not agree perfectly with this mutation model. Shriver *et al*. (1993) suggested a mutation model closely related to SMM but with infrequent multistep mutations. The mutation process of microsatellites gives rise to the possibility that two different ancestral alleles by mutation can produce two alleles, with the identical number of repeats without being identical by descent. This phenomenon is termed homoplasy. If there is a size constraint in operation at microsatellite loci as suggested by Garza *et al*. (1996), the frequency of homoplasic events will be much more abundant. If a population is large enough and the number of different alleles at a locus is limited, mutation will eventually create an allele, which either has been lost in the past or already is present in the population.

Autosomal microsatellites in shrews

Microsatellites have been developed for the common shrew and used in numerous studies of European populations (e.g. Wyttenbach *et al.* 1999a; Lugon-Moulin *et al.* 1999; Lugon-Moulin & Hausser 2002). In the present thesis I used six microsatellite loci (L9, L33, L45, L67, L68 and L92) for analysis. All microsatellites are dinucleotide repeats (AC_n) (Wyttenbach *et al.* 1997; Balloux *et al.* 1998).

Y chromosome

The Y chromosome in mammals can to some extent be viewed as the male counterpart of mtDNA. Most of the Y chromosome in mammals is haploid and only a small pseudoautosomal region recombines with the X-

chromosome. As a consequence the loci on the non-recombining part of the Y chromosome share the history of a single male lineage (Hurles & Jobling 2001). With a balanced sex ratio and identical variance of reproductive success for males and females, the effective population size of the Y chromosome equals that of mtDNA, which is a quarter of the effective size of autosomes (Petit *et al.* 2002)

In contrast to mtDNA the mutation rate of the Y chromosome is similar to other nuclear loci, being subject to the same repair processes as these loci. Furthermore the problem of heteroplasmy does not affect the Y chromosome because it only occurs in a single copy in the carrier (Hurles & Jobling 2001).

Y chromosome microsatellite in the common shrew

Microsatellites on the Y chromosome show an equivalent mutation rate compared to other part of the nuclear genome and as a consequence show similar diversity (reviewed in Hurles & Jobling 2001). In the common shrew the isolation of a microsatellite situated on the Y-chromosome, L8Y (Balloux *et al.* 2000a), have enabled specific studies of male population structure. L8Y is an interrupted trinucleotide microsatellite (CTT_n) situated on the non-recombining part of one of the Y-chromosomes in the common shrew (Balloux *et al*. 2000a).

DATA ANALYSIS

Genetic structure

F_{ST} can be regarded as the relative loss of heterozygosity from what is expected under random mating due to subdivision of the total population, and was first defined by Wright (1921). Slatkin (1991), among others, later expressed F_{ST} as

$$F_{ST} = (f_0 - f_1)/(1 - f_1)$$

where f_0 is the probability of identity by descent of two different genes drawn from the same subpopulation and f_1 is the probability of identity by descent of two genes drawn from a different subpopulation. For all microsatellite loci, genetic structure was estimated using overall and pairwise F_{ST} values, calculated according to Weir and Cockerham (1984). Initially, F-statistics were developed for loci following the infinite allele model. However, the high mutation rate of microsatellites together with the fact that the mutational mechanism may create alleles identical by state without being identical by descent may result in that F-statistics underestimate the genetic structure of populations.

An analogue of F-statistics, R-statistics was developed for loci mutating according to the single step mutation model and takes into account difference between microsatellite allele sizes (Slatkin 1995; Michalakis & Excoffier 1996). However, it has been shown lately that even under a strict stepwise mutation model F_{ST} seems to give better approximations of gene flow than R_{ST} due to the high level of variance attached to the calculation of R_{ST} (Gaggiotti *et al.* 1999; Balloux & Goudet 2002). Nevertheless, in the case of the Y chromosome microsatellite R-statistics will give valuable information about the genetic structure. Because F-statistics only are based on the frequency of each allele in each population, estimates of F_{ST} will be low even if no alleles are shared between two populations. In the case of the Y-chromosome microsatellite where almost no alleles are shared between some populations F_{ST} calculations are less informative than R_{ST}, regardless of the high variance attached to the estimation of R_{ST}.

The high mutation rate of microsatellites resulting in high degrees of polymorphism may also cause overestimations of absolute gene flow between populations when allele frequency based methods as for example F-statistics are used (Gaggiotti *et al.* 1999). The risk of overestimation may be even higher in hybrid zones where numbers of shared alleles are expected to be fewer compared to other population comparisons. However, comparisons of F_{ST} values calculated from microsatellites with similar levels of polymor-

phism between different hybrid zones are still relevant (Balloux *et al.* 2000b).

AMOVA

The genetic structure of populations was also examined with an analysis of molecular variance (AMOVA). An AMOVA tests the validity of a specific population grouping through its genetic structure. A hierarchical analysis divides the total variance into different parts of covariance, which are used to calculate different fixation indices (Excoffier 2000). The source of variation is determined as percentage of variation originating among groups, among populations within groups and among all populations. The variation among the hierarchical system of populations can be estimated in two ways, using conventional F-statistics to estimate structure only from allele or haplotype frequencies, or estimating structure using both the gene frequencies and the pairwise difference between the different alleles or haplotypes (Excoffier *et al.* 1992; Slatkin 1995). In analyses based on microsatellites, allele size differences were estimated using R-statistics (Michalakis & Excoffier 1996) whereas in analyses based on mtDNA the molecular distance between haplotypes was estimated using pairwise difference between the different haplotypes (Excoffier *et al.* 1992).

Network construction

To visualise the molecular distance between haplotypes median joining networks between mtDNA haplotypes was constructed (Bandelt *et al.* 1999). The median-joining algorithm constructs a network not only between observed haplotypes but also infer haplotypes that connects the observed haplotypes with each other. Network construction is suitable to data where many sequences may be derived from the same ancestral sequence and the numbers of nucleotide differences between haplotypes are small. In a network haplotypes can appear as nodes within the network rather than exclusively as terminal tips of a phylogenetic tree. The produced network can also be regarded as containing all most parsimonious trees for a given dataset (Bandelt *et al.* 1999).

RESULTS AND DISCUSSION

Paper I: No apparent reduction of gene flow in a hybrid zone between the West and North European karyotypic groups of the common shrew, Sorex araneus.

In this study we investigated the level of gene flow in the hybrid zone between the two karyotypically most divergent chromosome races, Abisko (Northern group) and Sidensjö (Western group) in Sweden, using microsatellite markers. These two races represent two karyotypic groups believed to have been separated in different refugia during the Last Glacial Maximum. In total 140 common shrews from 9 sampling localities were investigated with six microsatellite loci.

Analyses of genetic structure in the data were performed on three different levels. First, the samples from the nine localities were considered as nine geographic populations. Second, the nine geographic populations were grouped according to the chromosome race of the majority of the sampled individuals in each population. Finally, all individuals of a chromosome race (excluding individuals which had been identified as hybrids, N=26), irrespective of the geographic sampling location, were pooled to make one large population of each race, resulting in three chromosome race populations of different sizes.

We found surprisingly low levels of genetic structure of in the hybrid zone between the Northern and the Western groups in Sweden. The F_{ST} values at all three levels were significant and of the same magnitude (0.014-0.018) suggesting weak genetic structuring but not larger between the karyotypic groups than within. Furthermore, an assignment test using the software Geneclass (Cornuet *et al.* 1999) resulted in low assignment scores both to the geographic populations (24%) and to the source races (57%, excluding hybrids). Indeed, this low amount of genetic differentiation is equivalent to that found in several intra-racial studies of common shrew populations from western Alps (Wyttenbach *et al.* 1999a; Lugon-Moulin *et al.* 1999; Lugon-Moulin & Hausser 2002).

Similar levels of genetic structuring were also found in a microsatellite study of the hybrid zone between Hällefors and Uppsala chromosome races (Western karyotypic group) in southern Sweden (Wyttenbach *et al.* 1999b). No variation of reproductive characteristics between hybrids and animals of pure race has been observed in this hybrid zone (Narain & Fredga 1997). Thus, substantial reduction of gene flow between these two races was not to be expected. In contrast, significant variation for different reproductive characteristics was found in a study of spermatogenesis in the Abisko-Sidensjö hybrid zone (Narain & Fredga 1998). Surprisingly, the level of microsatellite

differentiation between the Abisko and Sidensjö chromosome races, is of the same order of magnitude as between chromosome races within the Western karyotypic group.

Despite limited genetic differentiation, the regression of $F_{ST}/(1-F_{ST})$ over geographic distance was significant, indicating isolation by distance (Mantel test, $P<0.01$) (Fig. 4).

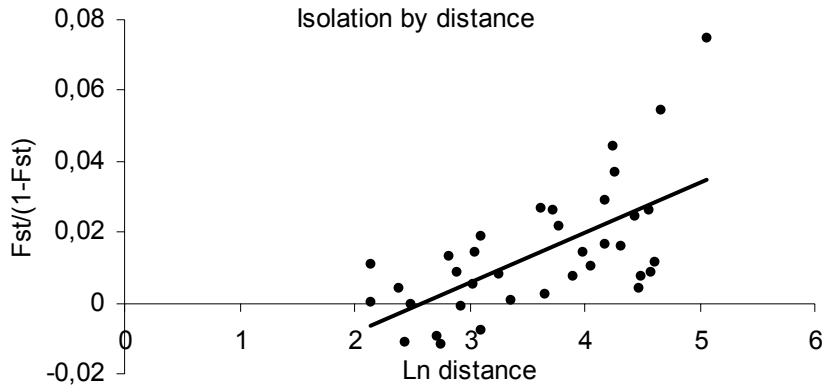

Figure 4. Isolation by distance graph between the geographic populations. The correlation was significant (Mantel test, $P<0.01$).

This suggests that the variation observed between the populations is a function of geographic distance rather than racial origin. Despite the chromosomal differences between the three chromosome races included in this study (Abisko, Sidensjö and Uppsala), no indications of decreased gene flow between the chromosome races could be found.

The pattern of microsatellite variation detected in our study in the hybrid zone between the two karyotypic groups could have arisen in different ways. Under the assumption that the Northern and Western karyotypic groups were separated in two separate glacial refugia followed by bi-directional recolonisation of Scandinavia, it is possible that nuclear genetic similarities between the karyotypic groups is a result of extensive gene flow in this secondary contact zone. Alternatively, the limited genetic differentiation could be explained by a recent divergence of the karyotypic groups. This could have occurred through either a bi-directional or a uni-directional colonisation process originating from a single glacial refugium. Based on the large observed karyotypic divergence between chromosome races, the scenario of bi-directional recolonisation from a single refugium requires rapid evolution of chromosome races during and after recolonisation, while not enough time elapsed for the corresponding differentiation to develop at nuclear loci. If common shrews recolonised Scandinavia from only one direction, all Swedish chromosome races would have a very recent common origin, and the

time since formation might be too short for genetic differences in neutral nuclear loci to appear. However, the considerable karyotypic differences between the two adjacent races (Sidensjö and Abisko) in the hybrid zone between the two karyotypic groups, compared to the chromosomal similarities existing between races within each karyotypic group, strongly dispute a uni-directional colonisation hypothesis. We suggest that the pattern of microsatellite variation in the present study most likely is a consequence of bi-directional recolonisation and extensive interracial gene flow. While a bi-directional colonisation process might have allowed for formation of differences in chromosomal morphology, it is difficult to explain how these chromosomal differences could be fixed in the advancing populations without showing differentiation in neutral nuclear loci.

In the light of all studies conducted over the hybrid zone between the Northern and Western groups, we are presently favouring the gene flow hypothesis. A theory of extensive gene flow does not contradict previous studies of allozyme variation, chromosomal morphology and hybrid fertility even if the magnitude of gene flow is surprising with respect to the considerable chromosomal differences between the Abisko and Sidensjö races.

Paper II: Lack of mitochondrial DNA structure between chromosome races of the common shrew, *Sorex araneus*, in Sweden. Implications for chromosomal evolution.

In spite of the karyotypic difference between the Western and Northern karyotypic groups of the common shrew in Sweden, Paper I showed that based on nuclear molecular markers gene flow does not appear to be reduced between the groups. Because the two karyotypic groups may have been separated in two refugia during the Last Glacial Maximum I further wanted to investigate if it is possible to distinguish between the six chromosome races or the two karyotypic groups in a molecular study, sequencing part of the mtDNA genome. I also wanted to investigate if molecular markers can reveal possible evolutionary scenarios for the common shrew chromosome races present in Sweden. A total of 150 common shrews from 27 localities in Sweden were included in the study.

I found no significant difference in mtDNA variation between the chromosome races as well as between the two karyotypic groups of common shrews present in Sweden. AMOVA analysis showed that most of the mtDNA variation (>77 %) was found within populations regardless of chromosome race or karyotypic group. Remaining variation could be found between populations within race and karyotypic group respectively.

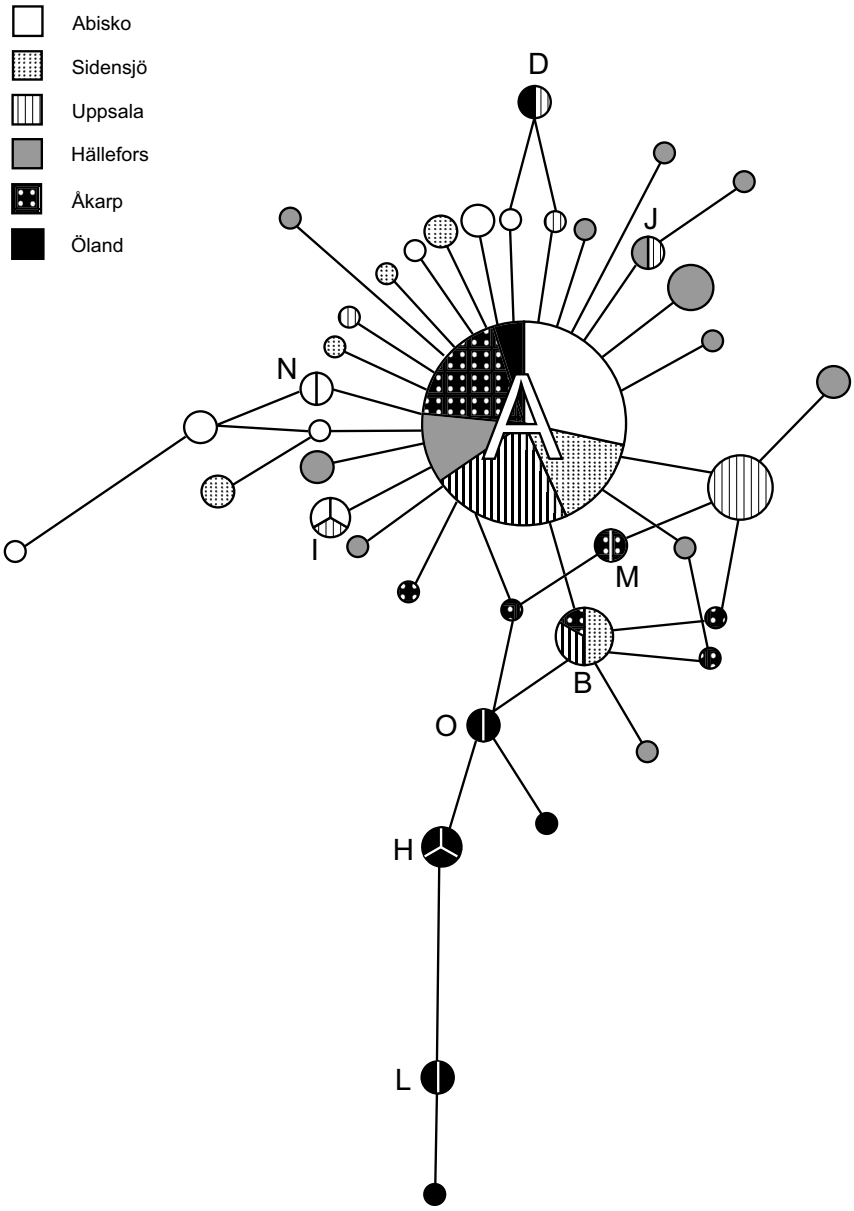

Figure 5. Median joining network (Bandelt *et al.* 1999) over the different mtDNA haplotypes displayed by the common shrew in Sweden. Circles are proportional to the frequency of haplotypes in the total sample and lines between circles are proportional to mutational steps. Patterns correspond to the different chromosome races. Haplotype designations are shown only for those haplotypes found in more than one locality. Haplotypes A, B, D, I and J are found in more than one locality in at least two chromosome races (shown as circle sectors with different patterns), whereas haplotypes H, L, M, N and O are found in different localities within the same chromosome race (shown as circle sectors with identical patterns).

Surprisingly, no mtDNA variation could be attributed to the grouping of populations into chromosome races or karyotypic groups ($\Phi_{CT} = 0$). Estimations of Φ_{ST} and Φ_{SC} were highly significant and approximately equal (Chromosome races, $\Phi_{ST} = 0.226$, $\Phi_{SC} = 0.255$; Karyotypic groups, $\Phi_{ST} = 0.215$, $\Phi_{SC} = 0.238$) showing that the mean coalescence time of two genes (haplotypes) drawn from different populations are the same even if the populations belong to different chromosome races or karyotypic groups.

I found 40 haplotypes of which 75 % were unique to the sampling locality, suggesting either that most of the haplotype variation actually arose *in situ*, or that most haplotypes only occur in very low frequencies and were missed due to insufficient sampling sizes. All chromosome races of the common shrew show strikingly similar haplotype distributions, with high frequencies of a central haplotype A. The remaining low frequency haplotypes are derived from the centre in a classical star-phylogeny pattern (Fig. 5). The genetic distance between haplotypes is short, often only a single mutation. Haplotype A, found in 20 of the 28 sampling localities, was present in 54% of all individuals absent only from two localities on mainland Sweden. However, on the Isle of Öland, haplotype A was absent from all but one locality. The relationship between the haplotypes showed no clear geographic structure, with the exception of haplotypes found among common shrews of the Öland race. A group of five haplotypes from Öland, separated from the central haplotype, showed the geographically most striking feature of the network (Fig. 5). In spite of the low sample size (n = 14), the average number of nucleotide differences, haplotype and nucleotide diversity were higher for the Öland race compared to other chromosome races. The minimum divergence times between the Öland race and the remaining Swedish races (11 000-20 000 years BP) also rendered higher estimations than in all other comparisons (0-2 000 years BP between all other races).

Two different scenarios can explain the obvious discrepancy between the absence of mtDNA variation on mainland Sweden and the chromosomal variation over the same area. First, the common shrews in Sweden may have originated from a single glacial refugium and recolonised Fennoscandia bidirectionally. The high observed frequency of haplotype A then suggest a rapid recolonisation by common shrews with low levels of mtDNA variation. The large amount of locality specific mtDNA variation implies that the majority of the mtDNA variation arose *in situ* after recolonisation. It is however possible that the chromosomal distinctness between the two karyotypic groups evolved in separate refugia during LGM as originally suggested. These two separate refugia would then represent two separate colonisation routes in Sweden, one from the northeast and one from the south. According to this scenario the segregation resulted in chromosome differentiation while it did not lead to (detectable) mtDNA divergence and the mtDNA variation

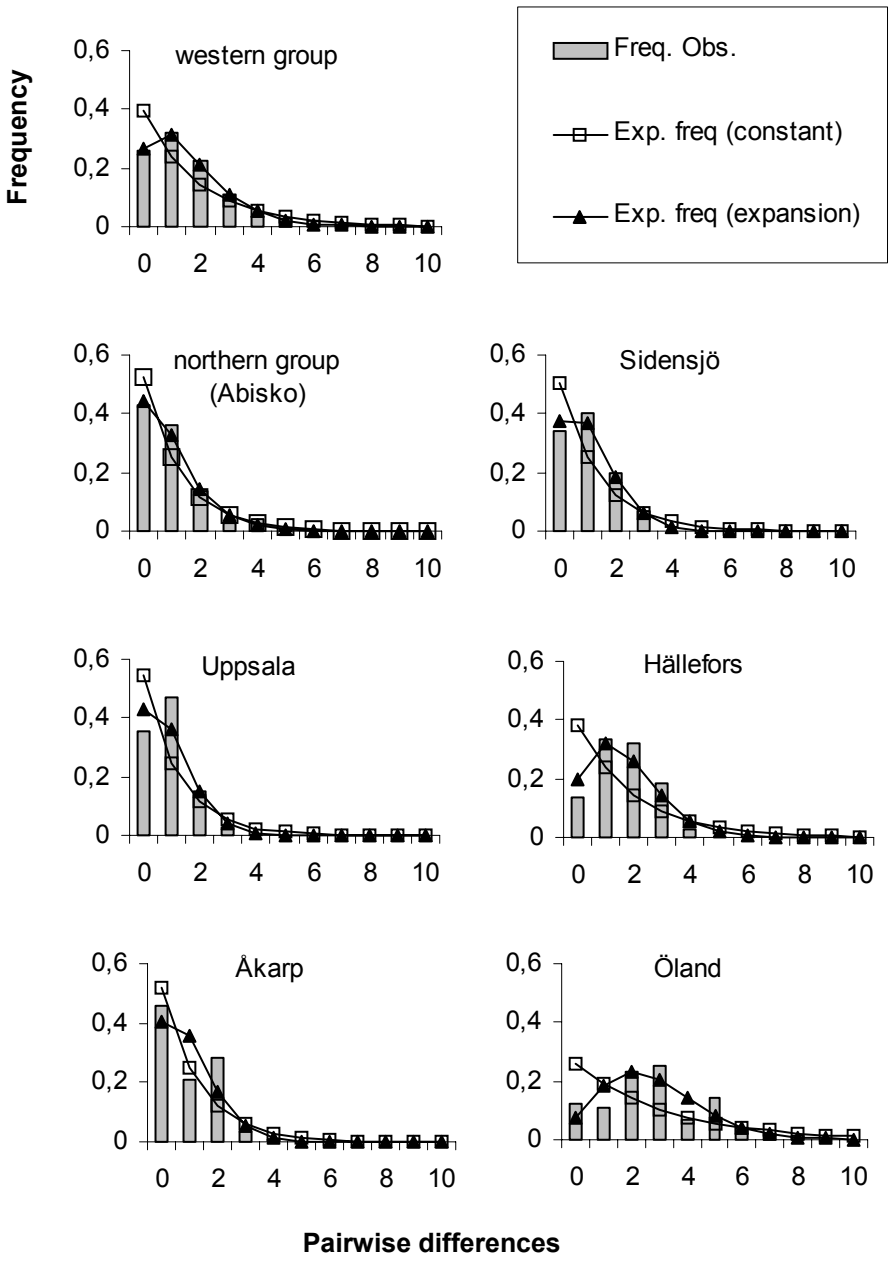

Figure 6. Mismatch distributions of the number of pairwise nucleotide site differences for the karyotypic groups and chromosome races. Bars denote the observed distribution, lines with open squares shows the expected distribution for a population of constant size and lines with filled triangles show the expected distribution for an expanding population. Mismatch analyses were performed with the software DnaSP 3.53 (Rozas & Rozas 1999).

would then reflect the variation present before the onset of the last glacial cycle as suggested by Ratkiewicz *et al.* (2002). If the mitochondrial mutation rate is slow the geographic pattern of mitochondrial haplotypes could be explained in this way. However, several studies have argued for the late formation of chromosomal variation in the common shrew, both between chromosomal races and karyotypic groups (Taberlet *et al* .1994; Fumagalli *et al.* 1999; Ratkiewicz *et al.* 2002). Stewart and Baker (1994) estimated the divergence rate of the hypervariable section of the control region in the mitochondrion of shrews to be high, implying that most mtDNA variation observed among common shrews in Sweden seems to have evolved *in situ*, in turn favouring the hypothesis of a common refugium.

I also found large deviations from selective neutrality. These results suggest either that the Swedish common shrew population have undergone a rapid population expansion or that the mitochondria of common shrews have been subject to a selective sweep. The mismatch distributions of pairwise nucleotide differences are also coinciding well with what is expected under theory of population expansion (Fig. 6).

The mtDNA variation found in the present study supports the theory that several chromosome races in Sweden and Denmark (Western karyotypic group) evolved through whole arm reciprocal translocations (WARTs) and founder events as described by Narain and Fredga (1996). The starlike haplotype network (Fig. 5) suggests a number of bottlenecks or that populations were founded by a small number of individuals. Furthermore, the chromosome races separately show mismatch distributions indicating recent population bottlenecks, as the distributions are slightly L-shaped, a pattern characteristic for population bottlenecks (Fig. 6) (Marjorham & Donnelly 1994). Mismatch distributions of all Swedish races except Öland are much more left truncated compared to the mismatch distribution calculated from cytochrome *b* variation of common shrews in Poland (Ratkiewicz *et al.* 2002). Notably, the formation of the Öland race, having a karyotype with several telocentric chromosomes, does not need a bottleneck event, and the mismatch distribution clearly deviates from the L-shaped form expected from strongly bottlenecked populations (Fig. 6) (Marjorham & Donnelly 1994).

In summary the mtDNA variation of the common shrew races on mainland Sweden concur well with what is expected under the WART model of karyotype evolution. Because no indications contradicting the WART hypothesis have been found in the present study, I consider this process to be responsible for the peculiar chromosomal variation of the races in the West European karyotypic group in Sweden.

Paper III: Fennoscandian phylogeography of the common shrew (*Sorex araneus*). Postglacial recolonisation - combining information from chromosomal variation with mitochondrial DNA data.

Paper II found no significant mtDNA differentiation between the Northern and Western karyotypic groups of the common shrew in Sweden. The incongruence between chromosomal and mitochondrial markers thus demanded an expanded study where populations from geographical areas surrounding Sweden are analysed for the same mtDNA sequence. The aim of Paper III is to combine available information on the chromosome morphology of the common shrew with mtDNA variation to improve the understanding of the postglacial recolonisation pattern of Fennoscandian common shrews. A total of 241 common shrews from 51 localities in Fennoscandia were included in the study.

In agreement with the results in previous studies we found no significant mtDNA structure between Northern and Western groups in Fennoscandia. The results from the AMOVA of the total sample showed that most of the variation (> 70%) was found within populations. No variation could be found among the karyotypic groups. To our knowledge, most studies have failed to detect differentiation of mtDNA between different groups of common shrews in Europe, either chromosomal or geographic (Taberlet *et al.* 1994; Fumagalli *et al.* 1999; Bilton *et al.* 1998; Ratkiewicz *et al.* 2002; Paper II). This has led to the conclusion that evolution of mtDNA variation is independent of chromosome race formation in the common shrew, the latter being a recent process (Taberlet *et al.* 1994; Fumagalli *et al.* 1999; Bilton *et al.* 1998). Although we found no differentiation between the Western and Northern karyotypic groups, we found significant variation (13%) between geographic areas within the Northern group. Evidently, there are significant mtDNA structure in Fennoscandia, but discordant with the major chromosomal grouping.

A majority of the haplotypes (80%) in the study area are unique to one sampling locality and most local haplotypes differ only by a single nucleotide substitution. Haplotype A was present in all geographic regions except south Finland and Denmark, and the frequency was consistently high throughout Sweden and the southern parts of Norway (Fig. 7). In contrast, in south Finland haplotype C was common and was observed in 31% of the individuals. Haplotype networks constructed for each geographic region separately resulted in star phylogenies with A as the central haplotype, except for Denmark and south Finland. The haplotypes found in south Finland are instead derived from haplotype C, the most common haplotype in this region. Haplotypes found in Denmark (n = 6) does not show a star-phylogeny, and are scattered throughout the major network based on all samples. Although haplotype B is predominant (33%) in north Norway, the observed haplotypes in this region appears to be derived from haplotype A.

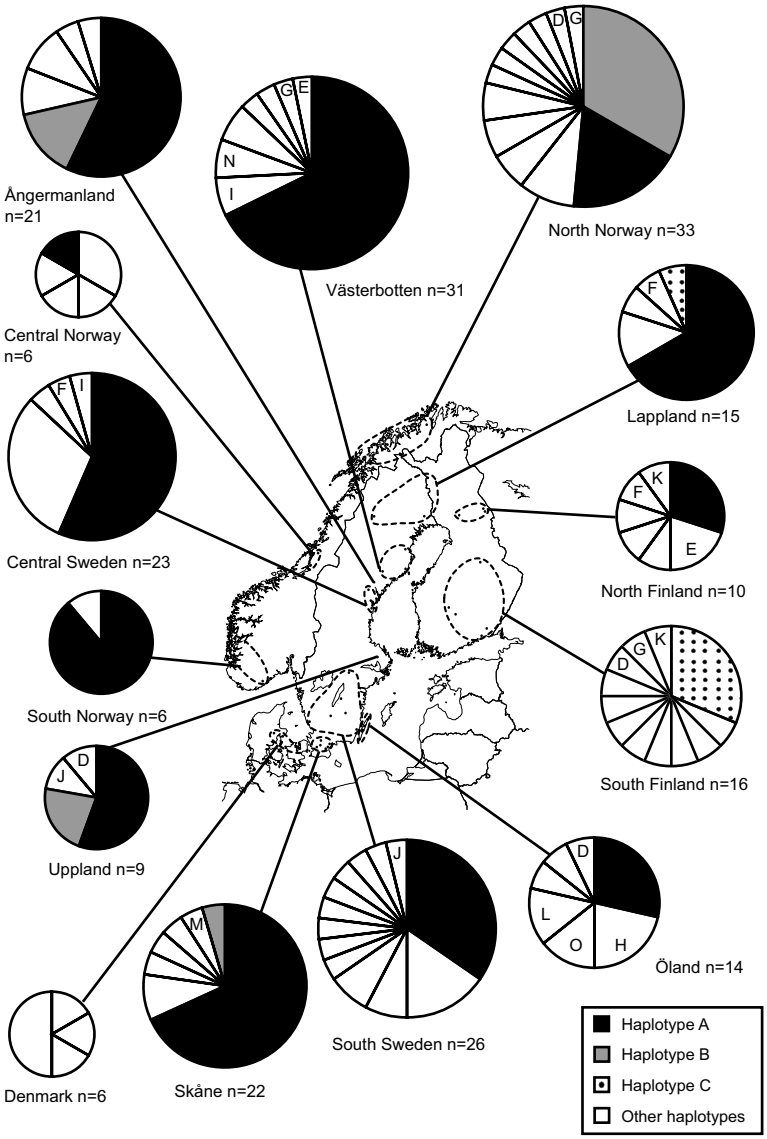

Figure 7. Distribution and frequency of the haplotypes (A-O), which are present in more than one sampling locality for the different geographic regions in Fennoscandia. Haplotypes H, L, M, N and O are present in different sampling localities of the same geographic region, the other in more than two geographic regions. The area of each pie chart is proportional to the sample size in each geographic region. The frequency of haplotype A is shown in black, frequency of haplotype B is shown in grey and the frequency of haplotype C is shown in white with black dots. Haplotypes D-O are shown in white with a capital letter for each haplotype. Haplotypes only found in one sample locality are not distinguished among each other in the pie-charts, and are consequently shown in white without descriptive letters. In total 72 haplotypes are represented.

The star-phylogenies and the large amount of unique haplotypes strongly suggest that most haplotypes arose *in situ*. In turn this implies that the analysed sequence has a high substitution rate, as most mutations must have arisen on location after the last glacial maximum (LGM). Based on the mtDNA variation of the common shrew in Fennoscandia we conclude that except for south Finland the entire area shares the same evolutionary history. South Finland appears to belong to a different mtDNA lineage, which diverged prior to the recolonisation of Fennoscandia. Thus mtDNA variation is not congruent with chromosomal variation over the same area. An estimation of minimum divergence time suggests that south Finland separated from the remaining Fennoscandian populations for 19 000 - 25 000 years ago, roughly coinciding with the LGM.

In isolation mitochondrial DNA variation does not confirm the prevailing view (Fredga 1996, Fredga & Narain 2000) that common shrews colonised Fennoscandia both from the south and from the northeast. However, to fully understand postglacial history of the common shrew it is necessary to include information on chromosomal morphology. Based on karyotypic variation, the northernmost of the Swedish races, Abisko, is closely related to the Sauvikoski race in northern Finland (Halkka *et al.* 1987), but distantly related to the Sidensjö race, which is distributed immediately to the south of the Abisko race (Fredga 1996). Hence, it is unlikely that the Abisko race has evolved by chromosomal rearrangements of the Sidensjö race, or vice versa (Fredga & Narain 2000). Thus, the major chromosomal differences among Fennoscandian common shrews unquestionably lie between the Northern and Western karyotypic groups (Halkka *et al*. 1987; Fredga 1996; Fredga & Narain 2000). The fact that the contact zone between the two karyotypic groups coincides with the major Fennoscandian suture zone strengthens the argument that Sweden was colonised by common shrews both from the south and the northeast. This in combination with the fact that no significant mtDNA variation was found between these groups supports theory of one refugium followed by bi-directional recolonisation. Furthermore, the high incidence of locality specific mtDNA variation suggest a high mutation rate, in turn meaning that most of the mtDNA variation arose *in situ* whereas the chromosomal distinctness between the Northern and Western group must have evolved during or at the onset of the proposed bi-directional recolonisation phase.

There is accumulating evidence of glacial refugia in central or eastern Europe which could have been utilised by small mammals both from genetic data (Bilton *et al.*1998; Jaarola & Searle 2002; Brunhoff *et al.* 2003), fossil data dating back to the LGM (Jánossy 1986; Nadachowski 1989; Markova *et al.* 1995) as well as indications of full-glacial forests (Willis *et al.* 2000). A refugium located in central or east Europe would have enabled a rapid recolonisation of northern territories. Several of the small mammals suggested to have utilised a central European refugia have earlier been proposed

to be early coloniser together with the common shrew (see Jaarola *et al.* 1999). Common shrews of this early colonisation wave used both the path in north Finland and the land bridge between Denmark and Sweden. The two colonisation fronts, with differentiated karyotypes, later met in north Sweden in the same area as several other early mammal colonisers meet. As discussed previously, this scenario is congruent with both chromosomal and mtDNA variation of common shrews in most of the Fennoscandian peninsula, with the exception of the region in south Finland, which based on mtDNA variation appears to belong to a different evolutionary mtDNA lineage. We therefore propose that south Finland were colonised in a second colonisation wave from a different source, perhaps situated further to the east. The geological record also in part corroborates this as the southernmost part of Finland was submerged under the Yoldia Sea for a longer time period than the northern parts of Finland (Andersen & Borns 1997). Furthermore, there are several examples of plant species with disjunct distributions between south and north Finland, with the populations in north Finland distinguishing themselves as early colonisers (see Nordal & Jonsell 1998).

Paper IV: Reduced levels of male gene flow in a hybrid zone between the North and West European karyotypic groups of the common shrew, *Sorex araneus*. Chromosomally based explanation for Haldane's rule?

Surprisingly, the previous studies (Paper II; III) of the Northern and Western karyotypic groups did not show mtDNA differentiation between the groups in spite of the karyotypic difference between them, implying recent evolution of these chromosomal differences. Further, autosomal microsatellite variation over the hybrid zone does not suggest that gene flow between the two karyotypic groups is more restricted than between populations within chromosome races (Paper I). However, a study of common shrew males in the Abisko-Sidensjö hybrid zone revealed significant differences of various reproductive characteristics (Narain & Fredga 1998), suggesting that the level of gene flow might vary between the sexes. In this study we revisited the hybrid zone between the two karyotypic groups aiming to elucidate potential differences between male and female gene flow. We used three molecular markers exhibiting three different modes of inheritance, autosomal microsatellites, mtDNA and a microsatellite situated on the Y chromosome. A total of 75 common shrews from four localities were included in the study.

We find a striking difference in the estimates of genetic structure based on the Y chromosome microsatellite variation compared to the other two genetic markers in our study. Only low levels of differentiation can be found over the study area using autosomal microsatellites (F_{ST} = 0.044, P<0.001; R_{ST} = 0.068, P<0.01) or mtDNA (F_{ST} = 0.051, p< 0.05; Φ_{ST} = 0.092, p<0.001). In contrast, a high level of structure is revealed by the Y chromosome microsatellite (F_{ST} = 0.054, p<0.05; R_{ST} = 0.82 p<0.0000). Further-

more, the AMOVA analysis using the Y chromosome microsatellite variation showed that approximately 80 % of the variation was found between the two karyotypic groups. In contrast, in the equivalent analyses using mtDNA only 0.7 % of the variation was observed between the Northern and Western groups. The distribution of Y chromosome microsatellite alleles was not uniform over the sampling area (Fig. 8). Adjacent populations may have overlapping allele size distributions, but only populations within each karyotypic group share alleles. Skellefteå and Bjurholm share 3 alleles (Northern group) whereas Björksjön and Bispfors share 2 alleles (Western group). Notably, Bjurholm and Björksjön do not share any alleles (Fig. 8). In summary, the results from the three different molecular markers suggest that the gene flow over the hybrid zone between the Northern and Western karyotypic groups is mainly female-mediated.

Is the discrepancy between the different molecular markers a result of reduced male gene flow or reduced total gene flow observed only in the male inherited marker? This is unlikely in the common shrew, which has a promiscuous mating system, leading to similar effective population sizes for both sexes (Searle 1990; Tegelström *et al.* 1991; Stockley *et al.* 1993). Furthermore, the total gene flow is not reduced (Paper I) together with the fact that both F1 hybrids and individuals, which by their karyotype can be verified as hybrid backcrosses to one of the ancestral races, are frequently found in this hybrid zone (Fredga & Narain 2000). We therefore conclude that the observed pattern is a result of reduced male gene flow over the hybrid zone between the Northern and Western groups.

Diverging patterns between female and male inherited markers could be explained by dispersal differences between the two sexes. In the common shrew this explanation is unlikely as both sexes disperse during immature stages (Hanski *et al.* 1991) and mature male common shrews extend their territories to overlap that of several females, thus moving to a greater extent than females (see Stockley & Searle 1998).

Haldane formulated (1922) a theory, which states that when one sex is absent, rare or sterile among hybrids between two races (species) it is the heterogametic sex (Haldane 1922). The most common explanation for the Haldane effect is based on gene expression, the "dominance theory" as first proposed by Muller (1940). This theory states that most genes that contribute to hybrid sterility or viability are recessive and if located on the X chromosome will be expressed only in the heterogametic sex (Orr 1997; Turelli & Orr 2000; see also Davies *et al.* 1997). In mice, it has been observed that chromosomal heterozygotes that form relatively short meiotic chains (chain of four) may demonstrate sterility limited to the male sex (Gropp *et al.* 1982), as male gametes seem to be more sensitive to the occurrence of an autosomal chain configuration during meiosis compared to female gametes (Forejt 1996; see Piálek *et al.* 2001).

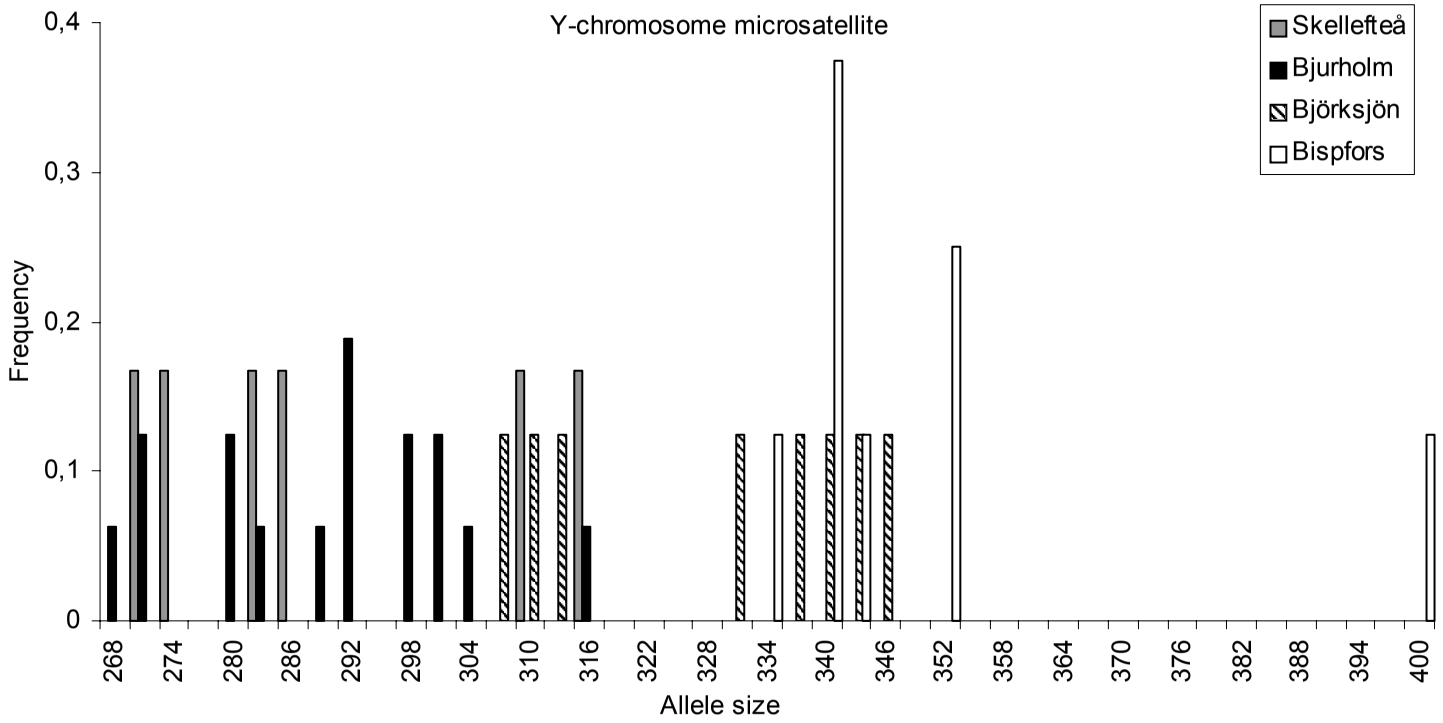

Figure 8. Allele frequency diagram over the Y chromosome microsatellite alleles observed in the different sampling localities. Different patterns correspond to different sampling localities, grey for Skellefteå, black for Bjurholm, black and white striped for Björksjön and white for Bispfors

The hybrid zone between the Northern and Western karyotypic groups separates two chromosomally divergent races, which show no indication of having diverged on a molecular basis apart from the Y-microsatellite marker. There is no mtDNA variation between the two karyotypic groups in Sweden (Paper II), and it is therefore possible that the two karyotypic groups actually survived the last glacial maximum in a common refugium (Paper III). In addition, no apparent reduction of gene flow between the two karyotypic groups was detected using autosomal microsatellites (Paper I). Hence we believe that a "genic" (Forsdyke, 2000) cause for the Haldane effect is less likely in the hybrid zone between the Northern and Western groups in Sweden. Because the only molecular marker that show divergence over the hybrid zone is Y-linked, we instead argue that the reduced fertility of the male common shrews might have a chromosomal basis for the Haldane effect as suggested for example for hybrids between chromosome races in mice (Forejt 1996; Gropp *et al.* 1982; se also Piálek *et al.* 2001). This is in part supported the study by Narain & Fredga (1998) where complex heterozygotes had lower testis weight and higher germ cell death compared to simple heterozygotes.

Paper V: Y-chromosome microsatellite variation among common shrews (*Sorex araneus*) in northern Europe.

Contrasting patterns between karyotypic differentiation and mtDNA diversity have previously been observed among Fennoscandian populations of the common shrew. Karyotypic variation implies that representatives of the Northern and Western karyotypic groups recolonised the area from two different directions after the Last Glacial Maximum (Fredga 1996; Fredga & Narain 2000). On the other hand, mtDNA variation over Fennoscandia does not coincide with either chromosome races or karyotypic groups (Paper II; III). In contrast, a distinct differentiation between the two karyotypic groups was found using the Y-chromosomal microsatellite L8Y, in a study restricted to the hybrid zone area in northern Sweden (Paper IV). Because variation of the Y-chromosome microsatellite in this area suggests that the two karyotypic groups are diversified at the Y-chromosome level, we wanted to examine genetic variation of this marker to detect potential genetic difference between the two karyotypic groups and/or chromosome races on a larger geographical scale. A total of 222 male common shrews from 24 localities from Fennoscandia and the Baltic region were included in the study.

Surprisingly, no significant variation (0%) was detected between the Western and Northern groups in the AMOVA analyses based on either F_{ST} or R_{ST} for the Y-microsatellite locus L8Y when populations distributed across Fennoscandia were included. Because, genetic differentiation of the L8Y locus might be an effect of geographic isolation and may have occurred

independently of chromosome race formation, we divided the populations into five distinct geographic areas. The AMOVA analysis showed significant variation, 29.9% ($p<0.03$) for the analysis based on R_{ST} but not based on F_{ST}. The explanation for these contrasting results at the different geographical scales probably lies in the mode of inheritance and the high mutation rate of the Y chromosome microsatellite. The lack of recombination and the strictly paternal mode of inheritance make Y chromosome linked markers extremely sensitive to genetic drift, leading to detectable genetic differentiation on a smaller geographical scale. The high mutation rate of microsatellites may on a larger geographical scale result in homoplasy, where some alleles are identical by state without being identical by descent. Homoplasy blurs the phylogeographic pattern so that genetic differentiation between regions no longer is detectable through analysis of molecular variance.

Despite non-overlapping allele distributions between the two karyotypic groups in the hybrid zone, we found no genetic differentiation between the Northern and Western karyotypic groups when all populations of Fennoscandia, Denmark and the eastern Baltic were included in the analysis. However, significant genetic differentiation was found when the whole region was subdivided into five geographic areas. We argue that given bidirectional recolonisation of Fennoscandia, this pattern would most likely only be observed if the two karyotypic groups originated in a common refugium (as opposed to two separate refugia). If recolonisation occurred from a single refugia we would expect to observe the highest level of genetic differentiation between populations on either side of the hybrid zone of the two recolonisation lineages, as these populations also are the temporally most divergent (most likely to have accumulated different mutations). By the same reasoning populations spatially closer to the refugium are therefore expected to be less genetically diverged from each other. Because such populations would be found in both the Northern and the Western group, we expect lower level of differentiation in comparisons where all populations for each karyotypic group are combined, in contrast to comparisons between populations in the hybrid zone area. Thus we would expect to find genetic divergence between geographic areas, rather than between karyotypic groups. In contrast, if the Northern and Western karyotypic groups originated from two separate refugia we would expect to find the highest level of genetic differentiation when all populations within each karyotypic group are combined. Hence, according to this scenario we would not expect to find the highest level of genetic differentiation between populations in the hybrid zone area as we observed in the present study.

CONCLUSIONS

The results presented in the thesis show several novel aspects of the post-glacial population history of the common shrew in Fennoscandia. In spite of the karyotypic divergence, I found surprisingly low levels of molecular differentiation between the chromosome races or karyotypic groups. No mitochondrial DNA (mtDNA) variation was observed between the Northern and Western karyotypic groups. The most straightforward explanation for the combined pattern of karyotypic and mtDNA variation of Fennoscandian common shrews, was bi-directional post-glacial recolonisation from a common glacial refugium. Y chromosome microsatellite variation over the same area supported this hypothesis. In contrast, significant mtDNA structure, discordant with the karyotypic variation, revealed that common shrews from south Finland belong to a different lineage than shrews from remaining Fennoscandian regions, implying postglacial recolonisation from a different source. The mtDNA variation of the chromosome races of the Western karyotypic group in Sweden supports the hypothesis that three of the chromosome races have been formed through whole arm reciprocal translocations (WARTs), as suggested by their mutual karyotypic variation and geographic distribution.

Both autosomal microsatellites and mtDNA variation revealed weak genetic structure over the hybrid zone between the Northern and Western groups, in Sweden. However, the genetic structure displayed by the Y chromosome microsatellite was orders of magnitude higher compared to the other markers. Hence, considerable chromosomal differences between the groups do not seem to prevent female gene flow, while male gene flow is reduced (cf. Haldane's rule). Furthermore, the results indicate that the Haldane effect in this hybrid zone may be caused by the chromosomal differences between the Northern and Western karyotypic groups. The variation of autosomal microsatellites, mtDNA and the Y-linked microsatellite in Fennoscandian common shrews clearly supports the theory of fast karyotypic evolution in this species. My data also suggest that even the karyotypic groups may have been formed more recently than previously suggested.

SUMMARY IN SWEDISH

Den vanliga näbbmusens (*Sorex araneus*) postglaciala populationshistoria i Fennoskandien.

Molekylära studier av återkolonisation, könsbundet genflöde och kromosomrasbildning.

Den vanliga näbbmusen tillhör ordningen insektsätare och är ett av våra vanligaste däggdjur. Den är vida spridd över den Europeiska kontinenten, från England i väster till Bajkalsjön i öster och så långt norrut som till den Arktiska kusten, medan den saknas på vissa öar som t. ex. Gotland och Irland. En av de märkligaste egenskaperna hos denna art är den har variabel karyotyp, vilket innebär att näbbmöss från olika populationer kan ha olika antal kromosomer. Kromosomtalet kan variera mellan 2n=20 till 2n=33, men antalet kromosomarmar är alltid konstant (40). Geografiskt närliggande populationer som uppvisar liknande karyotyp, d. v. s. samma uppsättning tvåarmade (metacentriska) och enarmade (telocentriska) kromosomer, brukar benämnas kromosomras. Hos vissa kromosomraser varierar kromosomtalet även inom rasen. En individ kan ha en eller flera metacentriska kromosomer vars homologer återfinns i den telocentriska formen. Individen är då en s. k. Robertsonsk heterozygot, i det ovan beskrivna fallet en "enkel heterozygot". Om en individ istället har två olika metacentriska kromosomer, som bara har en arm gemensam kallas den "komplex heterozygot". Individer med denna karyotyp återfinns ofta i hybridzoner mellan kromosomraser.

Baserat på specifika armkombinationer kan kromosomraserna ytterligare grupperas i större grupper, så kallade karyotypgrupper. Det finns 68 kromosomraser i världen idag, varav de flesta kan grupperas i någon av fyra karyotypgrupper. I Sverige återfinns sex raser, som representerar två karyotypgrupper, nordeuropeiska karyotypgruppen (norra gruppen) och västeuropeiska karyotypgruppen (västra gruppen). Den nordligaste rasen (Abisko) tillhör norra gruppen, medan övriga svenska raser (Sidensjö, Uppsala, Hällefors, Åkarp och Öland) tillhör västra gruppen. Karyotyp-variationen hos dessa grupper antyder att deras anfäder tillbringade den senaste istiden i två åtskilda istidsrefugier, varefter de återkoloniserade Skandinavien från olika håll, norrifrån via Finland samt söderifrån via landbryggor som periodvis förenade Sverige med Danmark och den europeiska kontinenten.

Kromosomvariationen hos den vanliga näbbmusen tros främst ha uppkommit genom en viss typ av Robertsonska translokationer som kallas Robertsonska fusioner, där två telocentriska kromosomer sammanfogas och bildar en metacentrisk kromosom. Den omvända processen, Robertsonska

fissioner, antas vara väldigt ovanlig hos denna art. Nya metacentriska kromosomer kan också bildas antingen genom att två metacentriska kromosomer utbyter armar med varandra eller genom att en metacentrisk kromosom utbyter en arm med en telocentrisk kromosom. Denna process kan benämnas "reciprok kromosomarmstranslokation" och tros ligga bakom bildandet av några kromosomraser i södra Sverige (Åkarp, Hällefors och Uppsala). I den här avhandlingen, som består av fem delstudier (I-V), har jag valt att fokusera på olika genetiska aspekter av näbbmusens postglaciala populationshistoria, både i Sverige och Fennoskandien. Jag använde mig av tre olika typer av genetiska markörer, autosomala mikrosatelliter, mitokondrie-DNA (mtDNA) samt en mikrosatellit belägen på Y-kromosomen. Mikrosatelliter är korta DNA-sekvenser (1-6 baspar) upprepade ett flertal gånger efter varandra, belägna var som helst i genomet. Jag använde mig dels av mikrosatelliter lokaliserade till de autosomala kromosomerna (icke könskromosomer), dels av en mikrosatellit som återfinns på näbbmusens ursprungliga Y-kromosom (Y_1) och som därför enbart nedärvs via den hanliga arvslinjen. MtDNA återfinns i mitokondrien, en organell som följer den honliga linjen via äggcellen. Jag sekvenserade ungefär 450 baspar som bland annat innefattar en mycket variabel del av mitokondriens icke-kodande kontrollregion.

I norra Sverige finns en hybridzon mellan Abisko- och Sidensjöraserna, som följaktligen också är en hybridzon mellan den norra och den västra karyotypgruppen. Eftersom den kromosomla skillnaden är mycket stor mellan dessa grupper borde genflödet vara reducerat mellan grupperna. Fertilitetsstudier hos hanar har också visat att egenskaper kopplade till reproduktionen hos näbbmusen (testikelvikt, spermatiddöd), varierar mellan enkla och komplexa heterozygoter i denna hybridzon. När jag studerade genflödet över den norra hybridzonen med autosomala mikrosatelliter (delstudie I), kunde jag sluta mig till att den genetiska strukturen över denna hybridzon var mycket låg, vilket innebär att genutbyte äger rum. Samma genetiska strukturnivå återfinns normalt mellan populationer som tillhör samma kromosomras. Trots de avsevärda karyotypskillnader som de båda raserna uppvisar verkar detta inte påverka genflödet, som är lika stort mellan som inom karyotypgrupperna.

I nästa steg av studiet av den norra hybridzonen (delstudie IV) använde jag mig av alla tre markörtyper beskrivna ovan. För att undvika att inkludera hybrider mellan karyotypgrupperna i studien, använde jag mig av fyra näbbmuspopulationer belägna utanför själva hybridzonen. De autosomala mikrosatelliterna och mtDNA-markören visade sinsemellan överensstämmande resultat, som återigen klart visade att genflödet var obehindrat. Y-kromosommikrosatelliten visade överraskande motsatt resultat. Genflödet mellan karyotypgrupperna var tydligt reducerat trots att populationer inom grupperna uppvisade obehindrat genflöde. Resultaten tyder på att hanliga hybrider inte är lika fertila som honliga viket är ett exempel på Haldanes regel. Vanligtvis förklaras denna fertilitetsskillnad med att det uppstår en

inkompatibilitet mellan genprodukter som enbart kommer till uttryck hos det heterogametiska könet (hannar hos däggdjur). I hybridzonen mellan norra och västra karyotypgrupperna finns dock indikationer på att den bakomliggande orsaken till det reducerade hanliga genflödet kan vara av kromosomal art.

En teori som nämndes ovan förklarar hur tre av kromosomraserna tillhörande den västra gruppen i Sverige har uppstått ur varandra genom en kaskad av reciproka kromosomarmstranslokationer. För att undersöka om man kunde hitta molekylärt stöd för denna hypotes studerade jag mtDNA-variationen hos alla svenska kromosomraser av vanlig näbbmus (delstudie II). Jag fann att mtDNA-variationen var oväntat låg, den huvudsakliga variationen återfanns inom populationerna, och ingen variation kunde upptäckas vare sig mellan de olika kromosomraserna eller mellan de två karyotypgrupperna. Över 50 % av alla individer hade samma haplotyp (A), medan de övriga haplotypernas utbredningsmönster antydde att de allra flesta uppstått på plats (75% av alla haplotyper återfanns enbart inom en provtagningslokal). Ölandsrasen uppvisar dock ett avvikande mönster med bland annat högre mtDNA-diversitet. Sammantaget överensstämmer mtDNA-variationen hos de svenska kromosomraserna mycket väl med hypotesen att tre av dem (Åkarp, Hällefors och Uppsala) har uppstått genom reciproka kromosomarmstranslokationer.

Eftersom ingen mtDNA variation hittades mellan de västra och norra karyotypgrupperna, kunde inte delstudie II stödja den kromosomalt underbyggda hypotesen om två postglaciala invandringsvägar till Sverige. För att kunna rekonstruera den vanliga näbbmusens postglaciala återkolonisationsmönster så undersöktes mtDNA-variationen hos ytterligare fennoskandiska näbbmuspopulationer, belägna i geografiskt angränsande områden (delstudie III). Genom att kombinera den redan existerande karyotypkunskapen om fennoskandiska näbbmöss med mtDNA variationen över samma område, har resultaten från delstudie III medfört att näbbmusens postglaciala populationshistoria i viss mån ha måst skrivas om. Den mest sannolika förklaringen till karyotypvariationen hos fennoskandiska näbbmöss är fortfarande att återkoloniseringen skedde via två invandringsvägar. Men jag fann att alla geografiska områden i Fennoskandien, undantaget södra Finland, uppvisade snarlik mtDNA-variation med en hög andel individer med haplotyp A, medan resterade haplotyper i stor utsträckning förmodligen uppstått senare och på plats. Detta antyder att näbbmöss från hela detta geografiska område har en gemensam evolutionär historia. Eftersom de två karyotypgrupperna uppvisar näst intill identisk mtDNA-variation förefaller det osannolikt att de tillbringade den senaste istiden i två separata refugier. Det genetiska mönstret tyder istället på att de delade istidsrefugium och att karyotypvariationen uppstått omedelbart före eller under återkoloniseringen. Södra Finland däremot tillhör en annan mtDNA-linje, som verkar ha uppstått innan återkoloniseringen, kanske i ett annat östligare beläget istidsrefugium.

Y-kromosommikrosatelliten uppvisade som beskrivits tidigare, skillnader mellan västra och norra karyotypgrupperna, i en delstudie (IV) begränsad till området runt hybridzonen. Jag ville ta reda på om samma mönster kunde iakttas över ett större geografiskt område och därför undersökte jag den genetiska variationen hos Y-kromosommikrosatelliten i hela Fennoskandien (delstudie V). Inte heller med denna markör kunde någon skillnad mellan de två karyotypgrupperna upptäckas, vilket följaktligen överensstämmer väl med mtDNA-variationen över samma område (delstudie III).

Resultaten från alla delstudier i denna avhandling överensstämmer väl med gängse forskning som tyder på att karyotypevolutionen är exceptionellt snabb hos den vanliga näbbmusen. Mina data antyder dessutom att den kromosomala variationen mellan karyotypgrupper kan ha uppstått senare än man tidigare trott. Sammantaget visar avhandlingen på ett flertal nya aspekter av näbbmusens postglaciala populationshistoria.

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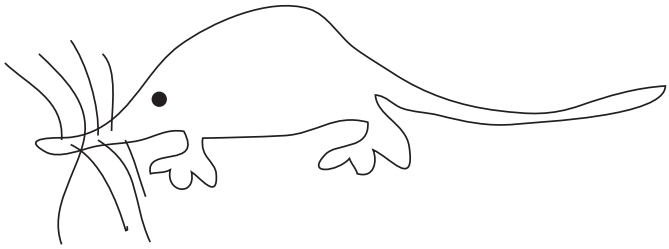

I know now how to tame a shrew.
I dreamt upon it all this night till now,
and thou hast waked me out of the best dream
that ever I had in my life.

Christopher Sly, a drunken tinker
in The Taming of the Shrew
by William Shakespeare

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