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THE ORIGIN OF SCANDINAVIAN MOUNTAIN HARES (*LEPUS TIMIDUS*)

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ABSTRACT

During the most recent glacial period, the Weichsel, Scandinavia was completely covered with ice. As the ice started to melt some 14,000 years ago, various organisms started to recolonise the newly deglaciated areas. Two alternative colonisation routes to Scandinavia became available, first one in the south and later one in the north-east. To determine if these two alternative colonisation routes have resulted in a genetic subdivision of mountain hares, Lepus timidus, *in Scandinavia, an approximately 400 base pair, highly variable mtDNA control region fragment from 15 mountain hares and two brown hares,* Lepus europeus, *was sequenced. The hares included represent mountain hare maternal lineages in Scandinavia detected in an earlier study, three different central European mountain hare subspecies, as well as mountain hares from Finland and Russia. One brown hare was included as an outgroup. The sequences obtained were used to estimate genetic distances between different lineages and to construct phylogenetic trees in order to evaluate relationships between mountain hares. Three of the Scandinavian mountain hares show close relatedness with mountain hares from Scotland, Ireland, and Russia, respectively. We suggest that the mtDNA variation observed among the Scandinavian mountain hares is due to a retention of old mtDNA haplotypes present in a continuous population of mountain hares throughout the last glaciation. The observed mtDNA relatedness may reflect a postglacial colonisation of the Scandinavian peninsula both from the south and from the north-east.*

I. INTRODUCTION

Northern Europe has repeatedly been affected by glaciations during the Pleistocene. The last glaciation, the Weichsel, lasted for approximately 80,000 years. At the height of the Weichselian glaciation, Fennoscandia, the Baltic, most of the British Isles and parts of Germany, Poland and Russia were covered with ice. The British Isles were connected to the European continent by a wide land bridge over the British Channel.

The first areas of land on the Scandinavian peninsula were relieved of the ice cover about 13,500 years ago. For several thousand years, Scandi-

navia was connected to Denmark and the continent by a land bridge (BJÖRK, 1995) which enabled colonisation of Scandinavia from the south. Later on, as the ice melted in northern Scandinavia, colonisation from the north-east, via Russia and Finland, was possible. These two alternative colonisation routes to Scandinavia have resulted in the occurrence of a northern and a southern form of several species. Karyotype investigations of the common shrew, *Sorex araneus* (FREDGA and NAWRIN, 1977), analysis of mitochondrial DNA (mtDNA) variation in bank voles, *Cletrionomys glareolus* (TEGELSTRÖM, 1987) and brown bears, *Ursus arctos* (TABERLET and BOUVET, 1994) as well as integrated studies of karyotypes and mtDNA of field voles, *Microtus agrestis* (JAAROLA and TEGELSTRÖM, 1995) all reveal a northern/southern differentiation of these species in Scandinavia.

The mountain hare, *Lepus timidus*, is well adapted to harsh habitats with limited food resources. Therefore, this species is among the first larger mammals to utilise the poor conditions of newly deglaciated land. Throughout the Weichselian glacial period, central Europe (including southern Britain) most likely had a continuous population of mountain hares (HANSTRÖM, 1972; STUART, 1974). The first mountain hares in Ireland, are believed to occur in the middle of Weichsel (STUART, 1985). Today, the mountain hare is geographically distributed in tundra and taiga habitats, ranging from the British Isles to Japan (ANGERBJÖRN and FLUX, 1995). In Europe, there are relict populations of mountain hares with subspecies status in Ireland (*L. t. hibernicus*), Scotland (*L. t. scoticus*), and the Alps (*L. t. varronius*).

In Scandinavia, the first documented records of mountain hares are dated 10,000 years BP (LEPIKSAAR, 1986). Historically, mountain hares inhabited all of the Scandinavian peninsula, but deforestation and competition from the introduced brown hare, *Lepus europeus*, has forced the mountain hare north of 56°N (ANGERBJÖRN and FLUX, 1995). BERGENGREN (1969) describes two morphologically distinct groups of mountain hares in Scandinavia and concludes that this grouping could be the result of differential colonisation. These groups are given subspecies status based on fur colour, form, size and behaviour (ANGERBJÖRN and FLUX, 1995). The northern mountain hare, *L. t. timidus*, occurs naturally in Scandinavia north of 59°N (ANGERBJÖRN and FLUX, 1995) while the southern mountain hare, the heath hare, *L. t. sylvaticus*, reaches the northernmost distribution in Sweden at 61°N (BERGENGREN, 1969). Between 59°N and 61°N there is an overlapping distribution, where both subspecies occur in various frequencies (BERGENGREN, 1969). Also, translocations of captive reared mountain hares has disturbed the former geographic separation of the two subspecies, so that presently *L. t. timidus* can be observed south of the natural range.

The maternally inherited mtDNA is a useful genetic marker when studying population history, genetic differentiation and taxonomy (AVISE, 1994). An earlier investigation of mtDNA variation among Scandinavian mountain hares shows a high degree of intraspecific genetic variation with five distinct maternal lineages (THULIN *et al.*, 1997). No geographic differentiation of these lineages could be observed, even though representatives of both Scandinavian subspecies were included. Also, one of the main mountain hare mtDNA lineages observed was only detected in four brown hares, *Lepus europeus*, a phenomenon interpreted as introgression due to interspecific

hybridisation between mountain hares and brown hares in Scandinavia (THULIN *et al.*, 1997).

To determine whether the five distinct mtDNA lineages observed in Scandinavian mountain hares reflect different origins due to alternative postglacial colonisation routes, a part of the highly variable mtDNA control region was sequenced in 15 mountain hares and two brown hares. The included individuals represent the main mountain hare mtDNA lineages described by THULIN *et al.* (1997), the three different western European subspecies of mountain hares and north-eastern mountain hares from Finland and Russia. Finally, one brown hare was included as an outgroup. The obtained sequences were used for phylogenetic inferences and estimates of genetic distances.

II. MATERIAL AND METHODS

Tissue samples from hares were collected by hunters and kept in low temperature freezers until used. The localities for the samples are shown in Figure 1. To obtain a suitable fragment of the mtDNA control region, we used the polymerase chain reaction (PCR) technique (SAIKI *et al.*, 1985). Purified mtDNA was used as a template in the reactions to minimize the risk of unspecific amplification of equivalent nuclear sequences as described by ZHANG and HEWITT (1996). Mitochondria were isolated from 0.5-1.0 g of kidney or heart by differential centrifugation (LANSMAN *et al.*, 1981; JONES *et al.*, 1988). MtDNA was purified by phenol/chloroform extraction as described by JAAROLA and TEGELSTRÖM (1995).

Two pairs of primers for the PCR were tested. The universal primers, designed by KOCHER *et al.* (1989) to amplify the complete control region, amplified several unspecific fragments and were therefore excluded. Primers designed by WILKINSON and CHAPMAN (1991) amplified one distinct fragment of approximately 400 base pairs. The primer sequences are L, 5'-TCCTACCATCAGCACCCAAAGC-3' and H, 5'-GTTGCTGGTTTCACGGAGG TAG-3'. The final PCR conditions were; 1X buffer (10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1% Triton X-100), 1.5 mM Mg^{2+}, 60 µM/nucleotide dNTP, 400 nM of each primer, 0.5 U polymerase (Dynazyme) and 20 µg/ml of spermidine. The PCR was initiated by denaturation at 94°C for 2 minutes, continued with 35 cycles of denaturation at 93°C for 1 minute, annealing at 50°C for 1 minute, elongation at 72°C for 2 minutes, and finished with an extended elongation at 72°C for 10 minutes. To check the success of the PCR, two µl of the product were applied to a 2% agarose gel, run for 140 Volt hours, stained with ethidium bromide and visualised with UV-light.

Approximately 80 ng of purified PCR product was cloned into the pCR™ 2.1 vector (Invitrogen). INVaF' competent cells were transformed with the cloned vector using the Original TA Cloning Kit (Invitrogen). Positive transformants were sequenced by the dideoxy chain termination method (SANGER *et al.*, 1977) using the T7Sequencing ™Kit (Pharmacia Biotechnology). We used the M13 reverse and the M13 (–20) forward primer as primers in the sequencing reaction. The recognition sequences for these

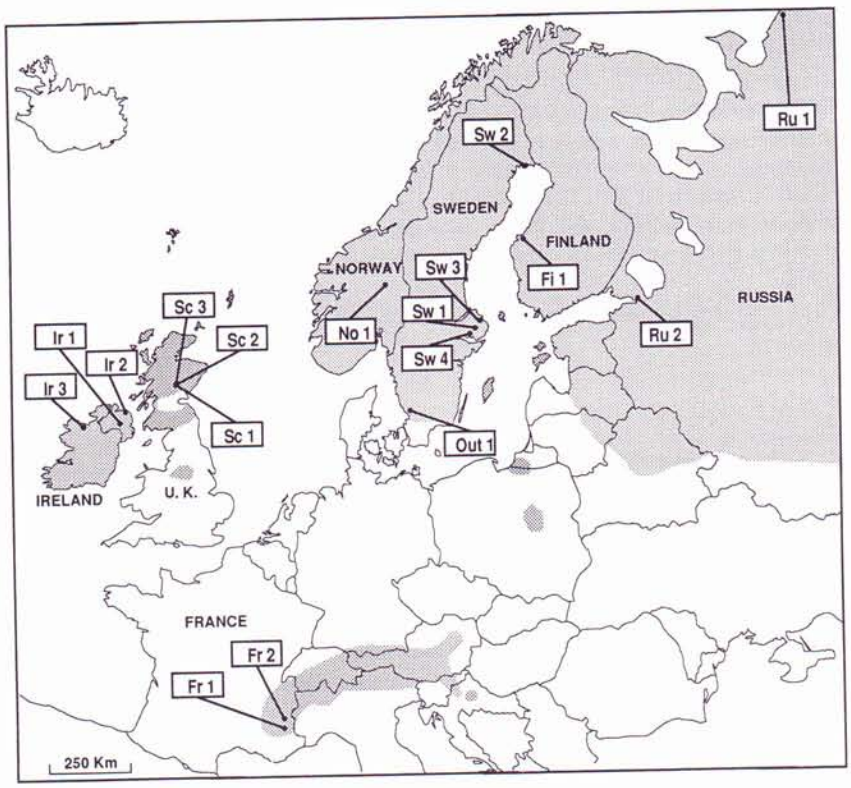

Figure 1: Map of northern and central Europe showing the localities for the samples. The distribution of mountain hares, *Lepus timidus*, is shaded (depicted from ANGERBJÖRN and FLUX, 1995).

Figure 1 : Localisation des sites de collectes d'échantillons. L'aire de distribution de *Lepus timidus* est représentée en grisé (carte d'après ANGERBJÖRN et FLUX, 1995).

primers are located in the pCR™ 2.1 vector approximately 100 base pairs from each end of the inserted fragment to ensure successful sequencing of the complete fragment. ^{33}P was used as the radioactive label. Each sequence reaction was run on 6% polyacrylamide gels in 1 × TBE buffer for 7,000 and 11,000 Volt hours, respectively. The gels were dried on a filter paper and exposed to autoradiographic film.

The sequence data were used to estimate genetic distance between taxa and to construct phylogenetic trees. To test if the data set contains any phylogenetic signal, we used a randomisation test developed by ARCHIE (1989). Only the sites in the sequences which were informative when searching for the most parsimonious trees were used in the test to shorten the time consumed. A site is informative when there are at least two different kinds of nucleotides at the site, each of which is represented in at least two of the sequences under study (LI and GRAUR, 1991). Furthermore, length

differences were treated as non informative and were also excluded. Transitions/transversions were weighted 1:1. In the test, the different characters in the informative sites were randomised within each site and used to build random trees. Randomisation was done with the shuffle option in MacClade 3.0. Heuristic searches were performed in the tree building process. The tree length, calculated as substitution steps, and the consistency index of KLUGE and FARRIS (1969) were recorded for each tree. Mean tree length, standard deviation, and skewness of the resulting tree length frequency distribution were calculated in Matlab 4.2c.1.

The construction of parsimony phylogenies and the calculation of genetic distances between taxa were performed in PAUP 3.0r (SWOFFORD, 1990). Two different state substitution weightings were tested, namely a 1:1 weighting of transition/transversion costs and a weighting matrix based on an empirically found pseudogene substitution step matrix (LI and GRAUR, 1991). The inferred frequencies of substitutions in the pseudogene matrix were multiplicatively inverted to represent costs in the phylogeny constructions and then logged to make the weighting more conservative. The resulting step matrix is shown in Table I. For the 1:1 weighting, branch and bound searches for the most parsimonious trees were performed. For the step matrix we used the less time-consuming heuristic search. Consensus trees were constructed to compare the general structure of the phylogenies derived from the different weightings. The length of the shortest tree found when using the 1:1 weighting was tested, with a one sample t-test, against the length frequency distribution of the random trees (see above). To test the phylogenies inferred from the pseudogene step matrix, 100 bootstrap replications were performed (EFRON, 1982). Finally, for comparative purposes, the obtained genetic distance values from the 1:1 and the pseudogene step matrix weightings were used to construct trees with the Neighbor-joining, UPGMA and Fitch and Kitch methods in the computer package Phylip 3.5 (FELSENSTEIN, 1993).

The nucleotide sequence data reported in this paper will appear in the EMBL, Genebank and DDBJ Nucleotide Sequence Databases under the accession numbers: Y15299-Y15315.

TABLE I

The pseudogene step matrix, used for weighting of substitutions, derived from LI and GRAUR (1991).

TABLEAU I

Fréquence de substitution des bases adénine, thymidine, cytosine et guanine, établie d'après la matrice de pseudogène de LI et GRAUR (1991).

	To:	A	T	C	G
From:	A	–	1.33	1.30	1.03
	T	1.36	–	1.09	1.44
	C	1.19	0.68	–	1.38
	G	0.68	1.14	1.28	–

III. RESULTS

The fragment sequenced was 407 base pairs long in the brown hare mtDNA and 410 base pairs in all investigated mountain hare mtDNAs. In total, 92 variable sites were detected, of which 44 were informative. Among the 16 mountain hare mtDNAs (of which one was found in a brown hare), there were 66 variable sites.

On average, the sequence divergence, calculated as the percentage of differing nucleotides, was 13.5% between the two species (range 12.0-14.6%) and 5.1% among mountain hares (range 0-7.3%). The obtained distance values, calculated in PAUP 3.0r (SWOFFORD, 1990), are shown in Table II. For the 1:1 weighting, distances between the species range from 0.121 to 0.149, and distances among mountain hares from 0 to 0.076. Using the pseudogene step matrix in the weighting, the obtained distance values increase, and vary from 0.202 to 0.239 between the species and from 0 to 0.095 among the mountain hares.

The lengths of the phylogenetic trees constructed in the randomisation test were approximately normally distributed (skewness – 0.46; mean length 151 ± 2 steps). The length of the shortest tree built from the original informative site data was significantly shorter than the trees constructed in the randomisation test (104 steps; t-test, $P < 0.001$). The consistency index for randomised trees was on average 0.352. For the shortest tree found, using the original data, it was 0.510.

The main clusters observed in the shortest tree (Figure 2) were retained throughout the phylogenetic inferences using different weighting matrixes and several tree building processes. The bootstrap analysis supports the basic structure of the phylogeny (see values in Figure 2) and we therefore consider our interpretations of the phylogenetic clusters reliable. The different clusters show that the mtDNAs of Scandinavian mountain hares are heterogeneous and branch out, with mtDNA from several representatives of the different subspecies and localities included in the present investigation. The mountain hares from Scotland (Sc 1-3) form a monophyletic cluster with No 1 from Norway added, and the mountain hares from Ireland (Ir 1-3) also form a separate monophyletic cluster, with Sw 3 from central Sweden added. The tightest connection between a Scandinavian and a non Scandinavian mountain hare is between Sw 2 from northern Sweden and Ru 1 from arctic Russia. Also, in the Neighbor-joining, UPGMA, Kitch and Fitch trees constructed from the distance data in Table II, Sw 1, representing a Scandinavian brown hare carrying mountain hare mtDNA, cluster with Ru 2 and Fi 1, representing mountain hares from Russia and Finland, respectively (not shown). Finally, mountain hare Sw 4 from central Sweden associates with the other mountain hares but shows no specific relationship to any of the subgroups described above.

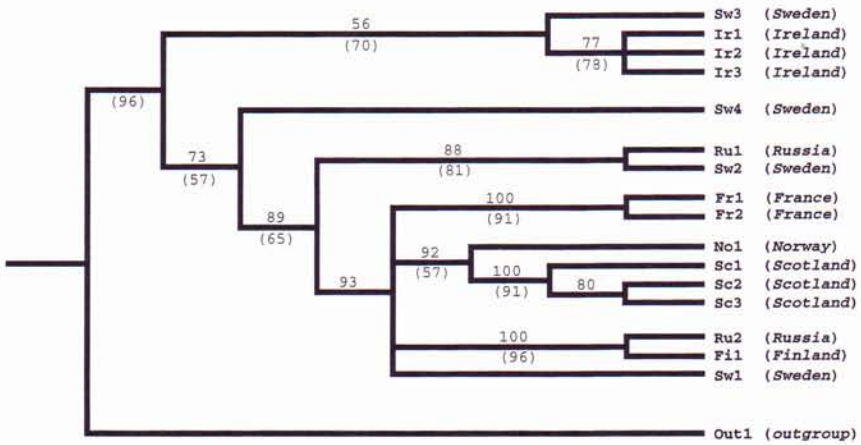

Figure 2: Parsimony phylogeny of the investigated mountain hare, *Lepus timidus,* mtDNAs showing a 50% majority rule consensus of the 200 shortest trees found by the program Paup 3.0r (FELSENSTEIN, 1993) using branch and bound search and weighting transition/transversion 1:1. The numbers above branches indicate percentage of the trees used to build the consensus which support that branch. Bootstrap values are given in parentheses below branches. The outgroup, Out1, consists of a brown hare from southern Sweden.

Figure 2 : Arbre phylogénique établi à partir des ADN mitochondriaux des lièvres variables, *Lepus timidus,* collectés. Les branches et les nœuds ont été établis dès lors que la structure était rencontrée dans au moins 50% des 200 arbres les plus parcimonieux fournis par le programme PAUP 3.Or (FELSENSTEIN, 1993). Les probabilités de transitions et de tranversions ont été considérées comme identiques. Les chiffres au-dessus des branches donnent le pourcentage d'arbres utilisés parmi les 200 fournis par le Logiciel pour établir la structure correspondante. Le groupe externe Out1 est constitué d'un lièvre européen, *Lepus europaeus*, provenant du sud de la Suède.

IV. DISCUSSION

For the purposes of the present investigation, we find the 407/410 base pair mtDNA control region fragment useful. The randomisation test shows that the fragment contains significant phylogenetically informative data, *i.e.* variant nucleotides are not randomly associated. In the evening bat, *Nycticeius humeralis*, this control region fragment is constructed by a variable number of 81 base pair repeats (WILKINSON and CHAPMAN, 1991). No repeats were detected in the hare mtDNA investigated, but the more conservative flanking regions were homologous to the sequence of the evening bat. BIJU-DUVAL *et al.* (1991) observed a variable number of repeats in the noncoding mtDNA control region in several taxa of Lagomorphs, including the brown hare. Because no repeats were detected in the hares included in the present investigation, we suggest that these repeats are located elsewhere in the mtDNA control region of hares. This conclusion is in accordance to CASANE *et al.* (1994), who determined the location of repeats in rabbits,

TABLE II

Genetic distances between hares, *Lepus timidus*, estimated from a part of the mtDNA control region. Distance values above the diagonal were calculated with a 1:1 transition/transversion weighting. Those below the diagonal were calculated with a pseudogene substitution step matrix (Table I). The first two letters in the sample number indicate the country of origin (see Figure 1), except for Out1, which refers to a brown hare outgroup.

TABLEAU II

Distances génétiques entre lièvres, *Lepus timidus*, calculées à partir d'un fragment d'ADN mitochondrial situé dans la région de contrôle. Les valeurs au-dessus de la diagonale ont été calculées avec une équiprobabilité des transitions et tranversions. Celles en dessous de la diagonale ont été calculées avec la matrice de fréquences de substitution (Tableau I). Les deux premières lettres du code de l'échantillon indiquent son pays de provenance, sauf pour Out1 qui représente un lièvre européen, *Lepus europaeus*, du sud de la Suède utilisé comme groupe externe.

Sample	Out1	Sw1	Sw2	Sw3	Sw4	No1	Ru1	Ru2	Fi1	Ir1	Ir2	Ir3	Fr1	Fr2	Sc1	Sc2	Sc3
Out1	–	0.136	0.131	0.131	0.124	0.144	0.134	0.141	0.146	0.124	0.129	0.121	0.149	0.144	0.134	0.136	0.136
Sw1	0.222	–	0.049	0.051	0.049	0.034	0.041	0.029	0.032	0.059	0.061	0.056	0.063	0.066	0.039	0.039	0.039
Sw2	0.227	0.059	–	0.054	0.054	0.049	0.027	0.049	0.046	0.051	0.054	0.054	0.068	0.056	0.059	0.049	0.049
Sw3	0.212	0.056	0.068	–	0.046	0.061	0.046	0.051	0.059	0.027	0.032	0.024	0.066	0.063	0.061	0.056	0.056
Sw4	0.204	0.051	0.066	0.054	–	0.054	0.041	0.054	0.056	0.054	0.051	0.051	0.068	0.061	0.051	0.049	0.049
No1	0.229	0.034	0.059	0.066	0.056	–	0.041	0.029	0.032	0.068	0.071	0.066	0.059	0.054	0.029	0.024	0.024
Ru1	0.222	0.046	0.032	0.056	0.049	0.046	–	0.051	0.044	0.044	0.051	0.046	0.059	0.054	0.051	0.046	0.046
Ru2	0.224	0.029	0.059	0.056	0.056	0.029	0.051	–	0.007	0.054	0.056	0.051	0.063	0.059	0.049	0.044	0.044
Fi1	0.234	0.037	0.061	0.068	0.063	0.037	0.054	0.012	–	0.061	0.063	0.059	0.071	0.061	0.051	0.046	0.046
Ir1	0.209	0.068	0.061	0.032	0.066	0.078	0.049	0.063	0.076	–	0.015	0.007	0.073	0.071	0.068	0.063	0.063
Ir2	0.214	0.071	0.073	0.037	0.063	0.080	0.066	0.066	0.078	0.024	–	0.012	0.076	0.073	0.071	0.061	0.061
Ir3	0.202	0.061	0.068	0.024	0.059	0.071	0.056	0.056	0.068	0.012	0.017	–	0.071	0.068	0.066	0.061	0.061
Fr1	0.239	0.073	0.088	0.080	0.080	0.068	0.073	0.073	0.085	0.093	0.095	0.085	–	0.051	0.073	0.073	0.073
Fr2	0.236	0.071	0.071	0.073	0.068	0.059	0.063	0.063	0.071	0.085	0.088	0.078	0.056	–	0.063	0.059	0.059
Sc1	0.219	0.039	0.068	0.066	0.054	0.029	0.056	0.049	0.056	0.078	0.080	0.071	0.083	0.068	–	0.010	0.010
Sc2	0.222	0.039	0.059	0.061	0.051	0.024	0.051	0.044	0.051	0.073	0.071	0.066	0.083	0.063	0.010	–	0
Sc3	0.222	0.039	0.059	0.061	0.051	0.024	0.051	0.044	0.051	0.073	0.071	0.066	0.083	0.063	0.010	0	–

Oryctolagus cuninculus, to the control region between the H strand replication origin and the 12 S rRNA, *i.e.* beyond 3'-end of the fragment investigated in the present study.

An earlier investigation (THULIN *et al.*, 1997) indicates that there is a relatively large intraspecific mtDNA variation among Scandinavian mountain hares. In the present study, the main maternal lineages described by THULIN *et al.* (1997) are included as representatives of the Scandinavian mountain hare mtDNA gene pool (haplotypes 1, 5, 6, 7 & 8, denoted Sw 1-4 and No 1 in this study). The phylogenetic associations between mtDNA haplotypes among Scandinavian mountain hares and mtDNA from several non Scandinavian mountain hares may be caused by the suggested (TEGELSTRÖM, 1987) postglacial colonisation routes to the Scandinavian peninsula, from the south and from the north-east. We interpret the cluster of mountain hares from Scandinavia (No 1 and Sw 3) with the Scottish and Irish representatives (Sc 1-3 and Ir 1-3 respectively), to be the result of a common origin of these mountain hares from a continuous population in central Europe during the last glaciation. The variation we observe among Scandinavian mountain hares would then be a retention of ancestral polymorphisms present in that continuous ice age population. The alternative colonisation route to Scandinavia from a north-eastern mountain hare population could explain the observed relationship between the mountain hare from northern Sweden (Sw 3) and the one from arctic Russia (Ru 1). Though, we can not rule out the possibility that the observed phylogenetic relationships are the result of sorting of ancestral polymorphism that once were present in a continuous source population, and not the effect of differential colonisation. Extensive studies of mountain hare mtDNA heterogeny throughout Europe is necessary to reach a definite conclusion.

In some of the phylogenetic inferences (not shown) we observe an association between the mountain hare mtDNA lineage represented by one brown hare (Sw 1) and the Russian and Finnish representatives (Ru 2 and Fi 1). This association may have a different background than the colonisation inferences described above. In the early 20th century, approximately 100 mountain hares, imported to central Sweden from Waldai in Russia, were used for breeding and for supplementing existing populations of mountain hares (LEWENHAUPT, 1929). Such a supplement could have an impact on the genetic structure of the natural population, and presently unknown introductions of mountain hares of various origins could affect the geographical distribution patterns of mtDNA variation and our interpretations concerning colonisation history. Release experiments with captively reared mountain hares show that the survival rate of released hares is low, with an average post release survival of only 11.2 days (LEMNELL and LINDLÖF, 1982), suggesting a minimal contribution of alien genes to the natural population. However, the lineages of the imported Russian mountain hares may very well have persisted in captivity. Because the mtDNA lineage of Sw 1 has not yet been detected in any wild mountain hare in Scandinavia, the observed association with the Finnish and the Russian hares could indicate releases of brown hares carrying mountain hare mtDNA as a result of hybridisation in captivity.

The observed phylogenetic clusters of mtDNA from mountain hares in Europe ought not to interfere with the present subspecies status of, for ex-

ample, Scottish and Irish mountain hares. Also, the mountain hare mtDNA variation in Scandinavia may very well reflect a natural division of the Scandinavian mountain hares in a northern and a southern subspecies. As denoted by PAMILO and NEI (1988), phylogenies of genes and phylogenies of taxa may differ. A subspecies may have all the mitochondrial lineages of the species, or only a few, depending on the population history. Furthermore, the population genetic structure of small populations at the front of colonisation will not necessarily be the same as in the original population because of repeated founder events (HEWITT, 1993). Thus, different mtDNA lineages may be retained in different populations, a process that may be comparably rapid and can have significant effects in the time perspective given by postglacial recolonisation of Scandinavia.

In conclusion, we suggest that the mtDNA variation earlier observed among the Scandinavian mountain hares (THULIN *et al.*, 1997) is due to a retention of old mtDNA haplotypes that were present in a continuous mountain hare population in central Europe throughout the last glaciation. The associations of the Scandinavian mtDNA lineages with Scottish, Irish and Russian lineages may reflect a differential postglacial colonisation of the Scandinavian peninsula, from the south and from the north-east.

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ORIGINE DU LIÈVRE VARIABLE (*LEPUS TIMIDUS*) EN SCANDINAVIE

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MOTS-CLÉS : Lièvre variable, *Lepus timudus,* historique de la colonisation, séquence de contrôle de l'ADN mitochondrial, séquençage de l'ADN, Scandinavie.

RÉSUMÉ

Lors de la période glaciaire la plus récente (Weichsel), la Scandinavie était complètement recouverte de glace. À la fonte des glaces il y a environ 14 000 ans, plusieurs espèces ont commencé à recoloniser les zones nouvellement dégagées. Deux voies de colonisation vers la Scandinavie étaient alors disponibles, la première par le sud et la deuxième, plus tard, par le nord-est. Pour vérifier si ces deux voies de colonisation ont abouti à une division génétique chez Lepus timidus, *nous avons séquencé un fragment très variable d'ADN mitochondrial, d'environ 400 paires de bases, chez 15 lièvres variables et 2 lièvres européens,* Lepus europaeus. *Les premiers provenaient d'une lignée maternelle détectée en Scandinavie lors d'une étude précédente, mais aussi de trois sous-espèces de lièvre variable d'Europe Centrale, de Finlande et de Russie. Nous avons aussi inclus un lièvre européen comme groupe externe. Les relations entre lièvres variables ont été établies à partir d'arbres philogéniques les plus parcimonieux construits sur la base de la distance génétique séparant les séquences. Parmi les lièvres variables de Scandinavie, trois individus étaient étroitement apparentés aux animaux de la même espèce provenant d'Ecosse, d'Irlande et de Russie. Ces résultats suggèrent que la forte variabilité observée dans les ADN mitochondriaux des lièvres variables de Scandinavie, est due à la conservation d'anciens haplotypes mitochondriaux issus des populations en contact au cours de la dernière glaciation. Les associations observées entre ADN mitochondriaux peuvent refléter une double colonisation de la Péninsule Scandinave par le sud et le nord-est.*

DIE ABSTAMMUNG DER SKANDINAVISCHEN SCHNEEHASEN (*LEPUS TIMIDUS*)

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SCHLÜSSELWÖRTER : Schneehase, *Lepus timidus,* Besiedlungsgeschichte, mtDNA-Kontrollregion, DNA-Sequentierung, Skandinavien.

ZUSAMMENFASSUNG

Während der letzten Eiszeit in Nordeuropa (Weichsel) war Skandinavien völlig von Eis bedeckt. Als dieses vor etwa 14 000 Jahren zu schmelzen anfing, begannen zahlreiche Organismen, die erneut freigelegten Areale wieder zu besiedeln. Es öffneten sich zwei alternative Besiedlungsrouten nach Skandinavien, die erste vom Süden und eine spätere vom Nordosten aus. Um festzustellen, ob diese zwei Alternativrouten zu einer genetischen Unterteilung der Schneehasen, Lepus timudus, *in Skandinavien führte, haben wir ein sehr variables Segment von Basenpaaren mit rund 400 mtDNA-Kontrollregionen von 15 Schneehasen und 2 Feldhasen, Lepus europaeus, sequentiert. Die Hasen stammten von einer mütterlichen Linie ab, die in einer früheren Studie in Schweden gefunden worden war, aber auch von drei verschiedenen mitteleuropäischen Schneehasenunterarten sowie von Schneehasen aus Finnland und Rußland. Ein Feldhase wurde als Außenseitergruppe mit einbeschlossen. Ausgehend von den erhaltenen Sequenzen schätzten wir die genetischen Entfermungen zwischen den verschiedenen Stämmen und erstellten phylogenetische Stammbäume, um die Beziehungen zwischen den Schneehasen zu klären. Drei der skandinavischen Schneehasen zeigten eine enge Verwandschaft jeweils mit Schneehasen aus Schotland, Irland und Rußland. Unsere Ergebnisse lassen vermuten, daß die starken mtDNA-Variationen der skandinavischen Schneehasen auf das Erhalten alter mtDNA-Halotypen auf während der letzten Eiszeit nicht erloschenen und in Kontakt gebliebenen Schneehasenpopulationen zurückzuführen sind. Die beobachteten mtDNA-Verwandschaften können eine nacheiszeitliche Besiedlung der skandinavischen Halbinsel sowohl vom Süden als auch vom Nordosten aus widerspiegeln.*

Übers K. Ebner