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SHORT COMMUNICATION

The origin of Swedish and Norwegian populations of the Eurasian harvest mouse (*Micromys minutus*)

Lars Råberg · Jon Loman · Olof Hellgren · Jeroen van der Kooij · Kjell Isaksen · Roar Solheim

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Abstract The harvest mouse (*Micromys minutus*) occurs throughout most of continental Europe. There are also two isolated and recently discovered populations on the Scandinavian peninsula, in Sweden and Norway. Here, we investigate the origin of these populations through analyses of mitochondrial DNA. We found that the two populations on the Scandinavian peninsula have different mtDNA haplotypes. A comparison of our haplotypes to published sequences from most of Europe showed that all Swedish and Norwegian haplotypes are most closely related to the haplotypes in harvest mice from Denmark. Hence, the two populations seem to represent independent colonisations but originate from the same geographical area. We discuss the age of the Swedish and Norwegian populations and suggest that both have been introduced recently.

Keywords Control region · Cytochrome *b* · Phylogeography

Introduction

The harvest mouse *Micromys minutus* (Pallas 1771) occurs throughout most of continental Europe, north of the Mediterranean, from France to Russia and northwards to central Finland (Mitchell-Jones et al. 1999). Recently, it has also been found to occur in Sweden and Norway. In 1985, an isolated population was discovered in the province of Dalsland in western Sweden (Loman 1986), and during the last decade, this population has been found to extend into the surrounding provinces as well as into Norway. The known distribution in Norway is limited to a relatively small area close to the Swedish border, in Eidskog, Hedmark (van der Kooij et al. 2001; van der Kooij et al., unpublished data). In 2007, another population was discovered in the province of Skåne in southern Sweden, about 500 km south of the Dalsland population, and during 2008, harvest mice were recorded at more than ten different sites throughout southern Skåne (Råberg and Holmqvist 2008). Finally, a few records have been made along the coast of the Gulf of Bothnia in the province Norrbotten in northern Sweden, close to the Finnish border, during 1996–2004 (Hallman 2005) (see Fig. 1 for a distribution map). While the sporadic records from northern Sweden obviously concern animals stemming from Finland, the origin of the populations in southern Sweden and Norway is still unclear. To investigate the origin of these populations, we compare here the mtDNA haplotypes of mice from Sweden and Norway with the haplotypes from other parts of Europe. Specifically, we sequenced parts of the control (ctrl) region and cytochrome *b* (cyt *b*) in the samples (skin biopsies and muscle tissue) collected in Skåne and Dalsland in Sweden, in Hedmark in Norway, as well as in Finland and Denmark.

Materials and methods

Danish samples originated from museum specimens collected in the 1960s and 1970s; all other samples were from mice collected during 2001–2008 (see Table 1 for details of the

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L. Råberg (✉) · J. Loman · O. Hellgren
Department of Biology, Ecology Building, Lund University,
223 62 Lund, Sweden
e-mail: lars.raberg@biol.lu.se

J. van der Kooij · K. Isaksen
Norwegian Zoological Society,
P.O. Box 102, Blindern,
0314 Oslo, Norway

R. Solheim
Agder Natural History Museum and Botanical Garden,
P.O. Box 1887, Gimlemoen,
4686 Kristiansand, Norway

Fig. 1 The distribution (*black spots*) of the harvest mouse in Sweden and Norway. The names of the provinces where the populations are located are indicated in *italics*

samples). We also obtained published sequences from other areas in Europe (Yasuda et al. 2005; GenBank accession numbers: AB125095, AB125097-AB125100, AB201981-AB201983, AB201975 and AB201976 for *cytb*, and AB202039.1, AB202040.1 and AB202048.1-AB202057.1 for the ctrl region). Museum specimens from Denmark had been stored in formalin, so DNA from these were extracted with QIAamp DNA FFPE tissue kit (Qiagen GmbH, Germany); using this method, we were able to amplify both cyt *b* and control region sequences from four out of six available specimens. DNA from all other samples was extracted by placing biopsies in lysis buffer and proteinase K, where after the DNA was precipitated in ethanol, as described in Laird et al. (1991). We amplified 1,140 bp of the cytochrome *b* region and 842 bp of the ctrl region using the primers and PCR conditions described by Yasuda et al. (2005). PCR products were sequenced in both directions using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit on an ABI 3100 sequencer (Applied Biosystems).

Sequences were aligned by eye using BioEdit 7.0 (Hall 1999). Phylogenetic reconstructions were performed using Bayesian inference as implemented by the software MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The run was partitioned to allow for ctrl and cyt *b* to have different models of molecular evolution. For the ctrl region, we allowed for six different substitution rates and a proportion of invariable sites (corresponding to the GTR+I model). For cyt *b*, we allowed for two different substitution rates and a proportion of invariable sites (corresponding to the HKY85 model). The models were chosen using MrModeltest v2 separately for each of the genes (Nylander 2004). Trees were rooted using ctrl and cyt *b* from the specimens collected in Russia (Yasuda et al. 2005).

Table 1 Harvest mouse samples used to obtain cytochrome *b* and control region sequences

Origin	Year of collection	Number	Sample name and origin of museum samples
Denmark, Jutland, Varde	1968	1	L968/2337 ZML
Denmark, Jutland, Fredrikshavn	1969	1	L969/7170 ZML
Denmark, Jutland, Tönder	1971	2	L971/0331, 0332 ZML
Sweden, Skåne, Revingehed	2007–2008	9	
Sweden, Skåne, Sösdala	2007	1	
Sweden, Dalsland, Ånimskog	2008	3	
Norway, Hedmark, Klanderud	2006	1	2076 ANM
Norway, Hedmark, Finnsrudtjennet	2003	1	
Norway, Hedmark, Klanderud	2001	1	11621 NHM
Finland, Luhanka	2004	2	1836, 1837 ANM

ANM Agder Natural History Museum and Botanical Garden (Kristiansand), *NHM* Natural History Museum (University of Oslo), *ZML* Zoological Museum, Lund

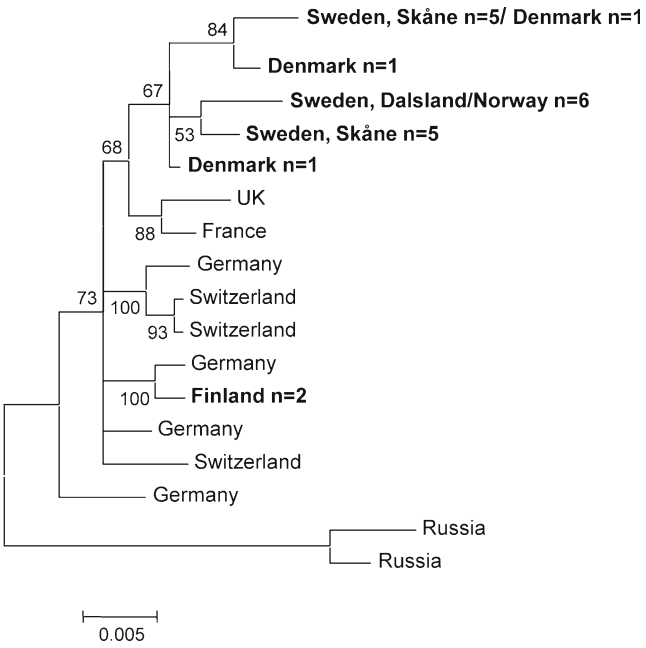

Fig. 2 Phylogenetic tree based on concatenated *cytb* and ctrl region sequences (1,940 bp). Sequences obtained in this study are in bold. Sequences from outside the Nordic countries are from Yasuda et al. (2005)

Results

Based on concatenated control region and cyt *b* sequences, we found two different haplotypes in Skåne (in equal frequencies), one in Dalsland/Norway, three in Denmark and one in Finland. One of the haplotypes from Skåne was identical to one of the Danish haplotypes. The sequences have been deposited in GenBank (accession nos. JX531446-50 for cyt *b* and JX531451-56 for ctrl region). A phylogenetic tree constructed from concatenated sequences showed that all the Scandinavian haplotypes formed a separate clade, although the statistical support for this clade was relatively weak (posterior probability value of 0.67; Fig. 2). The Finnish haplotype grouped together with the central European haplotypes.

Discussion

Based on these results, we conclude that the harvest mice in Skåne and Dalsland/Hedmark stem from different source populations. Moreover, since one of the haplotypes in Skåne was identical to the one found in Denmark, it is clear that the mice in Skåne share common ancestry with the individuals found in the Danish population. The origin of the mice in Dalsland/Hedmark is more uncertain, but the phylogenetic relationships shown in Fig. 2 suggest that they stem from Denmark rather than central or eastern Europe.

An intriguing question is how old are the populations in Skåne and Dalsland/Hedmark. One possibility is that the harvest mouse, like many other animals, spread to the Scandinavian peninsula from the south after the last glaciation, when there was a land bridge between Denmark and Sweden for about 400 years (Björck 1995). Under this scenario, the populations in Skåne and Dalsland/Hedmark should be remnants of a previously wider distribution on the Scandinavian peninsula. Alternatively, the harvest mouse may have been introduced by humans more recently, for example with transports of domestic animals or agricultural products. Unfortunately, it is not possible to decide whether the populations on the Scandinavian peninsula are 10 or 10,000 years old based on the currently available genetic data. However, as regards the population in Skåne, we favour the hypothesis that it has been founded recently because it seems highly unlikely that they could have avoided detection for so long in a region where intense rodent trapping has been performed from the 1970s until 1995 at the very same sites where harvest mice were discovered in 2007 (Sam Erlinge, personal communication). When it comes to the population in Dalsland/Hedmark, the jury is still out, although also in this case a relatively recent introduction seems most likely, for example because it appears unlikely that an ancient population of harvest mice would not have expanded into most of the favourable habitat in southern Sweden and Norway.

How were they introduced then? Small numbers of harvest mice were illegally imported from Denmark during the early 2000s and sold to herpetologists in Malmö (in Skåne) as food for small pet snakes (C. Sjöholm, personal communication). However, it seems unlikely that a few escapes from this source could have established a population covering large parts of the province of Skåne (at least 2,500 km^2) in just a few years. Instead, we suggest that harvest mice may have been repeatedly introduced by transports of domestic animals and/or agricultural products. The harvest mouse has apparently colonized new areas in the Netherlands and England in this way (Mostert 1992; Perrow and Jowitt 1995). There are also other examples that such transports can be an important dispersal mechanism for mammals that are normally not intimately associated with humans (in the same way as, e.g. the house mouse (*Mus musculus*) and the brown rat (*Rattus norvegicus*)). For example, the common vole (*Microtus arvalis*) was introduced to the Orkney islands from southwestern Europe during the Neolithic period, probably through transports of animal fodder (Haynes et al. 2003). More recently, a number of striped field mice (*Apodemus agrarius*) have arrived with potato transports from Jutland in Denmark to Ödeshög in south central Sweden (Björkman 2009); this constitutes the first documented records of the species in Sweden. We anticipate that also other small mammal species may colonize the Scandinavian peninsula in this way in the future, an obvious candidate being the common vole (*M. arvalis*).

The sudden appearance of the harvest mouse over large areas in Skåne highlights our surprisingly poor knowledge of the distribution of small mammals in Scandinavia, in particular, in comparison with other organisms such as birds. Since introduced species may compete with indigenous species (or subspecies) and carry new zoonotic diseases, it would be of interest to improve monitoring of small mammals.

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