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Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice

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Summary

Restriction enzymes were used to search for genetic variability at 162 cleavage sites in mitochondrial DNA (mtDNA) purified from 22 mice caught at seven Swedish localities. Although all of these mice bear the nuclear genes of *Mus musculus*, they bear the mtDNA of *M. domesticus* exclusively. Yet, some of the Swedish localities are 750 km away from the hybrid zone between these two species. Furthermore, only one type of mtDNA was found at the seven Swedish localities; this type was found before at an eighth locality in Sweden as well as in Jutland north of the hybrid zone. The apparent lack of mtDNA divergence in the mouse population of Sweden contrasts with the extensive divergence usually found within other geographic areas in Europe, Africa and North America. Electrophoretic analysis of proteins encoded by nuclear genes indicates that the Swedish mice have lower average heterozygosity than Danish and Central European populations of *musculus* mice. These findings lead us to suggest that the source of the commensal mouse population in Sweden was a small propagule that originated from a population situated only a few kilometres to the east of the point at which the hybrid zone on the European mainland meets the Baltic Sea, namely on East Holstein. Such a founder event may have been associated with the spread of farming from north Germany into Sweden about 4000 years ago.

Introduction

Mitochondrial DNA (MtDNA) of mammals has been extensively used to infer phylogenetic relationships (Avise *et al.* 1983; Brown & Simpson, 1981, 1982; Ferris *et al.* 1981, 1983*a*, *b*; Yonekawa *et al.* 1982). The recent discovery that mtDNA can move from one species to another, in the possible absence of nuclear gene flow, has important implications for the utility of mtDNA as a genealogical tool (Yonekawa *et al.* 1982; Ferris *et al.* 1983*a*; Powell, 1983). Since this type of transfer appears to be frequent and not limited to certain taxa (Yonekawa *et al.* 1984; Wilson *et al.* 1985) it is important to obtain a better understanding of the conditions under which nuclear and mitochondrial genes can move at different rates across a hybrid zone. To help explain this phenomenon, which was first recognized in wild house mice in Scandinavia (Ferris *et al.* 1982, 1983*a*), we have investigated the extent to which mtDNA from *domesticus* mice has intruded into the *musculus* populations of Sweden.

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Mus domesticus is the house mouse of Southern Jutland (Fig. 1), and most of Western Europe, whereas *M. musculus* is the house mouse in most of Scandinavia and Eastern Europe (Sage, 1981; Marshall, 1981; Marshall & Sage, 1981). The two species meet and form a narrow hybrid zone, across which there is little or no flow of nuclear genes (Hunt & Selander, 1973; Ferris *et al.* 1983*a*; Sage *et al.* 1986). The hybrid zone goes south from Denmark and North Germany (Fig. 1), through Austria to Southern Yugoslavia and then swings east through Bulgaria to the Black Sea (Boursot *et al.* 1984). Previous studies of 4 *musculus* localities in Scandinavia, not far from the hybrid zone (localities 8–11 in Fig. 1), revealed exclusively *domesticus* types of mtDNA (Ferris *et al.* 1983*a*; Moriwaki *et al.* 1984). The present paper extends knowledge of the mtDNAs (and of proteins encoded by the nucleus) in Swedish mice to include seven new localities as far as 750 km from the region occupied by *domesticus* mice. A single *domesticus* mtDNA lineage was found throughout the sampled area; yet as is evident both from morphology and protein electrophoresis, genes of these mice are predominantly, if not exclusively, *musculus* in character. To explain this finding we present a hypothesis based on geological and archaeological

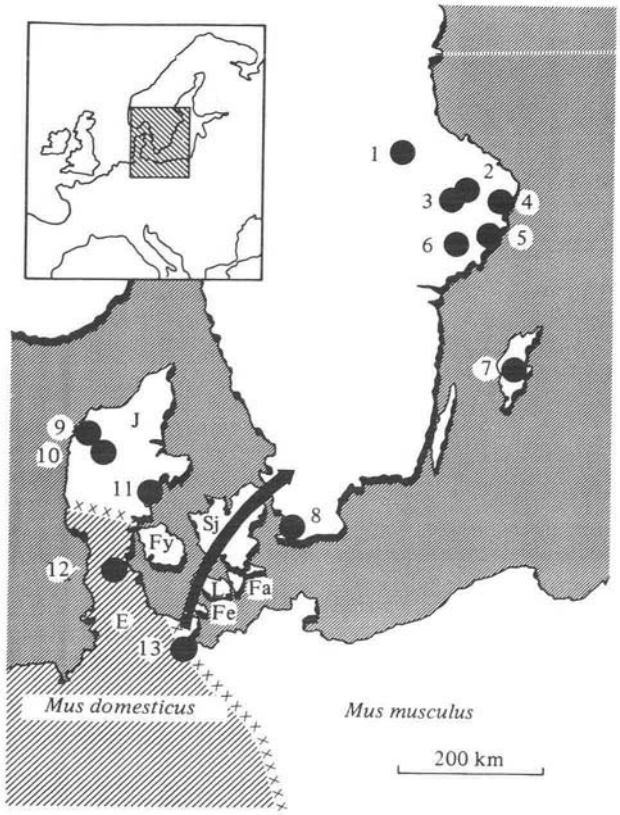

Fig. 1. Map of Scandinavia showing the sampling localities with *Mus domesticus* mtDNA (black circles). The results for localities 8–13 have previously been reported (Ferris *et al.* 1983*a*, *b*). The shaded area indicates the distribution of mice with nuclear genes of *domesticus* type and the × indicates the location of the hybrid zone between *M. domesicus* and *M. musculus*. The arrow shows the presumed way of colonization of Sweden by mice from Denmark and West-Germany: i.e. East Holstein (E) → Fehmarn (Fe) → Lolland (L) → Falster (Fa) → Själland (Sj) → Sweden. Fy, Fyn; J, Jutland.

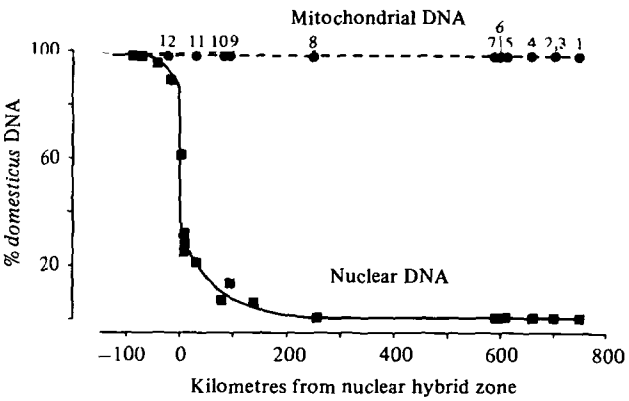

Fig. 2. The transition in allozyme frequencies (squares) and mtDNA type (circles) along a transect from the hybrid zone in Denmark and through southern Sweden. For each of 12 localities (shown in Fig. 1), the ordinate gives the percentage of genetic types or alleles that are of *domesticus* origin. The nuclear estimates for these and additional localities come from the allozyme data of Hunt and Selander (1973) and this work. The genealogical criteria used to classify the mtDNA types appear in Ferris *et al.* (1983*a*, *b*) and Wilson *et al.* (1985).

evidence concerning the history of this region, which was probably uninhabitable by commensal species of *Mus* until colonized by farmers about 4000 years ago.

Materials and methods

A total of 22 mice were trapped at seven Swedish localities: Grimsö *1*($n = 5$), Jumkil *2* ($n = 1$), Uppsala *3* ($n = 1$), Åkersberga *4* ($n = 1$), Tovetorp *5* ($n = 8$), Tyresta *6* ($n = 3$), Gotland *7* ($n = 3$). The location of these and an additional six localities (Malmö *8* ($n = 1$), Skive *9* ($n = 1$), Viborg *10* ($n = 1$), Hov *11* ($n = 22$), Haderslev *12* ($n = 1$), Lübeck *13* ($n = 3$)) previously described by Ferris *et al.* (1983*a*), is shown in Fig. 1. MtDNA from single mice was prepared to high purity by CsCl propidium diiodide isopycnic centrifugation by the procedure of Brown (1980). The mtDNA was cleaved with the following 11 type II restriction endonucleases: *Hin* dIII, *Xba* I, *Hin* cII, *Acc* I, *Ava* II, *Fnu* DII, *Hpa* II, *Hae* III, *Taq* I, *Mbo* I, *Hin* fI). The DNA fragments were labelled by filling the 3′ termini with the appropriate α^{32} PdNTP's in the presence of the large fragment of DNA polymerase I (Klenow) and separated by agarose and polyacrylamide electrophoresis (Brown, 1980). High resolution restriction site mapping (Cann *et al.* 1984) was possible using the mouse mtDNA of known sequence (Bibb *et al.* 1981) and previously mapped restriction site polymorphisms (Ferris *et al.* 1983*b*).

To confirm that Swedish mice were members of the species *Mus musculus* as well as to estimate the average heterozygosity for their nuclear genes, standard starch gel electrophoresis and histobiochemical staining techniques were used to analyze the allelic state at ten isozyme loci: *ADH*, *IDH-1,2*, *MDH-1,2*, *LDH-1,2*, *GPI-1*, *PGM-1,2*. The inbred strain C57BL/6 was used as authentic *domesticus* and the Brno strain used as authentic *musculus*.

Results

Restriction analysis of mtDNA

A total of 162 sites, distributed over all parts of the mouse mitochondrial genome, were studied using the 11 restriction enzymes but only a single mtDNA type was found at the 7 Swedish localities (Fig. 1). This mtDNA type, referred to here as *A*, was found before in southern Sweden (Malmo) and Denmark (Hov) and differs at three sites (nucleotide positions 2817, 8984 and 9505) from type *B* found at Viborg and Skive (Ferris *et al.* 1983*a*, *b*), all north of the hybrid zone. Using the proportion of shared sites to estimate the average number of nucleotide substitutions between mtDNA types (Nei & Li, 1979), Ferris *et al.* (1983*b*) calculated that type *A* differs by 0·2% from type *B* and by 0·08% from a related type in Italy but by 0·3–1·5% from other *domesticus* types examined

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Table 1. *Mitochondrial DNA sequence divergence among mouse populations within a geographic region*

Geographic region	No. of localities	Mean distance between localities (km)	Mean sequence divergence (%)[a]	No. of sites tested[b]
Mus domesticus mtDNA				
Sweden	8	225	0	162
Denmark, north of hybrid zone	3	60	0·13	160
S. Denmark and N.W. Germany	2	150	1·97	68
England	2	300	0·40	64
Shetland Is.	3	40	0·81	64
Orkney Is.	4	50	0·10	63
Switzerland	2	300	0·63	170
Italy	2	350	0·70	162
W. Yugoslavia	2	230	0·63	164
N. Morocco	2	250	1·31	162
N. Egypt	2	80	0·60	168
N. California	4	35	0·36	167
Mus musculus mtDNA				
Czechoslovakia	2	130	0·87	178
Czechoslovakia, Poland and E. Yugoslavia	4	450	0·82	77

[a] Based on this work and Ferris *et al.* (1983*a*, *b*).
[b] Number of restriction sites examined per mtDNA; at Swedish locality 8 (Fig. 1) only 90 sites were examined.

with all 11 enzymes. By contrast, the Swedish lineage has diverged by about 5% from that of authentic *musculus* mtDNA (Ferris *et al.* 1983*a*, *b*).

Electrophoretic analysis of proteins

The enzymes of the Swedish mice had electrophoretic mobilities identical with those of authentic *musculus* (from Brno) at all the studied loci. Fixed allelic differences were found between the Swedish mice and *domesticus* (represented by the laboratory strain C57BL/6) at the diagnostic loci *IDH-2* and *PGM-2* (Hunt & Selander, 1973). The Swedish mice thus appear to have mtDNA similar to that of *domesticus* although they have predominantly *musculus* nuclear genes. The transition in allozyme frequencies and mtDNA type going from the hybrid zone in Denmark and north through Sweden is illustrated in Fig. 2. This transect shows the very narrow hybrid zone for nuclear genes, with 90% of the transition occurring over a distance of about 50 km. By contrast, *domesticus*-like mitochondrial DNA differing only slightly from that in other parts of western Europe is found about 750 km into *musculus* territory.

Discussion

Age of hybrid zone

Mus domesticus and *Mus musculus* are presumed to have reached Europe by different paths, connected to the spread of human grain culture (van Zegeren & van Oortmerssen, 1981, and references therein). Assuming that *domesticus* came via North Africa and Spain with 'West-Mediterranean' cultures and *musculus* from East Europe and the 'Danube' cultures (Balkan), the hybrid zone in Central Europe could be 5000–6000 years old.

Colonization of Scandinavia by mice

The association between commensal mice and man was probably already established in preagricultural times (Ferris *et al.* 1983*b*). This makes it likely that the colonization of Sweden by mice is related to the spread of early farming into Sweden, 3500–4500 years ago (Clark, 1975).

To explain the presence of *domesticus* type mtDNA in the mice with *musculus* nuclear genes in Scandinavia we favor an hypothesis based on a founder effect during the first colonization of Sweden and Norway. This and another explanation for the interspecific transfer of mtDNA are discussed below.

Founder event

According to the founder hypothesis, agriculture moved north through Germany bringing both species of mice and the hybrid zone. As suggested in Fig. 1, the place at which the hybrid zone met the Baltic Sea was in East Holstein. This is exactly where the hybrid zone now meets the Baltic Sea, close to the island of Fehmarn (van Zegeren & van Oortmerssen, 1981). The nearby islands were quickly populated by *musculus* and hybrid mice from parts of East Holstein near Fehmarn (Fig. 1). Slightly later, we suppose, Southern Jutland was colonized by *domesticus* mice. At the same time, Northern Jutland and Sweden were colonized from these islands before *domesticus* populations could get there.

The colonization of Sweden would thus appear to have proceeded in several steps (from island to island, see legend of Fig. 1), and each step may have been associated with a founder event. Furthermore, this hypothesis involves the assumption that the female colonists were hybrids or, more specifically, backcross individuals carrying exclusively *domesticus* mtDNA and predominantly *musculus* nuclear DNA. Given the close location of these islands to the hybrid zone and the capacity for females from F_1 crosses between species to reproduce, this appears to be a reasonable assumption.

The presence of two lineages, type A and B, both members of the clade shown in fig. 11 of Wilson *et al.* (1985), in N. Jutland, indicates that at least two females were involved in the colonization event. The sequence difference (0·2%) between these types indicates that they diverged about 100000 years ago, well before these types entered Scandinavia.

The results of both the mtDNA and protein analysis render support for a founder effect. A comparison of the mtDNA sequence variation in populations of mice from 14 geographic regions of the world appears in Table 1. The total lack of mtDNA variation both within and among Swedish mouse populations is conspicuous and contrasts sharply with estimates for other European, African and North American populations, both for *domesticus* and *musculus*; the small divergence among Scandinavian mouse populations was also noted by Takahata & Palumbi (1985) based on the data of Ferris *et al.* (1983*b*).

The average heterozygosity for the 10 nuclear isozyme genes appears lower in the Swedish populations (only *ADH* polymorphic: $H = 0{\cdot}05$ for localities 1–7) compared to that of Danish *musculus* populations (the same set of loci; $H = 0{\cdot}156$, Selander *et al.* 1969). Although based on a small number of loci this difference between Danish and Swedish populations is indicative of a previous bottleneck in the Swedish populations.

The archaeological evidence indicates that agriculture was introduced to Scandinavia from the south and spread rapidly over a period of only a few human generations (B. Arrhenius, pers. comm.; Clark, 1975). The first farming populations were most certainly quite small, and may have originated from a single settlement. Thus, it is possible that only a limited number of mice were introduced, all descendants of a single female and thus monophyletic with respect to mtDNA.

It is not surprising to find that mtDNA can function on a nuclear background of another species. By backcrossing of hybrids between *Mus domesticus* and *Mus spretus*, we have constructed mouse strains that have mtDNA of one species on the nuclear background of the other (Gyllensten *et al.* 1985). No decrease in viability and fertility was noted in these backcross strains although the two species differ by about 2000 mutations in their mtDNA and 0·3 electrophoretically detectable substitutions per polypeptide encoded by nuclear genes. This indicates that mtDNA can function on the nuclear background of even very remote species.

Persistent asymmetric flow of mtDNA?

An alternative to the founder hypothesis is that mtDNAs have flowed persistently across the hybrid zone and largely in one direction. MtDNA from *domesticus* therefore slowly replaced the mtDNA of *musculus*, especially in areas where *musculus* is isolated from other populations of its own species, such on the Sweden–Norway peninsula. *Domesticus* nuclear genes have been found at a greater distance into *musculus* territory than vice versa (Hunt & Selander, 1973; Fig. 3, this paper) and *domesticus* have been shown to be more aggressive in confrontations between the two species under controlled conditions (van Zegeren & van Oortmerssen, 1981). Thus, *domesticus* appears to be more successful in spreading its genes into the neighbouring species. Could such an asymmetrical gene flow account for the observed mtDNA pattern?

This can be evaluated by calculating the migration rate from the apparent rate of flow of nuclear genes. Assuming equilibrium conditions the maximum possible migration rate (m) between species can be estimated from the relation $I = m/(m+v)$, where I is Nei's (1972) genetic identity and v the substitution rate per locus per generation for electrophoretic loci (Nei, 1975). The electrophoretic estimate of genetic identity ($I = 0{\cdot}836$, 41 loci, Selander *et al.* 1969) between the species in Denmark translates into a migration rate of the order of $5v$ using this equation. With a substitution rate (v) of 10^{-7} for electrophoretic loci (Nei, 1975), this means an absolute migration rate of 5×10^{-7} per generation or $4 \times 5 \times 10^{-7}$ per year assuming 4 mouse generations per year. The model for mitochondrial gene flow between species by Takahata (1985) suggest that the time for replacement of one mtDNA type by another is on the order of the inverse of the migration rate, or in our case $1/(2 \times 10^{-6}) = 500000$ years. This time span should be compared to the maximum age of mouse habitats in Scandinavia of 5000 years. Evidently, our model of mtDNA flow in one direction is inadequate to explain the observed pattern of mtDNA variation unless the rate of migration of mtDNAs exceeds that of nuclear genes by a factor of 100. The latter possibility receives no support from a study of the zone of contact between *domesticus* and *musculus* in southern Germany (Sage *et al.*, 1986).

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