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A steep cline for mitochondrial DNA in Danish mice

F. VANLERBERGHE[1], P. BOURSOT[1]*, J. T. NIELSEN[2] AND F. BONHOMME[1]
[1] *Laboratoire de génétique, Institut des Sciences de l'Evolution (UA. CNRS 327), U.S.T.L., Place E. Batailon, 34060 Montpellier Cedex France*
[2] *Institute of Molecular Biology and Plant Physiology, University of Aarhus, Aarhus Denmark*

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Summary

One hundred and ninety-eight mice trapped along a south–north transect through the Danish hybrid zone between *Mus musculus domesticus* and *M. m. musculus* were typed for mitochondrial DNA (mtDNA), the *Y* chromosome and ten autosomal loci encoding diagnostic proteins. The southern (*domesticus*) populations display two mtDNA variants (S1 and S2) and the northern (*musculus*) have a third mtDNA variant (N) of *domesticus* origin. Across the hybrid zone defined by ten autosomal loci, there is a steep cline between the southern and northern types of mtDNA. As well as confirming an earlier finding that Danish *musculus* all have a *domesticus* mtDNA (Ferris *et al.* 1983*a*, & *b*), our results show that this mtDNA takeover is not the result of a persistent mitochondrial gene flow between the two subspecies. While the coincident clines for the ten autosomal loci and the abrupt cline for the *Y* chromosome can be explained by selection, it is less likely to be the case for the mtDNA exchanges. We discuss the possible role of sex-linked migration and genetic drift to account for the distribution of the mitochondrial variants.

1. Introduction

Population geneticists are showing an increasing interest in mitochondrial DNA (mtDNA) because the molecular, genetic and evolutionary properties of this genome are now well known (for a review see Wilson *et al.* 1985; Avise, 1986; Boursot & Bonhomme, 1986). Comparisons of mtDNA are particularly valuable for studying introgression between differentiated gene pools because the evolutionary history of the variants can be traced. Interspecific mitochondrial introgression or complete transfer, accompanied by traces or no nuclear introgression, has been shown to occur in flies (Powell, 1983; Solignac & Monnerot, 1986), mice (Ferris *et al.* 1983*a*), crickets (Harrisson *et al.* 1986), voles (Tegelström, 1987), frogs (Spolsky & Uzzell, 1986; Lamb & Avise, 1986; Szymura & Barton, 1986) and deer (Carr *et al.* 1986).

The two European subspecies of mice *Mus musculus domesticus* and *M. m. musculus* are known to interact along a narrow zone of hybridization that crosses Europe from Denmark to Bulgaria (Hunt & Selander, 1973; Bonhomme *et al.* 1983; Boursot *et al.* 1984; Sage *et al.* 1986*a*, *b*). Restriction analysis of mtDNA showed that despite considerable polymorphism the mitochondrial variants fell into two distinct lineages that generally fitted the subspecies classification (Yonekawa *et al.* 1982; Ferris *et al.* 1983*b*; Boursot *et al.* 1984). An exception was found in Denmark and Sweden where mice which are typically *musculus* on the basis of nuclear markers have all fixed a mtDNA that belongs to the *domesticus* lineage (Ferris *et al.* 1983*a*). Gyllensten & Wilson (1987) showed that this mitochondrial introgression extends into Sweden for at least 750 km beyond the Danish hybrid zone. The low level of polymorphism both in the mitochondrial and nuclear DNA in this area led these authors to suggest that the transfer of mtDNA from *domesticus* to *musculus* was due to a founder event rather than persistent mtDNA flow. They speculated that this event was related to the spreading of farming about 4000 years ago.

We have devised a direct test of the founder event hypothesis as opposed to persistent mtDNA flow across the hybrid zone to explain the mtDNA transfer between the subspecies in Denmark. This was done by studying the types of mtDNA present in a transect across the Danish hybrid zone and comparing them with the allelic variation of the *Y* chromosome and ten autosomal loci coding for proteins that distinguished between the two subspecies. Although *domesticus*-like mtDNA prevails all over Scandinavia, we show that

* Corresponding author

there is a clearcut differentiation of this genome across the Danish hybrid zone defined on the basis of nuclear genes.

2. Materials and methods

(i) *Mice*

The 198 Danish mice examined were trapped in the wild in October 1984 and October 1985. Eleven of the sampling localities designated **a** to **k** (Fig. 1) lie along a transect across the introgression zone delineated by Hunt and Selander (1973), on the basis of nuclear genes. The twelfth sample (**l**) comes from Fyn island. The names of the localities appear in Table 1.

(ii) *Protein electrophoresis*

Standard techniques such as those described in Pasteur *et al.* (1987) were used for protein electrophoresis. We studied the ten following enzyme loci which are known to be diagnostic between the two subspecies (Hunt & Selander, 1973; Bonhomme *et al.* 1984; Boursot *et al.* 1984): *Es1*, *Es2*, *Pgm1*, *Sod1*, *Mpi1*, *Np1*, *Gdp1*, *Idh1*, *Amy1* and *Es10*. The Friedman two-way analysis of variance (Siegel, 1956) was used to compare the allele frequencies distribution at the different loci in the twelve localities. The samples are considered *domesticus*-like or *musculus*-like depending on the frequencies of alleles present at these ten loci. To do this we computed a hybrid index I for each locality sampled using $I = 100\ L^{-1}\ \Sigma fi$ where L is the number of loci and fi is the frequency of *domesticus* alleles at the ith locus. This hybrid index ranges from 100% for 'pure' *domesticus* populations to 0% for 'pure' *musculus* populations.

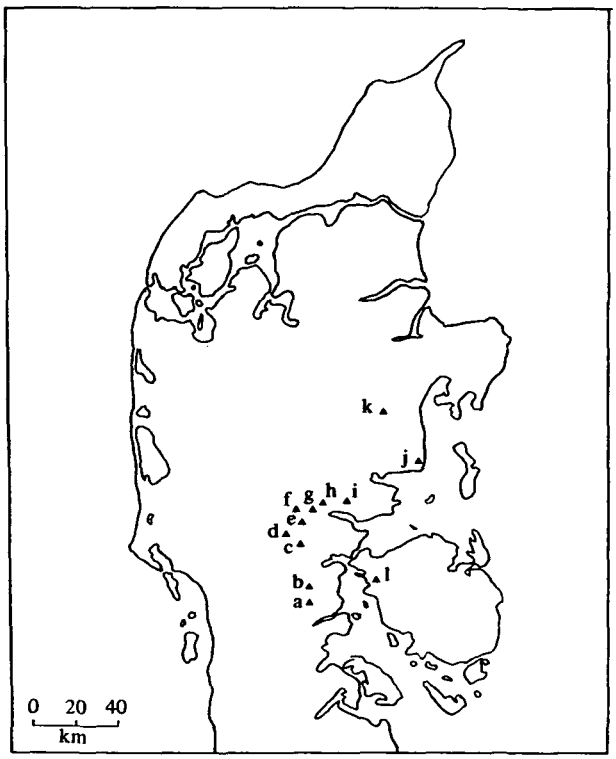

Fig. 1. Map showing the 12 sampling localities. Samples **a** to **k** represent a south–north transect in Eastern Jutland. **l** corresponds to a sample from the Fyn island. The names of localities are given in Table 1.

(iii) *mtDNA comparisons*

The mtDNAs of 161 mice from samples **a** to **k** were examined. They were extracted from single animals as described in Boursot *et al.* (1987) and then digested with the restriction enzymes *Eco*RI, *Pst*I, *Bam*HI, *Hae*II and *Hind*II (Boehringer Mannheim). These enzymes allow mtDNA variants that belong to the *domesticus* lineage to be distinguished from those belonging to the *musculus* lineage (Yonekawa *et al.* 1982; Boursot *et al.* 1984). The fragments were separated electrophoretically in 1% agarose gels and visualized under UV light after ethidium bromide staining and their size estimated by comparison with the known sizes of the fragments obtained when the λ phage DNA is digested with *Hin*dIII. The precise location of each cleavage site was inferred from the known sequence of mouse mtDNA (Bibb *et al.* 1981) and the published maps (Ferris *et al.* 1983*b*).

The correlation between the levels of mitochondrial and autosomal introgression was estimated by the Spearman rank correlation coefficient (Siegel, 1956).

(iv) Y *chromosome comparisons*

Extraction, preparation and digestion of nuclear DNAs, transfer (Southern blot), hybridization with the specific probe PY/353B (kindly provided by C. Bishop, Institut Pasteur) and distinction between *domesticus* and *musculus* variants were conducted as described in Vanlerberghe *et al.* (1986).

3. Results

(i) *Protein comparisons*

Table 1 gives the number of *domesticus* alleles observed at each locus in the different localities which are ordered along the south-north transect. All the loci show a sharp cline in allele frequencies. A non-parametric statistical test, the Friedman test, did not reveal any significant differences in the distribution of allele frequencies at these loci when the different localities were compared ($\chi^2 = 1{\cdot}17$, D.F. = 9). This means that at a given locality the variation of the level of introgression between loci is a matter of chance. So, we calculated the hybrid index I, which represents the degree of introgression of each sample averaged over the ten loci. The two samples **e** and **f** that show strong levels of introgression at each locus have an index of 49 and 51%, respectively, and correspond to the

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Table 1. *Number of* M. m. domesticus *alleles for each autosomal locus, and numbers of mtDNAs and* Y *chromosomes of each type found in every locality sampled.*

Locality (from south to north)	Number[a] of mice	Autosomal loci[b]											mtDNA[c]			*Y*[d]	
		Es1	*Es2*	*Pgm*	*Sod*	*Mpi*	*Np1*	*Gpd*	*1dh*	*Amy*	*Es10*	*I* (%)	S1	S2	N	d	m
a Simmersted	2	4	4	4	4	4	4	4	3	3	4	95	2	0	0	0	0
b Ødis	16 (2)	32	28	28	31	16	32	32	22	32	(4)	89	2	12	1	6	0
c Egtved	42 (21)	83	82	75	82	80	67	84	81	74	(42)	94	40	0	0	18	2
d Törskind	21 (15)	34	42	42	42	(30)	28	39	42	35	42	92	15	0	0	4	0
e Bredsten	5 (2)	6	4	6	6	(1)	7	(1)	(2)	7	(1)	49	1	0	1	1	0
f Södover	10 (6)	8	11	16	12	(4)	8	(2)	(7)	8	(4)	51	6	0	0	0	1
g Brandbjerg	4 (3)	0	1	1	3	(0)	1	(4)	(2)	3	1	22	0	0	3	0	1
h Vindelev	12 (6)	5	3	11	6	(5)	5	(6)	(3)	5	1	24	0	0	6	0	2
i Lösning	25 (8)	12	15	12	12	7	3	9	12	6	(5)	21	2	2	18	0	9
j Hov	25 (5)	1	2	1	1	0	0	1	0	0	(0)	1	0	0	24	0	17
k Klank	10	0	0	0	1	0	9	0	0	2	0	6	0	0	9	0	4
l Fyn	26 (17)	0	1	0	0	(0)	7	(0)	(0)	0	1	2	0	0	17	0	4

[a] The number of mice trapped in each locality and for which genotype was determined.
[b] Gives the number of *domesticus* alleles found at each locus. The numbers in parentheses indicate that for certain autosomal loci a smaller sample of mice was analysed.
[c] The number of mice with mtDNA variants N, S1 or S2.
[d] The number of mice with (d) a *domesticus* or (m) a *musculus Y* chromosome. The number of *Y* chromosomes studied does not represent the real number of males trapped in each sample because of a bias due to degraded DNAs. Data published in Vanlerberghe *et al.* (1986) are also included.

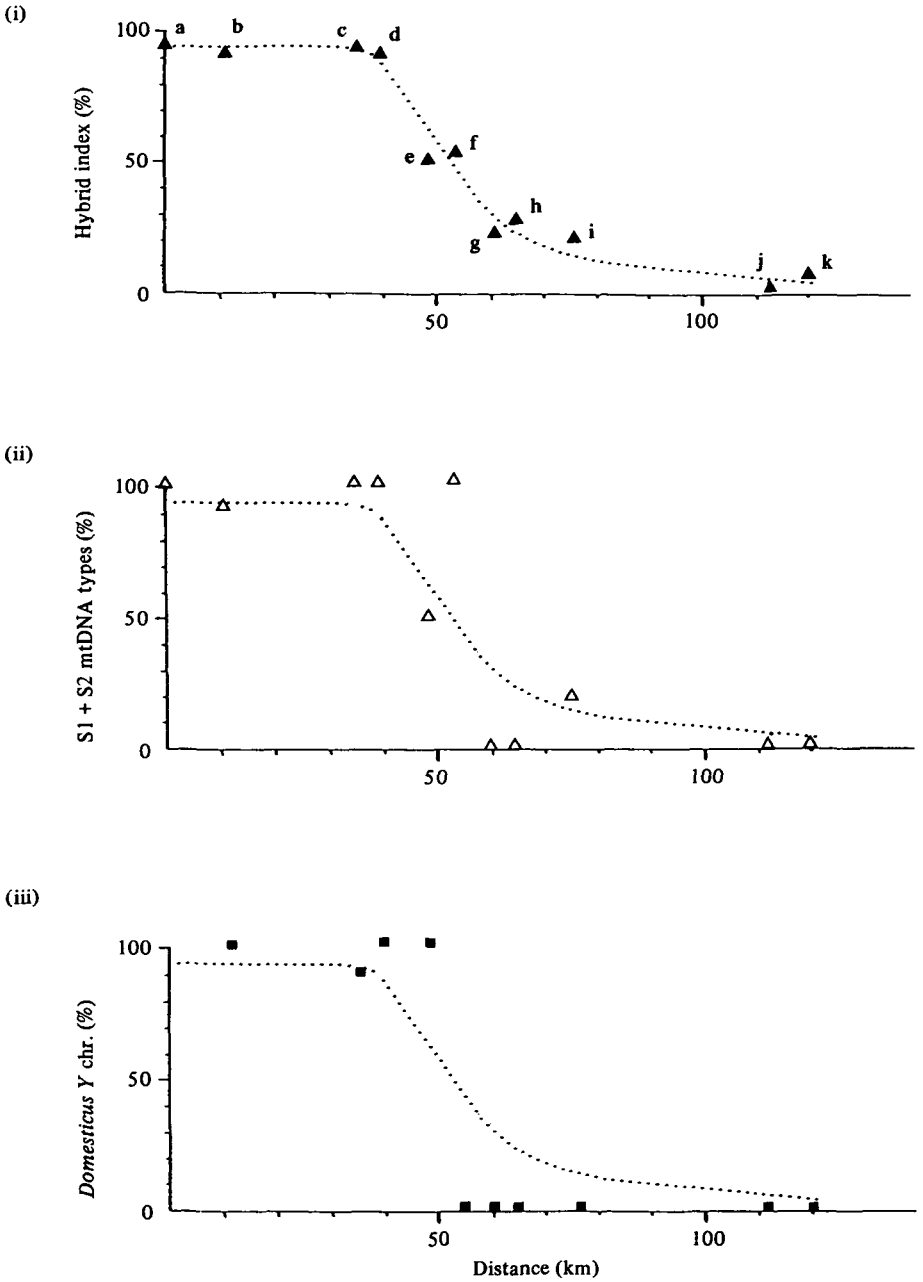

Fig. 2. (i) Cline showing the variation of the autosomal hybrid index *I* (black triangles) in localities **a** to **k**. Locality **l** is excluded from this figure as it is not on the south–north transect. (ii) Percentage of mtDNA types S1 and S2 (white triangles) and (iii) percentage of *domesticus* *Y* chromosome (black squares) for each locality superimposed on the autosomal hybrid index cline (dotted line).

centre of the hybrid zone. The other samples can be characterized as either *domesticus*-like (localities **a** to **d**) or *musculus*-like (localities **g** to **l**). The variations of the hybrid index along the transect form a stepped cline (Fig. 2) that shows that most of the allelic transition from *domesticus* to *musculus* occurs over a distance of 40 km. As this cline can be superimposed on that found earlier by Hunt & Selander (1973) in eastern Jutland, one can conclude that the position of the hybrid zone has not changed since their study.

Figure 3 shows the distribution of the introgression among the individuals making up each sample. The attribution of some individuals to either subspecies can be ambiguous, especially in the localities near the centre of the zone. We never found samples in which *domesticus* and *musculus* individuals, pure or nearly so, coexist, and no F_1 hybrid has ever been detected. All the individuals have highly recombined genotypes.

(ii) *mtDNA comparisons*

Only one morph can be detected with the restriction enzymes *Pst*I and *Eco*RI on the mtDNAs examined, and it corresponds to one which has been found in other regions occupied by *domesticus* populations (Yonekawa *et al.* 1982; Boursot *et al.* 1984). This confirms that the Danish *musculus* have a mtDNA which belongs to the *domesticus* lineage. The enzymes

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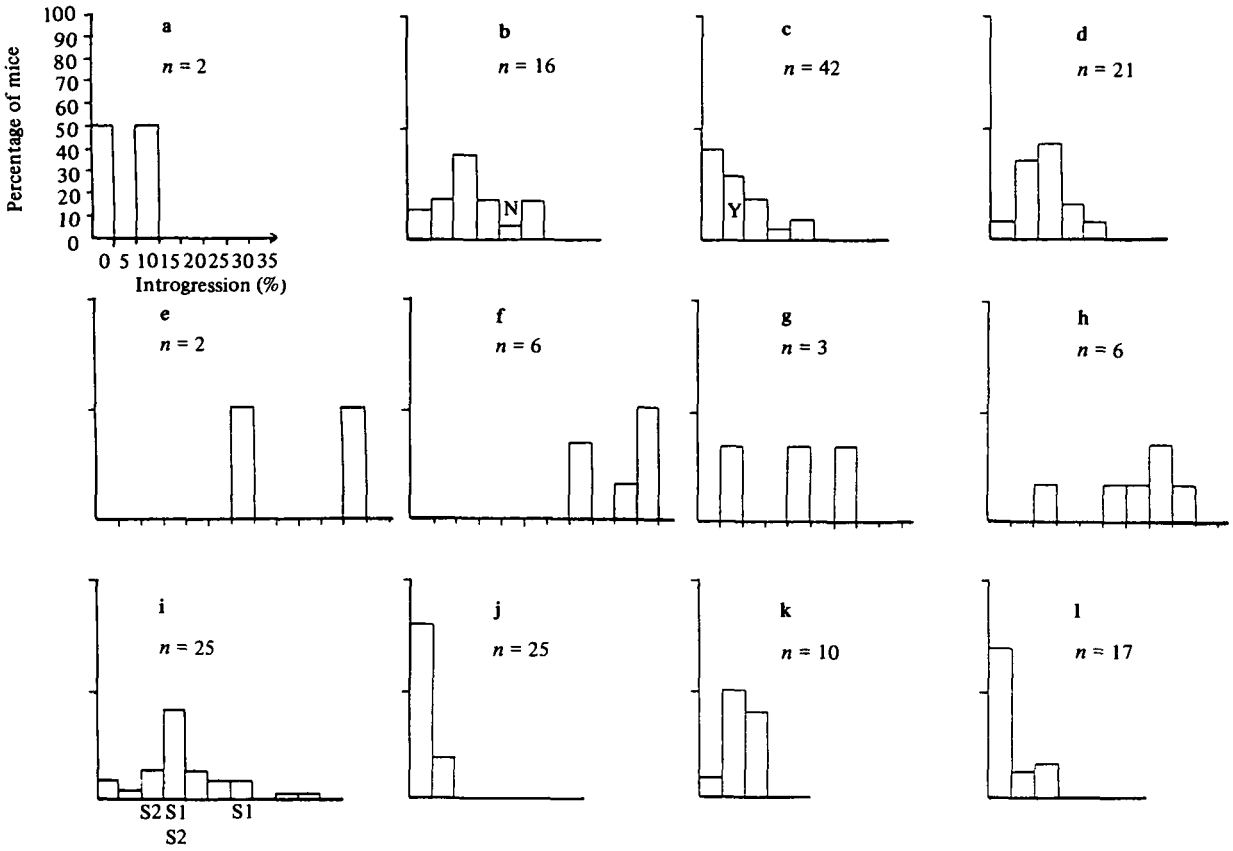

Fig. 3. Histograms showing the distribution of the individuals as a function of the percentage of autosomal introgression in each locality. For samples **a** to **f** the autosomal introgression is given as the percentage of *musculus* alleles and for samples **g** to **l** as the percentage of *domesticus* alleles. The degree of autosomal introgression of the few individuals which are introgressed for mitochondrial genome or the *Y* chromosome is indicated by S1, S2 and Y. (See Table 1 for details.)

*Bam*HI, *Hae*II and *Hin*dII revealed 5 polymorphic sites. We observed three patterns when mtDNAs were digested with *Bam*HI, two with *Hae*II and two with *Hin*dII. Table 2 lists the constant and variable sites for each restriction enzyme and the sizes of the fragments obtained. The observed combinations of these different patterns define the three mtDNA types found in our Danish sample (Table 2). It can be seen from Table 1 that in all the localities characterized as *musculus* on the basis of nuclear gene markers, one type, which we refer to as type N ('north'), predominates, while in the *domesticus* localities two other types, that we call types S1 and S2 (for 'south'), predominate. Type N is found in only one *domesticus* locality (Ødis, one out of 15 individuals). In the *musculus* territory, types S1 and S2 only occur in locality **i** (Lösning, two individuals each out of 22). The only locality at which S2 predominates is **b** (Ødis). The class of autosomal introgression to which the individuals carrying the 'wrong' mtDNA types belong is noted in Fig. 3.

Figure 2 shows that for most of the localities the mitochondrial frequencies do not fit the hybrid index well. The Spearman test reveals that the mitochondrial and autosomal introgression frequencies are not significantly correlated ($rs = 0{\cdot}45$, D.F. = 10, non-significant at the level $\alpha = 0{\cdot}01$).

(iii) Y *chromosome comparisons*

Table 1 shows that almost all the males have the *Y* chromosome variant belonging to the subspecies they are related to. As described in the previous study (Vanlerberghe *et al.* 1986), the *Y* chromosome introgression is very limited. Figure 2 shows that the cline for the *Y* chromosome is steeper than both mtDNA and autosomal clines. Among the 69 males, the two which come from the localities **e** and **f** in the centre of the hybridization zone cannot be related to either subspecies because their genome is a mixture of nuclear alleles from both subspecies. For instance, the male from locality **f** (Södover) has 11 *domesticus* autosomal alleles, mtDNA type S1 and a *musculus Y* chromosome (Table 1).

Out of the 67 remaining males only two from locality **c** (Egtved, *domesticus*) have a *Y* variant that does not correspond to their subspecies assignment. The level of autosomal introgression of these two males is noted in Fig. 3.

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Table 2. *Location of restriction sites and definition of the three mtDNA variants in Danish mice*

	Location of site				Presence of site[d]		
Enzyme	Site	Start[a]	Sequence[b]	Region[c]	South1	South2	North
					A	A	A
*Pst*I	1	8420	CTGCAG	ATPase 6	+	+	+
	2	12232	CTGCAG	ND 5	+	+	+
					A	A	A
*Eco*RI	1	1750	GAATTC	16S rRNA	+	+	+
	2	3803	GAATTC	Gln tRNA	+	+	+
	3	4013	GAATTC	ND 2	+	+	+
					A	E	I
*Bam*HI	1	3222	GGATCC	ND 1	+	+	−
	2	3566	GGATCC	ND 1	+	+	+
	3	4275	GGATCC	ND 2	+	−	+
	4	11167	GGATCC	ND 4	+	+	+
	5	14451	GGATC (A–C)	Cyt B	−	−	+
					A	A	D
*Hae*II	1	2603	AGCGCT	16S rRNA	+	+	+
	2	11471	AGC(C−G)CT	ND 4	−	−	+
					A	A	C
*Hin*dII	1	364	GTCAAC	12S rRNA	+	+	+
	2	1858	GTTAAC	16S rRNA	+	+	+
	3	5123	GTTAAC	Asn tRNA	+	+	+
	4	5452	GTCAAC	CO I	+	+	+
	5	7718	GTTAAC	Lys tRNA	+	+	+
	6	8637	GTTAA (T-C)	CO III	−	−	+

[a] Bases are numbered as in Bibb *et al.* (1981).
[b] Base changes are indicated in parentheses, the first base being replaced by the second one.
[c] Functional regions of the mouse mitochondrial genome where sites are located. ATPase 6, gene coding for the subunit 6 of the ATPase complex; cyt B for the cytochrome b; CO I, CO II and CO III, for the cytochrome c oxidase subunit I, II or III; ND 1 to 5 for components of the respiratory-chain NADH dehydrogenase (Chomyn *et al.* 1986); 12S rRNA and 16S rRNA, ribosomal RNA genes coding for RNA 12S or 16S; Gln tRNA Asn tRNA and Lys tRNA, transfer RNA genes identified by the amino-acid code.
[d] Each fragment pattern is designated by a capital letter. The sizes of the fragments in each pattern are *Pst*I (A) 12483, 3812; *Eco*RI (A) 14032, 2053, 210; *Bam*HI (A) 8350, 6892, 709, 344; *Bam*HI (E) 8350, 7601, 344; *Bam*HI (I) 6892, 5410, 3284, 709; *Hae*II (A) 16295; *Hae*II (D) 8868, 7427; *Hin*dII (A) 8941, 3265, 2266, 1494, 329; *Hin*dII (C) 8022, 3265, 2266, 1494, 919, 329.

4. DISCUSSION

The main result of our study is that although both the Danish *domesticus* and *musculus* mice have mtDNAs which belong to the *domesticus* lineage, there is a clearcut mtDNA differentiation across the hybrid zone. One should note that using the same five restriction enzymes we found type N mtDNA with a frequency of 9% inside an authentic *domesticus* sample from Bulgaria (Boursot *et al.* 1984; Vanlerberghe *et al.* in press). Similarly, Ferris *et al.* (1983*a*) found one of their Danish northern morphs in *domesticus* populations from Italy. We tried to situate the three morphs we found in Denmark within the *domesticus* mtDNA phylogeny constructed by Ferris *et al.* (183*a*, *b*) from a worldwide *domesticus* sample analysed with 11 restriction enzymes. Figure 4 shows that the three mtDNA types we found in Denmark could belong to three different major phylogenetic clusters defined by Ferris *et al.* (1983*a*). Therefore, the presence of these three mitochondrial types in Denmark cannot be the result of an *in situ* evolution from a local common ancestor but are more likely to reflect a polymorphism that already existed in the colonizers of this region.

The mouse settlements in Europe are presumed to have accompanied the establishment of human grain culture which spread in two directions: one from central Asia via eastern Europe along the Danube with the *musculus* mice and the other from northern Africa into Spain and south France carrying the *domesticus* mice. The contact between the two subspecies in Europe probably occurred when the Mediterranean and eastern European farming traditions met around 5000 B.C. Gyllensten & Wilson (1987) proposed that at that time the *musculus* populations had not yet reached Scandinavia. These authors also suggested that Swedish and Norwegian mouse populations were founded by a few backcross individuals carrying nuclear *musculus* genome and a

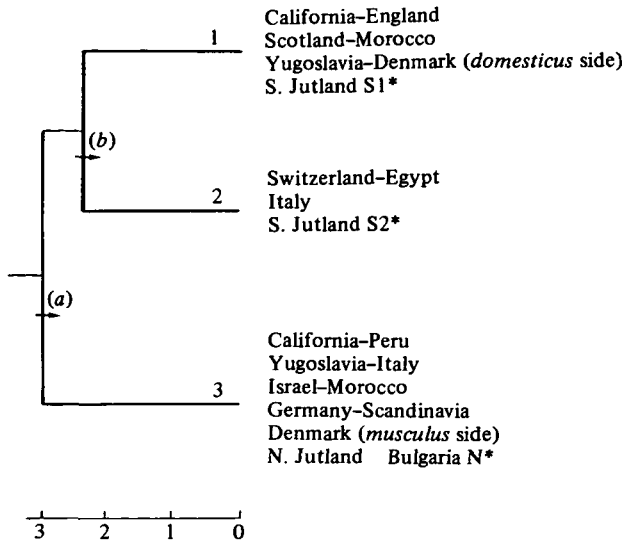

Fig. 4. Tree illustrating the possible relationships of our three mtDNA types (indicated by *) with respect to three mitochondrial *domesticus* phylogenetic lineages proposed in figs. 2 and 3 of Ferris *et al.* (1983*a*). Of the five mutational events we detected two can be placed on this tree. (*a*) Creation of a *Hin*dII site at position 8637 which puts the N mtDNA type into group 3. (*b*) Loss of a *Bam*HI site starting at position 4275 which presumably corresponds to the loss of the *Mbo*I site at position 4276 detected by Ferris *et al.* showing that the S2 type belongs to the group 2. We suggest that S1 type belongs to group 1 which includes all the mtDNA which possess the *Mbo*I site and lack the *Hin*dII site.

domesticus mtDNA, with the colonization proceeding step by step from East Holstein (Germany) via the Baltic islands to Sweden. In Denmark, no *musculus* type mtDNA has ever been found, either in our study or in Ferris *et al.*'s (1983) study. Moreover, there is a mtDNA differentiation south and north of the contact zone. So the most reasonable hypothesis is that the mitochondrial takeover in Northern Danish mice is not the result of a persistent gene flow across the hybrid zone, but rather the result of a founder event that happened before the establishment of the clearcut hybrid zone that now exists. Considering the predominance of the mtDNA types south and north of the contact zone, it is more parsimonious to admit that the low, local occurrence of the S1 and S2 types in the *musculus* sample **i** (both at a frequency of 2·5%) are the result of a recent mitochondrial introgression rather than the residual traces of an ancestral polymorphism related to the colonization event.

The concordance of the ten autosomal clines reflects the action of selection at many loci and can be considered to be the result of a balance between selection and dispersion (see Barton & Hewitt, 1985 for a review). The heterozygote combinations that were generated during the hybridization process between the two subspecies were probably under strong selective pressures because of the disruption of differentiated coadapted gene systems (Hunt & Selander, 1973; Vanlerberghe *et al.* in press). Sage *et al.* (1986) proposed that the increased susceptibility to parasitic infection of mice in the hybrid zone could be the result of the disruption of the co-adapted gene systems involved in resistance. As in our study their data on autosomal markers revealed only highly recombined genotypes. This suggests that the only genes that can 'escape' from the hybrid zones are those that are neutral or nearly so and have been excised from gene systems under selection after several generations of meiotic recombination. If this is the case the extent of introgression would depend on the migration rate. A good example of such a process is the two loci *Es1* and *Es2*. Although they are only 8 cM apart on chromosome 8, they are rarely introgressed together in the same individual. The non-introgression of the *Y* chromosome fits this hypothesis of coadapted gene systems. As it recombines very little the neutral genes that it might carry are prevented from introgression (Vanlerberghe *et al.* 1986).

The integration of a *domesticus* mtDNA variant in populations of mice with a predominantly *musculus* nuclear genome suggests that mtDNA is not directly involved in a system of interactions with nuclear genes responsible for the partial reproduction isolation (Barton & Jones, 1983). It is therefore difficult to believe that mtDNA exchanges are being strongly selected against across Danish hybrid zone. The major difference between mtDNA and autosomal markers is that mtDNA is only transmitted via the females. This maternal transmission could explain why mtDNA introgresses less than the autosomal DNA in Denmark. The study of Singleton & Hay (1983) showed that the reproductive *domesticus* females generally restrict their movements to within the territorial boundaries of a single dominant male, and Butler (1980) found that 75% of the animals that emigrated were males. These behavioural traits might contribute to the reduced mitochondrial introgression we observe. Another effect of female transmission is to reduce the effective population size as far as mtDNA is concerned and make it more sensitive to genetic drift than the autosomal genes. This could explain the predominance of the mtDNA type S2 (80%) in the *domesticus* locality **b**, and the presence of only the S1 type in the hybrid population from locality **f**. As at the southern end of the hybrid zone (Bulgaria), we found substantial *musculus* mtDNA introgression into the *domesticus* territory (Vanlerberghe *et al.* in press), the possibility that the distinctive patterns of introgression we observed are due to chance must be considered. The frequencies of mitochondrial introgression could change markedly over a small number of generations in populations of mice close to the hybrid zone simply as the result of drift. In the other cases where the dynamics of autosomal and mitochondrial DNA exchange across active hybrid zone have been studied (Szymura & Barton, 1986; Nelson *et al.* 1987), these

two types of markers were shown to have relatively similar patterns of introgression. Therefore, although mtDNA is more sensitive to founder effects and is supposed to be neutral, it is not necessarily more prone to flow across a hybrid zone than autosomal markers.

This raises the question of why the cases of takeover reported by population geneticists so far only concern the mitochondrial genome (see Introduction). It is perhaps related to the fact that if an mtDNA takeover between two closely related taxa exists, it is sure to be detected. If the methods which allow one to retrace the phylogeny of the mtDNA variants and to assess their taxonomic relationships are used for nuclear DNA it might be possible to show that certain nuclear alleles have also taken over as a result of a founder event.

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References

Avise J. C. (1986). Mitochondrial DNA and the evolutionary genetics of higher animals. In *The Evolution of DNA Sequences* (ed. B. C. Clarke, A. Robertson and A. J. Jeffreys). *Proceedings of the Royal Society of London*, **312**; 325–342

Barton, N. H. and Hewitt, G. M. (1985). Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16**; 113–148.

Barton, N. H. & Jones, J. S. (1983). Mitochondrial DNA: new clues about evolution. *Nature* **306**; 317–318.

Bibb, M. J., Van Etten, R. A., Wright, C. T., Walberg, M. W. & Clayton, D. A. (1981). Sequence and gene organization of mouse mitochondrial DNA. *Cell* **26**; 167–180.

Bonhomme, F., Catalan, J., Gerasimov, S., Orsini, P. & Thaler, L. (1983). Le complexe d'espèces du genre *Mus*. en Europe centrale et orientale. I. Génétique. *Zeitung für Säugetierkunde* **48**, 78–85.

Bonhomme, F., Catalan, J., Britton-Davidian, J., Chapman, V. M., Moriwaki, K., Nevo, E. & Thaler, L. (1984). Biochemical diversity and evolution in the genus *Mus*. *Biochemical Genetics* **22**, 275–303.

Boursot, P., Bonhomme, F., Britton-Davidian, J., Catalan, J., Yonekawa, H., Orsini, P., Gerasimov, S. & Thaler, L. (1984). Introgression différentielle des génomes nucléaires et mitochondriaux chez deux semi-espèces européennes de souris. *Comptes Rendus de l'Académie des Sciences, Paris (série III)* **299**, 691–693.

Boursot, P. & Bonhomme, F. (1986). Génétique et évolution du génome mitochondrial des Métazoaires. *Génétique Sélection Evolution* **18**, 73–98.

Boursot, P., Yonekawa, H. & Bonhomme, F. (1987). Heteroplasmy in mice with deletion of a large coding region of mitochondrial DNA. *Molecular Biology and Evolution* **4**, 46–55.

Butler, R. G. (1980). Population size, social behaviour, and dispersal in house mice: a quantitative investigation. *Animal Behaviour* **28**, 78–85.

Carr, S. M., Ballinger, S. W., Derr, J. N., Blankenship, L. H. and Bickham, J. W. (1986). Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west Texas. *Proceedings of the National Academy of Sciences, USA* **83**, 9576–9580.

Chomyn, A., Mariottini, P., Cleeter, M. W. J., Ragan, C. I., Matsuno-Yagi, A., Hatefi, Y., Doolittle, R. F. & Attardi, G. (1985). Six unidentified reading frames of human mitochondrial DNA encode components of the respiratory-chain NADH dehydrogenase. *Nature* **314**, 592–597.

Ferris, S. D., Sage, R. D., Huang, C. M., Nielsen, J. T., Ritte, U. & Wilson, A. C. (1983*a*). Flow of mitochondrial DNA across a species boundary. *Proceedings of the National Academy of Sciences, USA* **80**, 2290–2294.

Ferris, S. D., Sage, R. D., Prager, E. M., Ritte, U. & Wilson, A. C. (1983*b*). Mitochondrial DNA evolution in mice. *Genetics* **105**; 681–721.

Gyllensten, U. & Wilson, A. C. (1987). Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. *Genetical Research* **49**, 25–29.

Harrisson, R. G., Rand, D. M. & Wheeler, W. C. (1987). Mitochondrial variation in field crickets across a narrow hybrid zone. *Molecular Biology and Evolution* **4**, 144–158.

Hunt, W. G. & Selander, R. K. (1973). Biochemical genetics of hybridisation in European house mice. *Heredity* **31**, 11–33.

Lamb, T. & Avise, J. C. (1986). Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. *Proceedings of the National Academy of Sciences, USA* **83**, 2526–2530.

Nelson, K., Baker, R. J. & Honeycutt, R. L. (1987). Mitochondrial DNA and protein differentiation between hybridizing cytotypes of the white-footed mouse, *Peromyscus leucopus*. *Evolution* **41**, 864–872.

Pasteur, N., Pasteur G., Bonhomme, F., Catalan, J. & Britton-Davidian, J. (1987). Manuel technique de génétique par électrophorèse des protéines. In *Technique et Documentation*, 217 pp. Paris: Lavoisier.

Powell, J. R. (1983). Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila*. *Proceedings of the National Academy of Sciences, USA* **80**, 492–495.

Sage, R. D., Whitney, III, J. B. & Wilson, A. C. (1986*a*). Genetic analysis of a hybrid zone between *Domesticus* and *Musculus* mice (*Mus musculus* complex): hemoglobin polymorphisms. In *Current Topics in Microbiology and Immunology* Berlin, Heidelberg: Springer Verlag **127**, 75–85.

Sage, R. D., Heyneman, D., Lim, K. C. & Wilson, A. C. (1986*b*). Wormy mice in a hybrid zone. *Nature* **324**, 60–63.

Siegel, S. (1956). *Nonparametric Statistics for the Behavioural Sciences* 312 pp. New York, Toronto, London: McGraw-Hill Book Company.

Singleton, G. R. & Hay, D. A. (1983). The effect of social organization on reproductive success and gene flow in colonies of wild house mice, *Mus musculus*. *Behavioural Ecology and Sociobiology* **12**, 49–56.

Solignac, M. & Monnerot, M. (1986). Race formation, speciation and introgression within *Drosophila simulans*, *D. mauritiana* and *D. sechellia* inferred from mitochondrial analysis. *Evolution* **40**, 531–539.

Spolsky, C. & Uzzell, T. (1986). Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses. *Molecular Biology and Evolution* **3**; 44–56.

Szymura, J. M. & Barton, N. H. (1986). Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in Southern Poland. *Evolution* **40**, 1141–1159.

Tegelström, H. (1987). Transfer of mitochondrial DNA

from the northern Red-Backed Vole (*Clethrionomys rutilus*) to the Bank Vole (*C. glareolus*). *Journal of Molecular Evolution* **24**, 218–227.

Vanlerberghe, F., Boursot, P., Catalan J., Gerasimov, S., Bonhomme, F., Botev, B. A. & Thaler L. (1988) Analyse génétique de la zone d'hybridation entre les deux sous-espèces de souris *Mus musculus domesticus* et *M. m. musculus* en Bulgarie. *Genome* (In the Press.)

Vanlerberghe, F., Dod, B., Boursot, P., Bellis, M. & Bonhomme, F. (1986). Absence of *Y* chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. *Genetical Research* **48**, 191–197.

Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helm-Bychowski, K. M., Higuchi, G., Palumbi, S. R., Prager, E. M., Sage, R. D. & Stoneking, M. (1985). Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* **26**, 375–400.

Yonekawa, H., Moriwaki, K., Gotoh, O., Miyashita, S., Migita, N., Bonhomme, F., Hjorth, J. P., Petras, M. L. & Tagashira, Y. (1982). Origins of laboratory mice deduced from restriction patterns of mitochondrial DNA. *Differentiation* **22**; 222–226.