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Geographic localization of a contact zone between bank voles *Clethrionomys glareolus* with distinctly different mitochondrial DNA

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Fennoscandian bank voles *Clethrionomys glareolus*, Schreber 1780 show two distinctly different mitochondrial DNA (mtDNA) lineages, one occurring in the southern and central, and another in the northern parts. The mtDNA of northern bank vole populations is very similar or identical to that of the northern red-backed vole *C. rutilus*, Pallas, 1779 and the presence of this mtDNA lineage among northern bank voles probably is a consequence of hybridisation between the two species. To localise the geographic position of the contact zone between bank voles with the two types of mtDNA, we have analysed a transect through the area were both types of mtDNA have been found. The contact zone coincides with two other intraspecific mammalian contact zones, and is adjacent to a third zone. These zones constitute "suture zones", reflecting secondary contact between populations derived from separate glacial refugia and with synchronous timing of secondary contact. The width of the bank vole contact zone, approximately 30–60 km, is consistent with the hypothesis that it is a neutral contact zone. However, because mtDNA may penetrate reproductive barriers easier than nuclear genes it is necessary to analyse the zone with nuclear markers before drawing any definite conclusion regarding the extent of introgression.

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Introduction

The majority of extant species in northern Europe originate from ancestors who survived the last glacial period in refugia south of the ice, recolonising former distribution areas at the beginning of each interglacial (Yalden 1982, Aaris-Sørensen 1988). The last glacial period started about 110 000 years ago, covering at its maximum large parts of northern Europe and the whole of Fennoscandia with ice. Late glacial recolonisation of Scandinavia, starting some 12 000 years before present, may have occurred from two directions: south via a land-bridge connecting Denmark with southern Sweden, and northeast via Russia. As a consequence, Scandinavia may constitute a meeting place for European, Siberian and Arctic

populations, originating from different refugia. Based on geological and fossil data, as well as distribution patterns of present-day species and subspecies, it has been suggested that many mammals have used both recolonisation routes (Nilsson 1820, Ekman 1922, Siivonen 1982, Fredga 1987a, Tegelström 1987, Jaarola and Tegelström 1995) creating contact zones between taxa that had evolved genetic differences while geographically isolated. Such contact zones have long been an object of study for evolutionary biologists because they offer possibilities to evaluate the evolutionary significance of genetic differences between taxa (Hewitt 1988).

Studies of mitochondrial DNA (mtDNA) in bank voles *Clethrionomys glareolus,* Schreber, 1780 and field voles *Microtus agrestis* from northern Europe have demonstrated a macrogeographic pattern supporting the bi-directional colonising hypothesis (Tegelström 1987, Tegelström *et al.* 1988, Jaarola and Tegelström 1995). Among Fennoscandian bank voles there are two distinctly different mtDNA lineages, one occurring in southern and central Fennoscandia and the other in the northern parts. The two lineages show a 12.7% nucleotide sequence divergence (Tegelström 1987), a difference usually found only between distinctly different species. Analysis of mtDNAs from other *Clethrionomys* species present in Fennoscandia shows that the mtDNA of northern bank vole populations is very similar to that of northern red-backed vole *C. rutilus*, a species with a predominantly north Fennoscandian geographic distribution. Hence, the presence of two distinctly different mtDNA lineages within bank vole populations may be a consequence of hybridisation between the two species, probably during the postglacial recolonisation from the north-east 13.000–8.000 years ago.

Initially, hybrid females could have backcrossed to both parental species but interbreeding would have ceased as the two species migrated into different geographic areas. The successful interspecies introgression of mtDNA, from the northern red-backed vole to the bank vole indicates that the bank vole was the predominant species as consistent backcrossing to the paternal species is necessary to increase the probability for mtDNA transfer between species (Hale and Beckenbach 1985) and the number of mtDNA clones from the northern red-backed vole that survived in northern bank vole populations is high. Bank voles colonising from the north moved south until they met the bank voles colonising Scandinavia from the south, creating a contact zone between voles originating from different refugia. This secondary zone of contact thus can be defined by the presence of mtDNA from different species. Such hybrid zones often show selection against hybrids and can act as barriers to gene flow, thus maintaining the subdivision and isolation between the groups.

The present report describes the localization in Sweden of the contact zone between bank voles possessing the species-specific mtDNA and bank voles with the mtDNA from the northern red-backed vole. The position of the zone is compared with those of other Fennoscandian mammals that have used both recolonization routes.

Material and methods

For this study, a total of 52 bank voles were trapped north and south of Sollefteå, Sweden (Fig. 1). The first indication that the contact zone must be situated in this region came from three animals trapped earlier (area 2, site E, Table 1). Among these three animals, two were of the southern and one of the northern type. Thus, site E must be within the contact zone, which motivated collection of animals north and south of this site, as well as additional trapping of animals close to site E. The northernmost area 1 (Fig. 1) consists of four sites with a minimum distance of about 500 m between sites. One site was situated about 5 km east of the other three sites which were more close. Area 2 consists of two sites about 1 km apart. The most southern area 3 consists of four sites (G–J), with a distance of about 3 km between sites G and H, about 500 m between H and I, and about 3 km between I and J. Within each site, 24 traps were distributed in groups of four with a minimum distance of 30 m between groups. Trapps were visited twice a day (morning and evening) for 72 hours and all bank voles trapped were sacrificed and stored intact at –70°C until isolation of mtDNA.

Mitochondria were isolated from 1–2 g of liver, heart and kidneys by differential centrifugation (Lansman *et al.* 1981, Jones *et al.* 1988). We obtained adequately pure mtDNA samples by using a modified version of the phenol/chloroform extraction method described by Powell and Zúniga (1983) and Jones *et al.* (1988). The precipitated and dried mtDNA was resuspended in 30 to 50 µl of distilled, sterile water and stored at –70°C. Digestions of 10–50 ng mtDNA were performed for 1–2 hours in 10 µl of reaction mixture with 2 units of enzyme. The mtDNA of the two species were identified by restriction enzyme *Sau* 96I which gives restriction morphs which have almost no fragments in common in the two species (Tegelström *et al.* 1988). Three additional enzymes (*Hae* III, *Hpa* II and *Mbo* I) were used on a limited number of samples to resolve whether they represented different

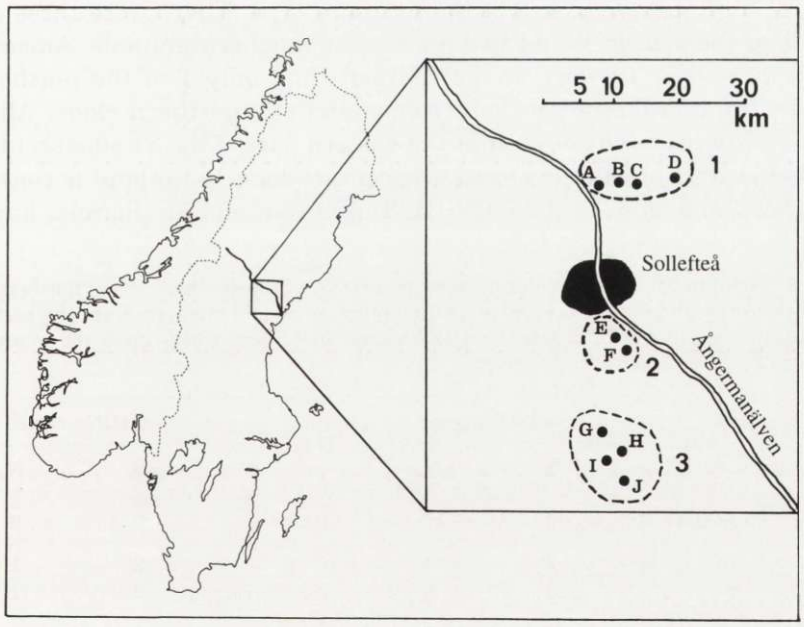

Fig. 1. Map showing the areas (1–3) and sites within areas (A–J) where bank voles *Clethrionomys glareolus* were collected along a transect of the Swedish contact zone between voles with different mtDNA.

mtDNA clones. Restriction fragments were separated by electrophoresis in 5% polyacrylamide gels (Tegelström 1986, 1992). Lambda DNA digested with *Bgl* I was used as a molecular size marker. The mtDNA fragments were visualized by silver-staining as described by Guillemette and Lewis (1983) and Tegelström (1986, 1992).

Results

The number of bank voles possessing mtDNA of the southern or northern type within the different areas and sites are shown in Table 1. Within area 1, which comprised four well separated trapping sites, all animals were of the northern type and three different mtDNA clones were identified within this area. Three of the sites showed only one clone while site A showed two different clones among the three animals investigated.

Within area 2, site E showed one animal of the northern type and two of the southern type. All the animals trapped at site F were of the northern type. These animals were investigated with *Sau* 96I only.

Area 3 is represented by four trapping sites which were geographically well separated. The 3 animals collected at site G all represent different females and 2 were of the northern and 1 of the southern type. Among the 6 animals from site H, 3 were of the southern and 3 of the northern type. There were two different clones among the southern and northern animals. Among the 8 animals from site I, 5 were of the southern and 3 of the northern type. There were three different clones among the southern and two among the northern animals. Among the 11 animals from site J, 10 were of the southern and only 1 of the northern type. There were three different southern clones and one northern clone. Altogether, the localities investigated shows that the eastern part of the Swedish contact zone is located close to the 63°N, whereas the contact zone in Finland is considerably more northern and close to the 64°N (H. Tegelström and M. Jaarola, unpubl.).

Table 1. The number of bank voles possessing mtDNA of the southern (S) or northern (N) type within the different areas and sites. Sampling areas and sites are from the north to the south. The number of different mtDNA clones identified, which gives the minimum number of female lines, are also given.

Area	Site	mtDNA-type		N-type (%)	mtDNA clones	
		S	N		S	N
1	A, B, C, D	0	12	100	0	3
2	E	2	1		2	1
	F	0	9	83 (E+F)	2	1
3	G	1	2		1	2
	H	3	3	56 (G+H)	2	2
	I	5	3	38	3	2
	J	10	1	9	3	1

Discussion

Cline widths are often measured as the distance between allele frequencies 0.2 and 0.8 (Endler 1977) where the steepest part of the allele frequency change occurs, but other criteria have also been used (ie Hunt and Selander 1973). To detect, with a 95% probability, a variant allele with a frequency of > 0.2 in a population size of over 200 individuals, a sample size of 12 specimens is required (Sjögren and Wyöni 1994, the program modified for haploid genomes). This sample size was achieved within the most northern area 1 where all 12 animals were of the northern type. The sample size of 11 specimens at the most southern site (J, area 3) is close to the number of animals needed to get a statistically reliable result and at this site only one northern type animal was found, indicating a low frequency and a predominance of southern type animals.

The cline width for the bank vole contact zone can only be roughly estimated because of the limited number of localities sampled and because we do not know whether the localities sampled are situated at a right angle to the geographic orientation of the contact zone. However, using the 0.2 and 0.8 criterion, we estimate the zone width to be roughly 30 km (the distance between sites E/F and site J), whereas if we use a 0.1 and 0.9 criterion (Hunt and Selander 1973), the width would be considerably wider, 40–60 km. Thus, our investigation indicates a zone width of approximately 30–60 km where we have a transition from the northern to the southern mtDNA type in the bank vole.

Geographic localisation of the bank vole cline in a suture zone

The Swedish contact zone between bank voles coming from the north and the south seems to have a north-west to south-east orientation, as indicated by a previous study (Tegelström 1987), roughly following the direction of northern Swedish rivers. However, as shown in the present study, the bank vole contact zone does not directly correspond to river Ångermanälven, but 10–30 km south of the river.

The bank vole contact zone in Sweden coincides with two other, previously studied, intraspecific mammalian contact zones, and is adjacent to a third zone. First, the bank vole contact zone overlap with the southern part of a comparably wide mtDNA contact zone between northern and southern field vole colonisers (Jaarola and Tegelström 1995, 1996). Second, the contact zone between two main chromosomal groups in the common shrew is found just north of the bank vole zone (Fredga 1987a, b). Third, contact between the two major mtDNA lineages in the brown bear *Ursus arctos*, adjoins the southern border of the vole zones (Taberlet and Bouvet 1994, Taberlet *et al.* 1995). Together these different contact zones in northern Sweden constitute a "suture zone", reflecting multiple, intraspecific secondary contact between populations derived from separate glacial refugia.

The conditions for small mammals were probably favourable soon after deglaciation, enabling the expanding edge of both the southern and northern

immigration contingents to closely follow the receding ice-fronts. Thus, differences in dispersal ability between the species would not greatly affect the positions of the contact zones. According to this scenario, the coincidence of the different zones reflects synchronous timing of secondary contact in the different species following deglaciation (cf Jaarola and Tegelström 1995).

The zone width for mtDNA indicates free gene flow between bank voles that colonised from different directions

The bank voles colonising Scandinavia from the north represent a distinctly different mtDNA lineage compared to those coming from the south, a consequence of hybridisation with northern red-backed voles. The two vole species are distinctly different in morphology, mtDNA sequence divergence and the difference in nuclear genes is similar to that of distinct species (allozyme Nei's D = 0.6 and 3% sequence divergence of single-copy nuclear DNA; Catzeflies *et al.* 1987, Tegelström *et al.* 1988). Thus, in northern bank voles mtDNA and nuclear genes from two different species interact without any apparent negative implications. When the southern bank voles with the species specific mtDNA came into contact with northern bank voles, the different origins of the voles, the difference in geographic distribution and the functional adjustment of the cytonuclear interactions created a situation where two different genepools that may not have been reproductively compatible met. An indication of such reproductive isolation is the level of gene flow between the groups, which may be investigated by analysing the shape and width of the contact zone.

The zone widths differ much among the three species in the suture zone: the field vole zone is 150–200 km wide, the contact zone for the common shrew is about 50 km, whereas the bank vole zone is 30–60 km. The width of a neutral secondary contact zone depends on the number of generations and the average rate of gene flow since contact. The time since contact can be estimated, based on Endler's (1977) neutral diffusion model, using the formula $T = 0.35\ (w/l)^2$, where T is the number of generations since contact, w is the width of the contact zone and l is the rate of gene flow (average dispersal distance). Bank voles have two generations per year under normal conditions, but yearlings may not reproduce under harder conditions. The width of the contact zone is 30–60 km. Dispersal distances in small mammals are very difficult to determine and as a consequence of the methods used, there is a paucity of concrete observations of long distance dispersal. Bank voles are no exception and only a few studies are available where average dispersal distances for both sexes have been determined. The rate of spread of bank voles in a non occupied area, like the situation during colonisation after the ice had disappeared, and a suitable habitat, may be very fast (2–4.5 km per year; Smal and Fairley 1984) but in an occupied area with normal density most voles do not move much more than within their home range. We have found only one report where the mean distance dispersal for juvenile and adult bank

voles have been determined, reporting an average of 272 m for females and 261 m for males per generation, roughly 2.5 times the diameter of the home range size (Watts 1970).

Thus, assuming two generations per year, an overall gene flow rate of 272 m per generation, a zone width of 60 km results in 8515 years since contact using Endler's (1977) equation. This estimate is very approximate because the outcome of the equation is very sensitive for the values included in the calculations (for example, if we choose 200 or 300 m instead of 272 m as mean dispersal distance, the result will be 15750 and 7000 calendar years since contact, respectively). However, our estimate of the time since contact (8500 calendar years) is very close to the time estimate for when the area was deglaciated (Björk 1995) about 9000–8900 ^{14}C BP (corresponding to 10000 calendar years). After that, the ice rapidly disappeared and opened up routes for small mammals to extend their distribution south.

If we instead use the lower estimate of the zone width (30 km) and the diameter of an average home range for bank voles (approximately 100 m, Watts 1970), we get an estimated time since contact of about 15 700 calendar years, whereas the average female dispersal distance used above (272 m) will give a time estimate of 2100 calendar years. Obviously, we can choose a value for mean dispersal that best will fit in with the result we desire. Because it is impossible to determine the true mean female dispersal during the first phase of contact between the populations or when populations had been established, we can only conclude that our zone width estimate for the bank vole mtDNA contact zone does not contradict the hypothesis that it is a neutral, secondary contact zone.

However, our conclusion should be regarded with caution, not directly indicating an absence of prezygotic isolating mechanisms or a decrease in hybrid fitness. First, our zone width estimate is based on a comparably small sample size, both in terms of number of individuals and localities along the transect. Secondly, the width of a contact zone may vary in different geographic regions (Hunt and Selander 1973). Thirdly, mtDNA is not directly linked to genes that are involved in reproductive isolation and may penetrate reproductive barriers easier than nuclear genes (Barton and Jones 1983, Takahata and Slatkin 1984). Also, mtDNA may have different selective values in different nuclear backgrounds (Ferris *et al.* 1983, MacRae and Anderson 1988, Ballard and Kreitman 1995, Kilpatrick and Rand 1995) resulting in non coincidence and/or differences in zone widths estimated by nuclear genes and mtDNA as markers. Consequently, an extensive analysis of the bank vole contact zone with nuclear markers is necessary to complement the results presented here.

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