Genetic structuring and gene flow in a hybrid zone between two chromosome races of the common shrew (*Sorex araneus*, Insectivora) revealed by microsatellites

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Hybrid zones between chromosome races are of interest because they offer the opportunity to investigate the role of chromosomes in speciation processes. Here we study gene flow and fine-scale genetic structure of a chromosome hybrid zone in the common shrew (*Sorex araneus*, Insectivora, Mammalia) in central Sweden using six polymorphic microsatellite loci. The centre of the hybrid zone closely follows a river towards which race-specific chromosomes sharply decrease suggesting a physical barrier to introgression. We examined 285 animals from 22 different localities and performed a hierarchical analysis for *F* and *R* statistics. Genetic structuring within and between the two chromosome races (Hällefors and Uppsala) was found to be weak, but significant. No positive relationship between geographical and genetic distance was detected, suggesting the absence of isolation by distance. Gene flow between the races is not reduced. Therefore, it does not seem that the river is an important barrier reducing introgression between races. The fact that the width of the hybrid zone is narrow and that its centre follows the river despite high levels of migration is discussed.

Keywords: common shrew, gene flow, genetic structure, hybrid zone, microsatellites, *Sorex araneus*.

Introduction

Hybrid zones are of particular interest because they offer a unique opportunity to study introgression, maintenance of genetic integrity and thus the process of speciation (Harrison, 1990). Chromosomal hybrid zones are well represented in mammals (Searle, 1993) and relevant to study if chromosomes play a role in speciation processes (e.g. King, 1993). In the common shrew (*Sorex araneus*, Insectivora, Mammalia), an immense chromosomal variation is found which is caused by Robertsonian (centric) fusion mutations within and between populations (Wojcik & Searle, 1988). So far, 50 well-defined chromosome races have been described in this species (Zima *et al.*, 1996). Inter-racial hybrid zones have been reported in Britain, the Czech Republic, Sweden, Switzerland, Poland and Siberia (Searle,

1993 and references therein; Banaszek, 1994; Lukacova *et al.*, 1994). Therefore, the common shrew is of great interest for the study of karyotypic evolution in mammals. At present, a growing number of *Sorex araneus* chromosomal hybrid zones are under investigation, but most of these have been examined using chromosomal markers only (e.g. Fedyk *et al.*, 1993; Lukacova *et al.*, 1994; Narain & Fredga, 1996; Szalay *et al.*, 1996). Three hybrid zones were studied using allozymes and/or mtDNA markers (Frykman & Bengtsson, 1984; Ratkiewicz *et al.*, 1994; Brünner & Hausser, 1996). With the exception of the contact zone between the Cordon and Valais races in Switzerland (Lugon-Moulin *et al.*, 1996), no informative results on gene flow and genetic structuring within these hybrid zones are available because nuclear markers used so far for analysis (allozymes) generally show weak genetic variation (Hausser, 1991). Microsatellite markers are now widely used in population genetics and have been shown to provide a sensitive measure to detect patterns of hybridization (e.g. Roy *et al.*, 1994). The recent development of

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polymorphic microsatellites for *S. araneus* (Wyttenbach *et al.*, 1997; Balloux *et al.*, 1998) offers the possibility of studying the genetics of hybrid zones in this species in much more detail. In *S. araneus*, microsatellite loci have been shown to be highly polymorphic, both within and between chromosomal races (Wyttenbach & Hausser 1996a; Wyttenbach *et al.*, 1997).

The distribution patterns and zone of contact between two chromosome races of *S. araneus* (Uppsala and Hällefors races) in the central part of Sweden were recently described (Narain & Fredga, 1996). A transect covering ≈ 21 km across the hybrid zone showed a sharp decrease of race-specific markers (distinct metacentric chromosomes with monobrachial homologies fixed in each race) towards a river. In this way it was demonstrated that the centre of the hybrid zone was closely following the river Kolbäck (Fig. 1). This river is generally 50–60 m wide and sometimes widens into small lakes (see Fig. 1). Around 70% of the river freezes regularly in winter. The observed cline width calculated from race-specific metacentric chromosomes (*ko* and *kp*) was as narrow as 2300 m. These observations lead to the question: to what extent might a geographical barrier (river) affect the level of introgression between the two chromosome

races? Generally, narrow hybrid zones (such as between the Uppsala and Hällefors races) can be the result of several factors. Selection against hybrids, different habitat preferences of the races, recent secondary contact between the parental forms or the presence of a geographical barrier are all reasons that, alone or in combination, affect the evolution and location of contact zones (Hewitt, 1988). In our case, fertility reduction of hybrid males between the two parental types was shown to be insignificant in a study of some reproductive characteristics (Narain & Fredga, 1997). However, anaphase I disjunction studies were not carried out and the authors did not consider fertility reduction in females. The habitat in the study area is dominated by forest interspersed by agricultural fields and grassland. It seems that the habitat is homogeneous in the distribution range of both races, particularly in the small area where the two races are hybridizing. Therefore, recent secondary contact or the presence of a geographical barrier (river) are likely factors affecting the width of the hybrid zone between the Uppsala and Hällefors races.

We estimated the level of gene flow between the Uppsala and Hällefors races, analysed the importance of isolation by distance and the impact of the geographical barrier that separates the races

Fig. 1 The sampling area of Surahammar in central Sweden. The centre of the *Sorex araneus* chromosomal hybrid zone is indicated, as is the river Kolbäck. Numbers above each sampling locality correspond to numbers given in Table 1.

(Kolbäck river), using six polymorphic microsatellites. In addition, by analysing the genetic structure within and between chromosome races, using a hierarchical *F*- and *R*-statistics approach, we tried to unravel the fine-scale genetic configuration of the hybrid zone in order to understand better its present geographical structure and evolution.

Materials and methods

Biological material

[For details of the distribution patterns of the two chromosome races and hybrid zone, see Fredga (1996) and Narain & Fredga (1996).] Tissue samples were obtained from a total of 286 shrews. These animals were previously trapped at 23 localities covering an area of about 400 km² in the hybrid zone as described by Narain & Fredga (1996). Individuals collected in 1994–96 were used for analysis. The number of animals scored from each locality ranges from nine to 44 with the exception of locality 4 (*N* = 6) (Fig. 1 and Table 1). Individuals were karyotyped and their race identified by G-banding (Narain & Fredga, 1996). In Table 1 the number of

animals and their karyotypes is given for each locality.

DNA isolation, amplification and electrophoresis

DNA samples were obtained by cutting the tip of a digit from frozen animals. Tissue samples were digested in an extraction medium (200 µL) consisting of Chelex-100 (5%), Proteinase K (10 mg/mL) and DTT/NaAc (1 M/0.08 M) at 56°C overnight. The mixture was then boiled for 8 min and centrifuged at 2000 *g* for 3 min. The supernatant containing DNA was removed and 1 µL used for each polymerase chain reaction (PCR). Six microsatellite loci (L9, L14, L16, L45, L67, L92) were used for analysis. Amplification conditions for each locus were performed following Wyttenbach *et al.* (1997) and Balloux *et al.* (1998). After amplification, aliquots of the 10 µL reaction mixture were mixed with 0.5 volume of formamide loading buffer, heated to 80°C for 2 min and run on denaturing polyacrylamide gels (6%, 8 M urea). Drying and autoradiography were performed following standard procedures. A sequencing reaction was used as a size marker for reading the band polymorphisms.

Table 1 Number of individuals of the Hällefors race, Uppsala race and hybrids of *Sorex araneus* analysed from each locality in the area of Surahammar

No. in Fig. 1	Locality	Number of specimens			
		Hällefors	Hybrids	Uppsala	Total
1	Rölö	10	0	0	10
2	Lappkärrsmossen	8	1	0	9
4	Hemlinge north	5	1	0	6
5	Djupmossen	11	0	0	11
6	Skyttorp	10	1	0	11
7	Baståsen	10	1	0	11
8	Seglingbo	3	6	2	11
9	Nybron/Trugsbo	15	3	0	18
10	Elingbo	10	3	0	13
11	Färmansbo	0	1	13	14
12	Laggarsjön	8	4	1	13
13	Ståltorp	6	4	1	11
14	Hedvallsbron	4	6	3	13
15	Rävnäs	4	19	20	43
16	Hallsta mosse	0	1	9	10
17	Framnäs	0	2	8	10
18	Svenskbyn	0	2	12	14
19	Stingsmossen	0	1	12	13
20	Timmermossen	0	2	10	12
21	Bårhuset	0	1	9	10
22	Klockartorp	0	1	9	10
23	Svartkärret	0	0	12	12

Statistical analysis

Allele frequencies and Hardy–Weinberg exact tests were calculated and performed using the software package FSTAT 2.3 (Goudet, 1995) and GENEPOP 3.1a (Raymond & Rousset, 1995). Both programs compute F -statistics according to Weir & Cockerham (1984). Observed heterozygosity (H_o) were calculated by a direct count of band polymorphism, whereas the expected heterozygosity (H_e) was measured as the gene diversity after Nei (1987). Race-specific microsatellite variants, defined as alleles occurring exclusively in one race, were calculated for each locus, each race and overall.

Temporal variation at microsatellite loci was computed for localities 10 and 20 (Fig. 1) where individuals from two different generations (spring and autumn 1994) were collected. Values of F_{ST} were used to measure the differentiation between generations and tested for a significant departure from zero with FSTAT. In this way, the distribution of the null hypothesis (F_{ST} not > 0) is obtained and compared to the observed distribution. The same procedure was applied for localities 14 and 15, where individuals were available from two different years belonging to the same generation (autumn 1994 and spring 1995). Fisher's procedure (Sokal & Rohlf, 1995) was applied combining the P -values across populations. The quantity $-2\sum \ln(P_i)$ was calculated; this quantity is distributed as χ^2 with $2k$ degrees of freedom. P_i is the i th value of k observations. To test for genotypic linkage disequilibrium we used GENEPOP 3.1a (Raymond & Rousset, 1995).

To assess genetic structuring between populations, F_{IS} (inbreeding coefficient), F_{ST} (fixation index) and F_{IT} (overall inbreeding coefficient) were calculated using FSTAT. To test for a significant departure from zero (see above) we used 5000 permutations of alleles within samples (F_{IS}) and 5000 permutations of genotypes among samples (F_{ST}). Migration can then be estimated following $Nm = \{(1/F_{ST}) - 1/4\}$. This is the relationship between Nm and F_{ST} expected for neutral alleles in an island model of subdivided populations at migration–drift equilibrium (Wright, 1951). N is the effective size of each population, which is assumed to be constant, and m the proportion of migrants arriving each generation in a population.

Mutational mechanisms associated with microsatellite evolution are debated (for review see Goldstein & Pollock, 1997). Variation at microsatellite loci can be explained by either an infinite allele size model (IAM) or a stepwise mutation model (SMM). R_{ST} is an F_{ST} -like estimator of population differentia-

tion, derived by Slatkin (1995) and based on the variance in the number of repeats of alleles; that allows a better estimation of genetic differentiation when the mutation pattern of microsatellite loci follows a SMM. Therefore, we also calculated R -estimates (R_{IS} , R_{ST} and R_{IT}) using GENEPOP. Estimates for F - and R -statistics were first calculated for each chromosome race separately. For this purpose, sites with animals showing karyotypes of both races were excluded from the analysis. Thus, for the Hällefors race we considered localities 1–10 excluding site 8, and for the Uppsala race we included localities 11 and 16–23.

A hierarchical approach using F -statistics (Weir, 1996) was chosen to estimate the number of migrants (Nm) among localities within and between races using the formula $F = [1/(4Nmd/(d-1)+1)]$ (Slatkin & Voelm, 1991). The correction d for the number of demes (localities in each race) is not squared because in our case F -estimates are based on the variance component method (see eqn 13, Rousset, 1996) (J. Goudet, pers. comm.). In this way, the heterozygote deficits within samples (localities) (F_{IS}), between samples within a race (F_{SR}) and between samples among races (F_{RT}) were estimated. Here the number of migrants among samples within races is calculated from F_{SR} and gene flow between the two races from F_{RT} . For all calculations the computer package S-PLUS (S-Plus, 1995) was used (J. Goudet, pers. comm.). Levels of significance for F_{SR} were estimated by running FSTAT for both races independently, combining the P -values of F_{ST} for each locus and overall applying Fisher's procedure (see above). For F_{RT} , individuals of each race were pooled to one population and the P -value of F_{ST} taken. In both cases, 5000 permutation steps were performed. Hierarchical R -estimates were obtained by an analysis of variance where alleles are analysed according to their number of repeats (Rousset, 1996). R -statistics generally show a great variance and therefore no test of significance was carried out.

To investigate further the effect of the river on genetic differentiation we estimated F_{ST} for five pairs of localities separated by the river (8/11, 9/15, 14/15, 13/17 and 12/16) and compared it with the values of five pairs of localities which are not separated by a geographical barrier (5/6, 6/7, 10/14, 15/18 and 18/19). The geographical distance between each locality ranged from 0.7 to 2.0 km. Overall values for F_{ST} were obtained by taking the mean, and Fisher's procedure was applied for P -values.

The associations between geographical distances and values of either F_{ST} or R_{ST} for pairs of samples can be tested for significance by performing

two-matrix Mantel tests (Mantel, 1967). In this way, it is possible to test if genetic differentiation increases with geographical distance (Slatkin, 1993). We used a method of data analysis involving the regression of $F_{ST}/(1-F_{ST})$ (or R_{ST}) estimates for pairs of populations on the logarithm of geographical distance for populations in two-dimensional habitats (Rousset, 1996). Mantel tests were performed using 5000 randomization steps.

Results

Genetic polymorphism, temporal changes in allele frequencies and linkage disequilibrium

Most loci display high levels of variation ranging from two to 35 distinct alleles (Table 2). With the exception of locus 16, the observed heterozygosity for each locus is high (0.58–0.89). The observed average heterozygosity over all microsatellite loci is 0.66. These values are close to the expected ones (gene diversity: 0.63–0.94; average: 0.71). The highest number of race-specific alleles is found for locus 9 ($N = 6$). Out of 97 alleles detected for both races, six alleles (6.2%) are exclusively found in the Hällefors race and eight (8.6%) in the Uppsala race. The total number of race-specific alleles is 14 (14.4%).

Animals collected at the same site over two years, encompassing two generations, were available for only two localities (nos 10 and 20). No significant gene frequency changes over time were observed and the overall change was not significant ($P > 0.1$, d.f. = 4). The same is true for localities 14 and 15, where individuals belong to the same generation, but two different years ($P > 0.5$, d.f. = 4). Genetic

variation over time at four of the six microsatellite loci used in this study (L9, L16, L45 and L67) was similarly analysed for common shrew populations in France, and no significant gene frequency changes over time were detected (N. Lugon-Moulin, unpubl. data). Therefore, samples from different years were pooled for analysis. Analysis for genotypic linkage disequilibrium resulted in nonsignificant values, indicating no genetic linkage between the six markers used in the present study.

Genetic structuring and migration

The Hardy–Weinberg exact test for loci over all localities gave significant results for loci 14 and 16. Tests for single localities resulted in nonsignificant P -values, except for site 15. Locality 15 showed a highly significant deviation from Hardy–Weinberg equilibrium which was, however, caused by loci 9 and 16 only (data not shown). In a first F -statistics analysis over all localities irrespective of the chromosome race ($N = 22$), a relatively high value for the within-sample heterozygote deficit ($F_{IS} = 0.052$) was observed and was highly significant. A closer inspection of F_{IS} values for the six loci revealed that locus 16 showed a much higher heterozygote deficit ($F_{IS} = 0.54$, $P < 0.001$) as compared to the rest of the microsatellites employed in this study (data not shown). This might indicate the presence of null alleles or selective constraints at this locus. A similar pattern for this locus was reported in a previous study (Wyttenbach & Hausser, 1996b). Therefore, we excluded locus 16 from further analysis. The low but significant F_{ST} value over all localities (0.017) suggests weak genetic structuring.

Estimates of F - and R -statistics for each locus over all populations in each chromosome race are given in Table 3. Values of F_{IS} for each locus are close to zero and nonsignificant in both races with the exception of locus 14. The overall value of F_{IS} for the Hällefors race ($F_{IS} = 0.037$, $P < 0.05$) becomes nonsignificant if locus 14 is excluded ($F_{IS} = 0.013$, $P > 0.263$). Again, R_{IS} for locus 14 displays the highest values of all loci in both races. Estimates of F_{ST} are similarly small in both races (Hällefors: $F_{ST} = 0.011$; Uppsala: $F_{ST} = 0.009$), significantly different from zero and close to the overall F_{ST} (0.017). In the Hällefors race, F_{ST} values for each locus and overall are lower compared to estimates of R_{ST} , except for locus 14. This trend is absent in the Uppsala race, where F_{ST} for two loci and the overall value is higher than R_{ST} (Table 3).

The results of the hierarchical F - and R -statistics analyses are presented in Table 4. Intraspecific struc-

Table 2 Number of alleles (k , total), specific alleles in each race (k , Uppsala and Hällefors) of *Sorex araneus*, observed heterozygosity (H_o), expected heterozygosity (H_e , measured as $1 - \sum(p_i)^2$) and number of individuals analysed at each microsatellite locus; race-specific alleles are defined as occurring exclusively in one race

Locus	k (Total)	k (Uppsala)	k (Hällefors)	H_o	H_e	n
L 9	35	3 (8.6%)	3 (8.6%)	0.89	0.94	274
L 14	23	—	2 (8.7%)	0.82	0.91	268
L 16	2	—	—	0.08	0.18	280
L 45	16	—	1 (6.3%)	0.84	0.84	265
L 67	10	3 (30%)	—	0.77	0.76	282
L 92	11	2 (18.2%)	—	0.58	0.63	272
All loci	97	8 (8.6%)	6 (6.2%)	0.66	0.71	273.5

Table 3 Estimates of F - and R -statistics for each locus over all populations calculated separately for each chromosome race (Hällefors and Uppsala) of *Sorex araneus*

Locus	F_{IS}	F_{ST}	F_{IT}	R_{IS}	R_{ST}	R_{IT}
Chromosome race Hällefors						
L 9	0.035	0.040	0.039	-0.091	0.045	-0.042
L 14	0.118**	0.011	0.127***	0.162	-0.003	0.159
L 45	-0.049	0.007	-0.042	-0.011	0.009	-0.002
L 67	0.012	0.032*	0.044	-0.190	0.081	-0.093
L 92	0.063	0.001	0.063	-0.217	0.033	-0.177
All loci	0.037*	0.011*	0.047**	-0.028	0.030	0.003
Chromosome race Uppsala						
L 9	0.017	0.003	0.019	0.158	-0.016	0.145
L 14	0.123**	0.014	0.135***	0.192	0.022	0.210
L 45	-0.058	0.012	-0.046	-0.158	0.032	-0.122
L 67	-0.014	0.010	-0.004	0.021	0.029	0.050
L 92	-0.057	0.006	-0.051	0.024	-0.038	-0.013
All loci	0.009	0.009*	0.018	0.099	0.001	0.101

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for F -statistics.

turing for F -statistics is highly significant ($F_{SR} = 0.0207$) and close to R_{SR} (0.0214). Three out of five loci (L9, L14 and L67) give significant results for F_{SR} . Surprisingly, both values for inter-racial genetic structuring ($F_{RT} = 0.0026$; $R_{RT} = 0$) are lower than estimates for within-race genetic differentiation. Here, two loci (L 9 and L 14) and the overall value for F_{RT} are significant. This indicates less pronounced genetic differentiation between the two chromosome races compared to genetic subdivision within a race. We estimated the number of migrants from F - and R -statistics, respectively. The migration rate between populations within a race gives a value of 11 migrants for F_{SR} and 10.6 for R_{SR} . The number

of migrants exchanged between the two races is 92 animals per generation using F_{RT} . Nm cannot be estimated from R_{RT} because its value is zero (Table 4).

Effect of river and isolation by distance

The mean F_{ST} value for sites separated by the river is 0.0152 and combined P -values gave a high overall significance ($P < 0.001$). Similarly, the average F_{ST} value for pairs of populations not divided by the river is 0.0226 and significant ($P < 0.001$). Mantel tests on either pairwise F_{ST} or R_{ST} matrices against the geographical distance matrix did not reveal any

Table 4 Values of a hierarchical F - and R -statistics analysis carried out on *Sorex araneus* for each locus and over all loci; letter subscript I stands for individuals, S for samples, R for races and T for total

Locus	Hierarchical values for F				Hierarchical values for R			
	F_{IS}	F_{SR}	F_{RT}	F_{IT}	R_{IS}	R_{SR}	R_{RT}	R_{IT}
L 9	0.0442	0.0135*	0.0022*	0.0592	0.0564	0.0130	-0.0046	0.0644
L 14	0.0801	0.0201***	0.0146**	0.1118	0.1992	0.0321	0.0084	0.2314
L 45	-0.0027	0.0034	-0.0006	0.0001	0.0092	0.0075	-0.0045	0.0122
L 67	-0.0154	0.0148*	0.0001	-0.0003	-0.0242	0.0368	0.0041	0.0176
L 92	0.0086	0.0625	-0.0074	0.0637	0.0128	0.0175	-0.0035	0.0268
All loci	0.0258	0.0207***	0.0026**	0.0232	0.0507	0.0214	0.0000	0.0705

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for F_{SR} and F_{RT} .

significant positive association. The observed matrix correlation using F_{ST} ($r = -0.2$, $P = 0.97$) or R_{ST} ($r = 0.05$, $P = 0.31$) is nonsignificant.

Discussion

The high genetic variability at, and absence of linkage between, the five microsatellite markers employed, make them an excellent tool to study genetic structuring at the small geographical scale where the Hällefors and Uppsala races meet and hybridize. Stockley *et al.* (1993) showed that female common shrews are highly promiscuous and regularly mate with close relatives. Based on this result they argued that multiple mating in the common shrew may be a strategy to reduce inbreeding. In our study, values over all loci for F_{IS} are never significantly different from zero, suggesting random mating within localities.

Considering each race separately, the reduction of heterozygosity as a result of geographical subdivision is weak, but nevertheless significantly different from zero ($F_{ST} = 0.009$ – 0.011 , $P < 0.05$). This indicates that the samples for each race cannot be regarded as collected from a panmictic population. We find no isolation by distance at the geographical level sampled.

Looking at the hierarchical analysis, we find that genetic differentiation on an intraracial scale is higher than on an inter-racial level. The number of migrants exchanged within chromosome races is about 11 animals per generation, estimated from F_{SR} and R_{SR} . The Nm value between the chromosome races is as high as 92, estimated from F_{RT} . This result is surprising because migration between races is not expected to be higher than within races, but similar, assuming no geographical barrier or mating preferences. However, Nm estimates from F_{ST} made for each chromosome race separately vary between 22 and 28 immigrants per generation. This indicates a three- to fourfold difference in migration within compared to between races, a degree of variation that can be expected for rough estimates such as Nm . These results suggest that the river is not a serious barrier to gene flow reducing introgression between the two chromosome races. This is also supported by the direct comparison of sites separated by the river with sites that are not, where similar F_{ST} values are found.

These findings are congruent with a study on the contact zone of the common shrew between the Cordon and Valais races in the French western Alps, where rivers did not have a significant effect on reducing gene flow (Lugon-Moulin *et al.*, 1996).

But then, how is extensive gene flow between the Hällefors and Uppsala race compatible with the steep cline of race-specific chromosomes decreasing towards the river? Hybrid zones are likely to be 'attracted' to a local geographical barrier or a region of low density (Barton & Hewitt, 1981). For mammals, chromosomal races commonly meet at local geographical barriers, such as rivers (Searle, 1993). A slight reduction in dispersal across a river is perhaps sufficient to 'trap' a race-specific chromosomal front. However, such a scenario is difficult to imagine in our study because of the high values for Nm found between races. The higher between-races Nm could be explained by some aspects of the reproductive behaviour or migratory habits of shrews (e.g. shrews are good swimmers), but not the narrow width of the cline.

A possible reason for this discrepancy is that some animals showing a karyotype of a pure parental form carry microsatellite alleles from a 'hybrid genome'. If an F_1 hybrid between the Uppsala and Hällefors races backcrosses with an individual exhibiting a karyotype of a pure parental form, 50% of the backcross offspring will show a race-specific karyotype although carrying 50% of the F_1 hybrid genome. Apparent F_1 hybrids between the Hällefors and Uppsala races were detected at very distant localities from the centre of the hybrid zone (Table 1 and Fig. 1). Thus at these sites, individuals showing for example a Hällefors karyotype, but carrying genetic material from the Uppsala race, are expected to be frequent. Therefore, the chromosomal cline is steeper than expected from the high level of migration found in the hybrid zone. Lower fecundity in heterozygous females compared to homozygotes could be an alternative explanation for the maintenance of the chromosomal hybrid zone. In a study on fertility in female common shrews, Wallace & Searle (1994) concluded that fecundity (the total number of offspring) could be lower for heterozygotes (having fewer oocytes and a shorter lifespan than homozygotes).

The low number of race-specific microsatellite alleles (14 out of 97, 14.4%) found in the present work indicates that the two races are genetically close, and contrasts with the study on the contact zone between the races Valais and Cordon in Switzerland (Lugon-Moulin *et al.*, 1996). There, the total number of alleles detected in both races was lower ($N = 78$), but 59% were race-specific (Lugon-Moulin *et al.*, 1996). Indeed, the Cordon and Valais chromosome races are known to be genetically very distinct, as shown for morphological characters (Hausser, 1991), by two protein markers (Brünner & Hausser,

1996) and mtDNA clones (Taberlet *et al.*, 1994). However, because of the limited number of animals analysed in the study of Lugon-Moulin *et al.* (1996) ($N = 77$), it is possible that some specific alleles of one race were missed in the other.

Chromosomal hybrid zones are often believed to be the result of secondary contact. If we advocated a secondary contact between the Uppsala and Hällefors races after isolation and fixation of race-specific chromosomes in allopatry, microsatellites would be sensitive markers to detect genetic differentiation between the two races. Then, the low level of genetic differentiation on an inter-racial level would suggest short isolation in the past. Alternatively, high gene flow across the hybrid zone could have allowed 'homogenization' of the two genomes, which were genetically more distinct when they met in the past. However, keeping in mind that our microsatellite analysis was carried out in a zone of strong hybridization, only animals of pure races should be analysed to infer the true genetic relationship between the races. Such animals are expected to occur more distantly from the centre of the hybrid zone and could be detected by race-specific markers other than chromosomes (e.g. mtDNA).

Under a stepwise mutation model (SMM), values of F_{ST} are expected to be smaller compared to values of R_{ST} and hence do overestimate migration (Slatkin, 1995). F - and R -statistics are now estimated and compared with their theoretical expectations in more and more studies of natural populations (e.g. Ross *et al.*, 1997; Viard *et al.*, 1997). So far, microsatellites do not seem always to follow an SMM, supporting the unique use of R -statistics. Here, we found that on an intraracial scale, F_{ST} is, as expected, smaller than R_{ST} in the Hällefors race but not in the Uppsala race. For the hierarchical analysis, values for within-race differentiation (F_{SR} and R_{SR}) are almost identical, whereas for the estimates between races F_{RT} is higher than R_{RT} . The variation among loci for R_{ST} values in each race shows a higher variance compared with values for F_{ST} , something that is expected when migration is high (Slatkin, 1995). This is, however, not the case for estimates of the hierarchical analysis. It is likely that these discrepancies are caused by the underlying mutation patterns that can differ from locus to locus. In a population genetic study by Wyttenbach *et al.* (in press) using the same loci (four out of six), neither the stepwise mutation model (SMM) nor the infinite allele size model (IAM) accounted for the variation detected at each locus.

In conclusion, the simultaneous use of hierarchical F - and R -statistics allowed us to estimate migration

across the hybrid zone. We found no evidence that the river is a barrier to gene flow, something that would have been expected considering the steep cline of race-specific chromosome markers (Narain & Fredga, 1996). It seems that the chromosomal cline is 'trapped' by the river despite a high level of introgression between races. Sampling at more distant localities and the use of race-specific genetic markers other than chromosomes will be necessary to understand the phylogenetic history of this hybrid zone.

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