

mtDNA comparison of the Alpine chromosomal races and species of the *Sorex araneus* group : preliminary results

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mtDNA comparison of the Alpine chromosomal races and species of the *Sorex araneus* group: preliminary results¹

BY

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Summary.—TABERLET P., FUMAGALLI L. and HAUSSER J., 1991. mtDNA comparison of the Alpine chromosomal races and species of the *Sorex araneus* group: preliminary results. *In*: J. HAUSSER, ed. The cytogenetics of the *Sorex araneus* group and related topics. Proceedings of the ISACC's Second International Meeting. *Mém. Soc. vaud. Sc. nat.* 19.1: 107-118.

279 base pairs of the Cytochrome *b* gene were sequenced for 16 individuals belonging to the various chromosomal forms of *Sorex araneus* of the western Alps, to *S. coronatus* and to *S. granarius*, which retains a primitive karyotype. Three main clones have been identified: *CC* corresponds to *S. coronatus*, *CV* to the chromosomal race Valais of *S. araneus*, except individuals from Les Houches near Chamonix, and *CA* is common to every other *S. araneus*. *S. granarius* shows only few differences with the *CA* group, what is in contradiction with the karyological data. That *CA* clone characterizes some of the representatives of the Valais race at Les Houches, while a clear congruence between karyotypic race and mtDNA clones is observed in the contact zone between Vaud race (clone *CA*) and Valais race (clone *CV*), suggests that Valais chromosomes entered Acrocentric populations by introgression, whereas in the Haslital, Valais race progressed by displacing Vaud populations, without genetic exchanges.

Résumé.—TABERLET P., FUMAGALLI L. et HAUSSER J., 1991. Comparaison du DNA mitochondrial des espèces et races chromosomiques alpines du groupe *Sorex araneus*: résultats préliminaires. *In*: J. HAUSSER, dir. The cytogenetics of the *Sorex araneus* group and related topics. Proceedings of the ISACC's Second International Meeting. *Mém. Soc. vaud. Sc. nat.* 19.1: 107-118.

279 paires de bases du gène du Cytochrome *b* ont été séquencées pour 16 individus appartenant aux différentes formes chromosomiques de *S. araneus* des Alpes

¹This work was undertaken as part of the agreement of collaboration in research between the University of Lausanne (Switzerland) and the University Joseph-Fourier of Grenoble (France).

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occidentales, à *S. coronatus* et à *S. granarius*, laquelle a conservé un caryotype primitif. Trois clones principaux ont été identifiés: *CC* correspond à *S. coronatus*, *CV* caractérise la race chromosomique Valais de *S. araneus*, à l'exception des individus capturés aux Houches près de Chamonix, et *CA* est commun à tous les autres *S. araneus* analysés. *S. granarius* ne montre que de très faibles différences avec le groupe *CA*, ce qui est en contradiction avec les données de la caryologie. Le fait que le clone *CA* soit caractéristique d'individus de la race Valais aux Houches, alors qu'une correspondance claire entre race chromosomique et clone de mtDNA est relevée dans les zones de contact entre la race Vaud (clone *CA*) et la race Valais (clone *CB*), suggère que les chromosomes Valais ont pénétré les populations Acrocentriques par introgression, tandis qu'au Haslital, la race Valais a progressé en repoussant la race Vaud sans qu'il y ait eu échange génétique.

INTRODUCTION

The genetic relations of the chromosomal races and species of the shrews belonging to the *S. araneus* group in the Western Alps are still poorly known, although a coarse general outline can be drawn. To summarize the situation, it is supposed that after the last glaciation, the Western parts of the Alps were first recolonized by a primitive form of *Sorex araneus* ('Acrocentric' form) in which all the chromosome arms involved in the Robertsonian polymorphism, namely arms *g* to *r* (SEARLE *et al.* 1991), were presents as acrocentric chromosomes. These primitive populations would then have been progressively invaded by a set of metacentrics progressing westward along the north slopes of the Alps, –the 'Vaud' race, connected to the West European phylogenetic group (WEPG) according to SEARLE (1984)–, and eventually their distribution was fragmented by the sibling species *Sorex coronatus* invading the lowlands. As the climatic conditions improved, a southern race ('Valais' race) crossed the mountain passes and settled in Valais, between the Penine and Bernese Alpine ranges. Representatives of this race can presently be found on the north slope of the Alps in the Haslital (upper valley of the Aar River, Switzerland), where they are in close contact with the Vaud race, and in the upper valley of the Arve River near Chamonix, France, where they meet the primitive Acrocentric form (HAUSSER *et al.* 1986, 1991).

Although the existence of polymorphic populations karyotypically intermediate between Vaud and Acrocentric forms support the idea of the progressive introgression of metacentrics in a previously acrocentric population, the sharp limits between Valais and both Vaud race and Acrocentric form rather suggest population displacement by competition. In order to determine the relative importance of these mechanisms for the present distribution of these forms, we compared a partial sequence of the Cytochrome *b* gene located in mitochondrial DNA (mtDNA) extracted from representatives of each of them.

The strictly maternal inheritance of mtDNA, without recombination, and its relatively high substitution rate in Vertebrates (WILSON *et al.* 1985, AVISE

1986, AVISE *et al.* 1987, MORITZ *et al.* 1987, HARRISON 1989) give us the opportunity to investigate the congruity between relatively independent evolutionary processes, namely the pattern of divergence of female clones and the course of karyotypic evolution, as well as to reconstruct the phylogenetic relationships of the clones studied. For this last reason we used *S. granarius*, an endemic species of the Iberic Peninsula, as an outgroup in our analysis, because it bears what is considered the ancestral karyotype of the *araneus* group (VOLOBOUEV and CATZEFLIS 1989, WÓJCIK and SEARLE 1988). The results presented here are based on a very small sample and thus have to be considered as preliminary, but nevertheless provide interesting and valuable information.

MATERIAL AND METHODS

16 individuals were used in this analysis (Table 1). Their geographical origin is shown on the Figure 1.

DNA extraction, Polymerase Chain Reaction (PCR) and sequencing were performed according to KOCHER *et al.* (1989), with some minor modifications. Liver samples preserved in 70% alcohol were first digested with proteinase K for 4 hours at 37 °C. The DNA was then extracted twice with phenol/chloroform, once with chloroform and once with ether. The sample was de-salted and concentrated by ethanol precipitation (SAMBROOK *et al.* 1989).

PCR was performed with a "GeneAmp" kit (Perkin-Elmer/Cetus) in 25 µl of reaction mixture and using primers (L14841, H15149) designed by KOCHER *et al.* (1989), which amplify 307 base pairs of the Cytochrome *b* gene. A fraction of the extracted DNA was subjected to 35 cycles of amplification. Each cycle consisted of denaturation for 1 min at 93 °C, hybridization for 1 min at 50 °C, and extension for 2 min at 72 °C.

Amplified mtDNA was purified in a 2% low melting agarose gel and used as the template in a second polymerase chain reaction with primer ratio L14841=100 and H15149=1, to generate single-stranded DNA for sequencing with the primer that had been limiting in the second chain reaction (GYLLENSTEN and ERLICH 1988).

The mtDNA was sequenced using a "Sequenase" kit (United States Biochemical).

For the phylogenetic parsimony analysis we used the PAUP program (SWOFFORD 1990). In order to compare mtDNA and karyotypic phylogenies, we also performed an analysis based on chromosome mutations. The karyotype of *S. granarius* was considered as ancestral. Each mutation, i.e. Robertsonian fusions, as well as the non-Robertsonian mutations in *S. coronatus* (see VOLOBOUEV and CATZEFLIS 1989), was coded as 0 if absent of the population, as 1 if present but not fixed, and as 2 if present and fixed. These characters have been considered as ordered, that is, we excluded the possibility of centric fissions.

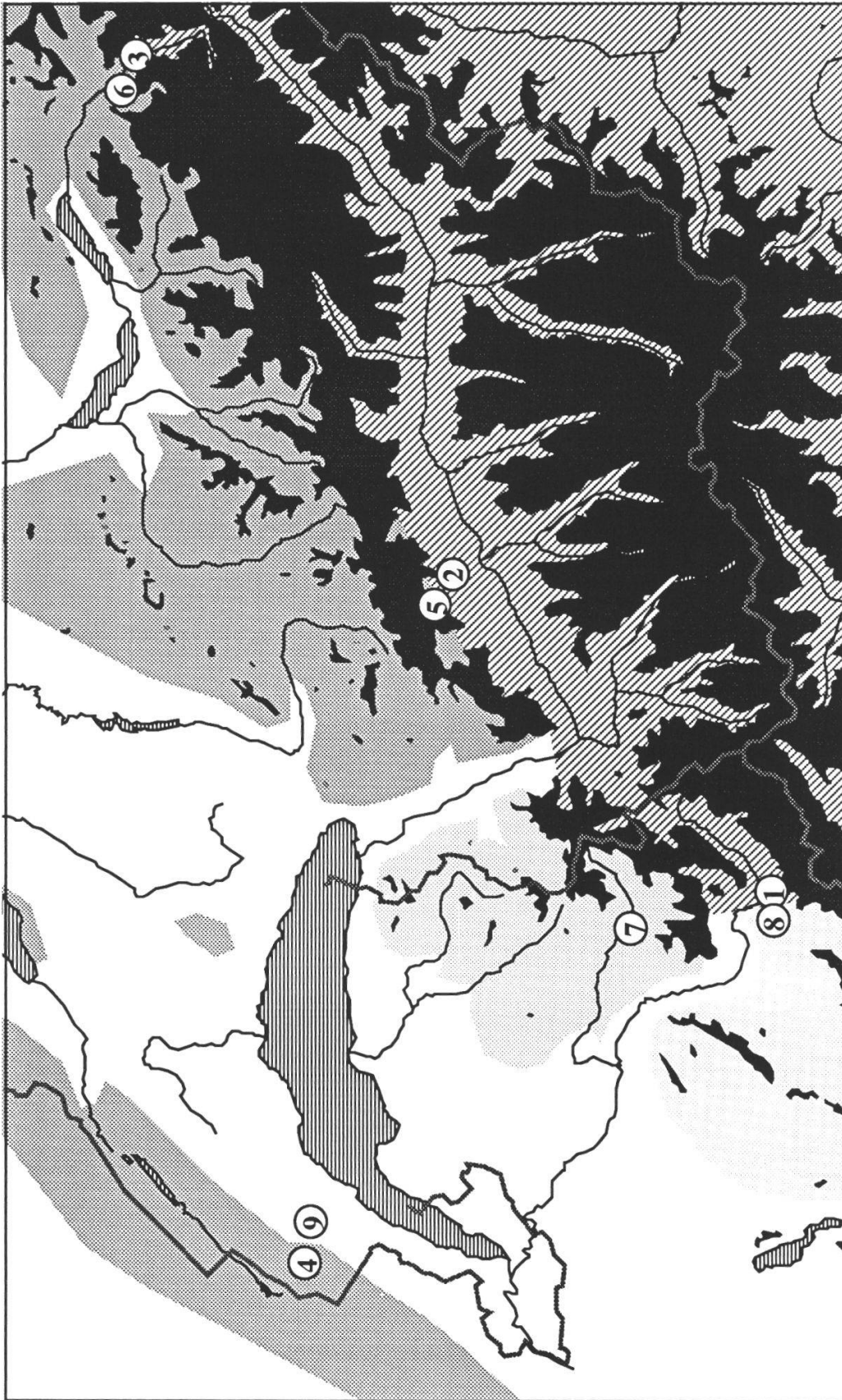


Figure 1.—Geographical distribution of the chromosomal forms studied and localities sampled. Distribution of the karyologic forms according to HAUSSER *et al.*, 1991. *S. araneus*: Dark grey: Vaud race; medium grey: Intermediate form; light grey: Acrocentric form; hatched: Valais race. *S. coronatus*: white; Black: mountains above 2000 m.

Table 1.—Geographical origin of the shrews studied. See Figure 1 for site names.

Shrew n ^o	Species	Chr. Race	Locality
3334	<i>S. araneus</i>	Valais	1 Les Houches, Hte Savoie, France
3339	<i>S. araneus</i>	Valais	1 Les Houches, Hte Savoie, France
3748	<i>S. araneus</i>	Valais	1 Les Houches, Hte Savoie, France
3303	<i>S. araneus</i>	Valais	2 Savièse, Valais, Switzerland
3720	<i>S. araneus</i>	Valais	3 Innertkirchen, Bern, Switzerland
3719	<i>S. araneus</i>	Vaud	4 Bassins, Vaud, Switzerland
3302	<i>S. araneus</i>	Vaud	5 La Tzandra, Valais, Switzerland
3691	<i>S. araneus</i>	Vaud	6 Innertkirchen, Bern, Switzerland
SAM	<i>S. araneus</i>	Intermediate	7 Samoëns, Hte Savoie, France
3383	<i>S. araneus</i>	Acrocentric	- Bauges, Savoie, France
3749	<i>S. araneus</i>	Acrocentric	8 Les Houches, Hte Savoie, France
3751	<i>S. araneus</i>	Acrocentric	8 Les Houches, Hte Savoie, France
3374	<i>S. coronatus</i>	coronatus	- Vercors, Isère, France
3717	<i>S. coronatus</i>	coronatus	9 Bassins, Vaud, Switzerland
E24	<i>S. granarius</i>	granarius	- Candelario, Salamanca, Spain
E69	<i>S. granarius</i>	granarius	- Rascafria, Madrid, Spain

RESULTS

Maximally, 279 base pairs of the Cytochrome *b* gene were sequenced. For 2 individuals, the sequences were only partial, but compatible with the complete sequences of other individuals of the same taxon. 17 variable sites were found, all of them represented transition mutations at silent positions. The consensus sequence for the *Sorex* races and species concerned is presented in Table 2. The maximum nucleotide sequence divergence between two individuals, a *Sorex coronatus* from locality 9 and a *Sorex araneus* "Valais" from locality 3 (Fig. 1) was 5.3 % (14 substitutions for 264 nucleotides)

A preliminary analysis of the data convinced us to combine identical or almost identical (one substitution) individuals of the same karyotypic group together in the same OTU. The only 'Intermediate Vaud–Acrocentric' *S. araneus* we analysed shows a sequence identical with one of the Vaud individuals, and therefore it was grouped with the Vaud race. The consensus sequences for these OTUs was used in the parsimony analysis. Table 3 shows the composition of the 17 variable sites for each of them.

The phylogenetic analysis provided 5 most parsimonious trees of length 18 and consistency index 0.944 from a total of 10395 evaluated (max. length tree: 31). A bootstrap test indicated that the separation of Valais 2 and 3 and of *S. coronatus* 1 and 2 are highly significant. Thus we can recognize 3 main and very distinct clones: CV, represented by the Valais 2 and 3 individuals,

CA, regrouping every other *Sorex araneus*, and CC represented by *S. coronatus* individuals. The *S. granarius* clone does not differ significantly from the CA clone.

In order to choose between the 5 suggested trees, we compared them with the (unique) most parsimonious tree obtained by karyotypic analysis, which has a consistency index of 0.973. The chosen mtDNA tree and the karyotype tree are presented in Figure 2. An unrooted tree (Fig.3) better illustrates the phyletic distances between mtDNA genotypes.

Table 2.—Consensus sequence for the region of the Cyt *b* gene studied in *Sorex* races and species, organised into triplets with amino acid in abbreviated form indicated.

AAC	TTC	GGC	TCC	CTC	CTA	GGT	GTC	TGC	YTA	ATY	ATY	CAA	ATY	CTT	45
N	F	G	S	L	L	G	V	C	L	I	I	Q	I	L	
ACA	GGA	CTC	TTT	YTA	GCA	ATA	CAT	TAC	ACA	TCA	GAC	ACA	ATR	ACY	90
T	G	L	F	L	A	M	H	Y	T	S	D	T	M	T	
GCT	TTC	TCA	TCA	GTY	ACA	CAC	ATC	TGC	CGA	GAT	GTA	AAC	TAC	GGR	135
A	F	S	S	V	T	H	I	C	R	D	V	N	Y	G	
TGA	YTA	ATC	CGA	TAC	CTT	CAT	GCA	AAC	GGA	GCA	TCA	ATA	TTC	TTC	180
W	L	I	R	Y	L	H	A	N	G	A	S	M	F	F	
ATT	TGY	CTA	TTC	CTC	CAC	GTC	GGA	CGA	GGY	CTT	TAC	TAC	GGR	TCY	225
I	C	L	F	L	H	V	G	R	G	L	Y	Y	G	S	
TAY	ATA	TAY	TTA	GAA	ACA	TGA	AAT	ATC	GGC	GTA	TTA	TTA	YTA	TTC	270
Y	M	Y	L	E	T	W	N	I	G	V	L	L	L	F	
GCA	GTA	ATA													279
A	V	M													

Within the nucleotide sequence: Y = C or T, R = A or G. Base 1 in this sequence corresponds to base 14 839 of the standard human mtDNA sequence (ANDERSON *et al.*, 1981).

Table 3.—Variable sites of the partial Cyt *b* gene sequence studied in *Sorex* races and species.

<i>S. araneus</i> Valais 1 (3334,3339,3748)	C	T	T	T	C	A	T	C	A	C	C	T	G	C	C	C	C
<i>S. araneus</i> Valais 2 (3303)	C	C	T	C	C	A	T	T	A	T	C	C	G	T	C	T	C
<i>S. araneus</i> Valais 3 (3720)	C	T	T	C	C	A	T	T	A	T	C	C	G	T	C	T	C
<i>S. araneus</i> Vaud (3719,3302,3691,SAM)	C	T	T	Y	C	A	T	C	A	C	C	T	G	C	C	C	C
<i>S. araneus</i> Acro (3383,3749,3751)	C	T	T	T	C	A	T	C	A	C	C	T	G	C	C	C	C
<i>S. coronatus</i> 1 (3717)	C	T	C	T	T	G	C	C	G	C	T	T	A	C	T	T	T
<i>S. coronatus</i> 2 (3374)	T	T	C	T	T	G	T	C	A	C	C	T	A	C	T	T	?
<i>S. granarius</i> (E24,E69)	C	T	C	T	C	A	T	C	A	C	C	T	G	C	C	C	?

Y = C or T; ? = not sequenced. These sites correspond to the positions 28, 33, 36, 42, 58, 87, 90, 105, 135, 139, 186, 210, 222, 225, 228, 234 and 265 of the sequence in Tab. 2

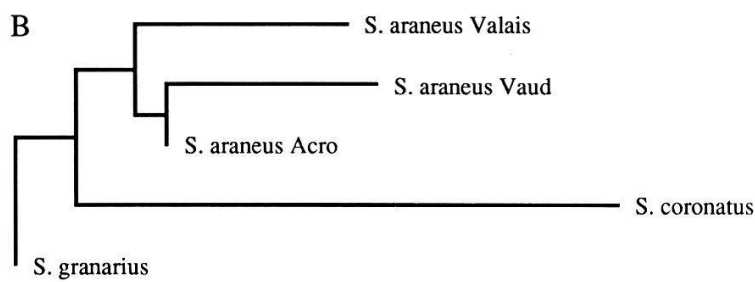
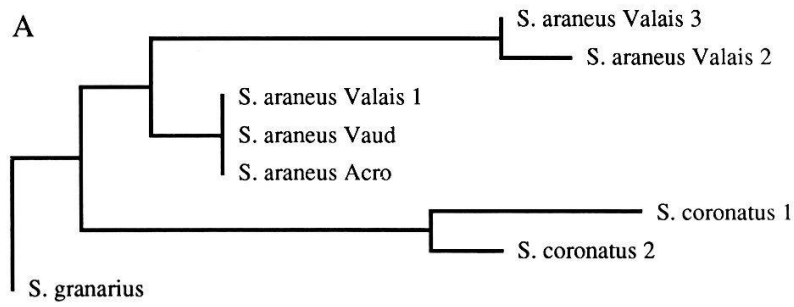


Figure 2.—Most parsimonious phylogenetic trees (Fitch parsimony) obtained from mtDNA (A) and karyotypic (B) data. *S. granarius* was chosen as an outgroup because it bears a primitive karyotype. See text for further explanations.

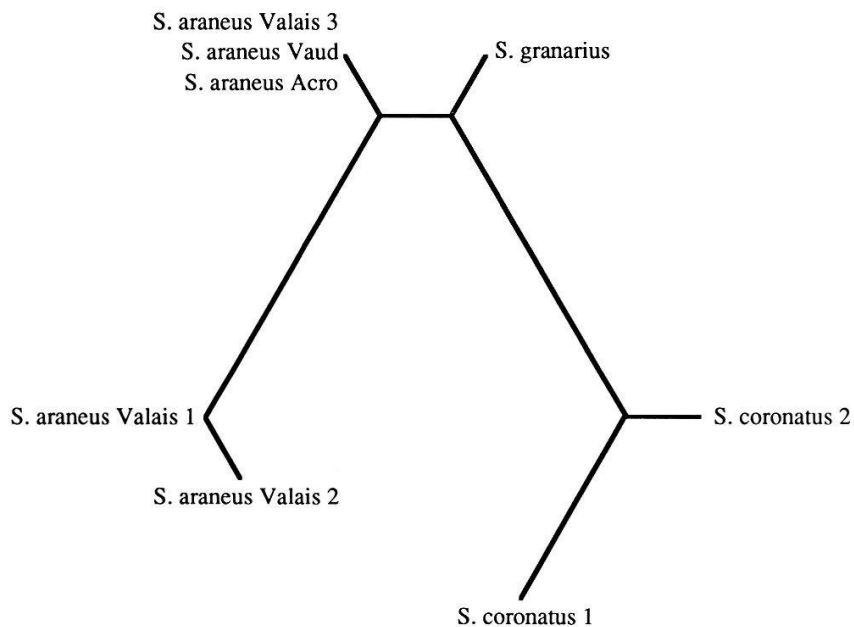


Figure 3.—Unrooted most parsimonious phylogenetic tree obtained from mtDNA data.

DISCUSSION

The maximum nucleotide sequence divergence of 5.3 % recorded is particularly high for a group of such closely related OTUs. The current estimations of mtDNA sequence divergence rate in mammals is roughly 2 % per million years (BROWN *et al.* 1979, FERRIS *et al.* 1983, HIGUCHI *et al.* 1987), what would set the common maternal ancestor of the shrews considered at least 2.5 millions years ago. This evaluation is nevertheless questionable. First, the divergence rate is not constant along the mtDNA molecule (HARRISON 1989). Secondly, the mtDNA tree of the Figure 2 as well as the unrooted tree of the Figure 3 suggests different evolutionary rates between lineages. Such differences in rates of mtDNA evolution have been reported by WAYNE *et al.* (1990) for East African black-backed jackals.

It is enticing to advocate isolation of small populations suffering frequent bottlenecks during the glaciations to explain the rapid divergence of *CC* and *CV* from the the *CA* clone. But it is far less easy to explain why *S. granarius*, which should have experienced an analogous situation, is still very close from *CA*, in which it should actually be included. This mtDNA proximity confirms the results of previous biochemical genetics analysis, in which *S. granarius* was not separated from *S. araneus* (CATZEFLIS *et al.* 1982, CATZEFLIS 1984). These data are rather difficult to reconcile with the current cladistic view of the chromosomal evolution of these species, since *S. granarius* should represent the outgroup of all other forms examined here, which share the same metacentric *af*. We should maybe consider that *S. granarius* has lost this metacentric secondarily, by a Robertsonian fission; note that this mechanism, even if of less importance than fusion in the evolution of this group, was observed in *S. coronatus* by OLERT (1973) and also suggested for *S. araneus* by HALKKA *et al.* (1987). Nevertheless, the large repartition of the *af* metacentric, which is shared by 3 species at least (see ZIMA 1991), and for which polymorphism was never reported, suggests instead a great stability of this fusion. Alternative models of the phylogenic relations of this taxa need to be developed. The case of *S. granarius* demonstrates however the great homogeneity of Cytochrome *b* clones over large populations assemblages, and it is therefore likely that the other recorded clones are also representative of the karyologic taxa to which they are related.

In fact, there is clear similarity between the karyotypic and the mtDNA trees (Fig. 2). If we temporarily disregard the Valais 1 OTU, we observe exactly the same branching structure in both trees. Considering the *CA* group, the Acrocentric form and Vaud race are noticeably different in karyotype (Vaud being characterized by five pairs of metacentrics, of which only one, *jl*, is shared by the Acrocentric form, despite its name), but identical from the mtDNA point of view. If one remembers the very existence of the Intermediate populations, characterized by a Robertsonian polymorphism for every Vaud metacentric except *no*, this situation strengthens the suggestion of a progressive introgression of the Vaud metacentrics into previously Acrocentric populations.

The most striking result we obtained is the obvious heterogeneity in mtDNA clones among representatives of the Valais race. While individuals collected in Valais (locality 2 on Figure 1) and in Haslital (locality 3) are both clearly related (*CV* clone) and clearly separated from the remaining OTUs, the Valais shrews from Les Houches are not different from the Vaud or Acrocentric OTUs (*CA* clone). If we postulate that the *CV* clone represents the original clone of the Valais race, which was probably isolated in Italy during the last glaciation, we face two quite different processes. Let consider the contact of Valais race with the Acrocentric forms first.

Progressing westwards from the Simplon Pass along the Rhone Valley and the low pass between Savoy and Valais, the Col des Monteyts, 1461 m, the Valais populations of *CV* clone met Acrocentric populations in the Upper Arve valley. There was a transmission of metacentrics from the Valais race to the Acrocentric form, as indicated by the Valais individuals which bear a *CA* mtDNA clone at Les Houches. This data therefore confirm the hypothesis of hybridization suggested by HAUSSER *et al.* (1991). It is quite possible that in such a case the males, which are nomadic during the reproduction season in *Sorex* (CANTONI 1990), are responsible for a rapid spreading of metacentrics, while the strict territoriality of the females accounts for a greater geographical stability of the mtDNA clones. Thus, as well as the case of Vaud metacentrics, we have here another good indication for the metacentric introgression mechanism.

Note however that in the actual contact zone between Valais and Acrocentric chromosomal forms (both forms with the same *CA* clone), we never found any intermediate karyotype, but a clear-cut limit corresponding to a river torrent (HAUSSER *et al.* 1991). This suggests that an individual being heterozygous for three or four metacentrics suffers a strongly reduced fertility. Thus the transmission of the plain set of Valais metacentrics to Acrocentric populations should be a relatively rare event. The problem would be less important if the metacentrics were transmitted from a highly polymorphic population, which suggests that the Valais metacentrics *ig*, *jh* and *nk* reached their present homozygous status only recently.

The contact between the Valais and Vaud races on the Bernese Alps shows a quite different situation: the Vaud individuals still bear the *CA* mtDNA clone, while the Valais ones are characterized by their own *CV* clone, which we have already postulated to be the original one. As far as we can see from our limited sample, the limit of the clones fits the limit of the chromosomal races at two different places, Haslital and Central Valais, south of the Sanetsch Pass. It is likely that shrews could not cross the Bernese Alps until recently, their passes (Grimsel: 2165 m, Sanetsch 2251m) being far higher than the ones connecting Valais and Savoy. We suggest that, when the concerned races did actually meet, their karyotypic divergence was already too important to allow any gene flow. (See also NEET and HAUSSER 1991). Thus, the colonisation of the upper Haslital by the Valais race bearing the *CV* clone, as well as the settlement of Vaud populations bearing the *CA* clone south of the Sanetsch, should be due to competition inducing population displacement.

In conclusion, our preliminary results suggest that both population displacement and introgression of metacentrics played their part in the actual distribution of the Alpine chromosome races. The key factor determining the actual mechanism should be the presence of largely incompatible metacentric sets in the populations making contact, which should efficiently prevent gene flow between them. That simple Robertsonian fusions are sufficient to actually stop gene flow between two populations of *S. araneus* was recently questioned by BENGTTSSON and FRYKMAN (1990). They state that good indications for selective pressure decreasing the isolation of karyotypic races in contact zone are available, but that they did not find any convincing evidence for a crucial role of Robertsonian mutations in speciation processes. That the Valais chromosome race invaded the upper Haslital by population displacement rather than by introgression of metacentrics in CA populations would be at least the beginning of such evidence, but still needs to be confirmed by further analysis.

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