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Biochemical analysis and determination of living individuals of the Alpine karyotypic races and species of the *Sorex araneus* group

BY

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Summary.–NEET C.R. and HAUSSER J., 1991. Biochemical analysis and determination of living individuals of the Alpine karyotypic races and species of the *Sorex araneus* group. *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*: 97-106.

Living individuals of *Sorex coronatus* and of the Vaud, Valais and acrocentric karyotypic races of *S. araneus* have been analysed by urinary pepsin and serum albumin electrophoresis. The sample analyzed includes 81 individuals taken from various swiss and french populations and covers a wide Alpine distributional range.

S. coronatus appears to be characterized by a unique and specific pepsin band, while *S. araneus* has three different bands, occuring in different relative proportions, according to the karyotypic race. A slow serum albumin form is found in *S. coronatus* and in the Valais race, while the Vaud and acrocentric races both have a rapid albumin. There are clear indications that urinary pepsin electrophoresis is a new biochemical determination method that permits to distinguish living individuals of *S. coronatus* from those of *S. araneus* over a wider range than the currently used technique of serum albumin electrophoresis.

Differences in albumin patterns and in frequencies of pepsin bands suggest that gene flow is interrupted or at least seriously limited between Vaud and Valais karyotypic race of *S. araneus*.

Résumé.–NEET C.R. et HAUSSER J., 1991. Analyse biochimique et détermination d'individus vivants des races chromosomiques et des espèces alpines du groupe *S. araneus*. *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*: 97-106.

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Des individus vivants de *Sorex coronatus* et des formes chromosomiques Vaud, Valais et Acrocentrique de *S. araneus* ont été analysées par électrophorèse des pepsines urinaires et de l'albumine du sang. L'échantillon analysé comprend 81 individus de différentes populations suisses et françaises et couvre une large zone des Alpes.

S. coronatus est caractérisée par une bande unique et spécifique pour les pepsines, alors que *S. araneus* montre trois bandes, qui présentent des proportions différentes dans chaque race chromosomique. *S. coronatus* et la race Valais possèdent une albumine à migration lente, alors que la race Vaud et la forme Acrocentrique ont une albumine à migration rapide. Il apparaît que l'électrophorèse des pepsines urinaires fournit une nouvelle méthode de détermination biochimique qui permet de distinguer les individus vivants de *S. coronatus* de ceux de *S. araneus* de façon plus générale que la technique courante de l'électrophorèse de l'albumine.

Les différences enregistrées pour les albumines et pour les fréquences des différentes bandes des pepsines suggèrent que le flux génétique est sans doute interrompu ou du moins sérieusement limité entre les races chromosomiques Vaud et Valais de *S. araneus*.

INTRODUCTION

To undertake ecological field studies on karyotypic races and species of the *Sorex araneus* group, one should be able to identify living individuals. In the case of the studies performed on the interspecific interactions between *Sorex coronatus* and the 'Vaud' race of *S. araneus*, a biochemical technique consisting in the electrophoretical analysis of serum albumins, was successfuly applied (NEET and HAUSSER 1989, 1990). However, in the more general context of the Alpine taxa of the *Sorex araneus* complex, this technique is of a somewhat limited use, since it does not permit to separate *S. coronatus* from the 'Valais' race of *S. araneus* (NEET 1989).

In this paper, we present results obtained while applying the method of albumin electrophoresis to individuals belonging to *S. coronatus* and to the Valais, Vaud and 'Acrocentric' populations of *S. araneus* recognized by HAUSSER *et al.* 1991. We also analyze results obtained while exploring another biochemical method that was expected to be of great potential interest to attempt to determine living individuals, *i.e.* the electrophoretical analysis of urinary pepsins. This second approach was suggested to us by Dr J. Szymura during the 1987 meeting in Oxford on "The Population and Evolutionary Cytogenetics of *Sorex araneus* ", organized by Dr J.B. Searle, Department of Zoology, University of Oxford.

MATERIAL AND METHODS

Shrews were caught by live trapping in various sites of the Swiss and French Alps between 1988 and 1989 (see Table 1 for a detailed list of localities). All the individuals were brought to the laboratory where urine and blood samples were taken. Except for some individuals of *S. coronatus* that were only determined by albumin electrophoresis, all the individuals analyzed in this study were karyotyped.

For karyological analyses, classical G–banded mitotic chromosome preparations were used (SEABRIGHT 1971, HAUSSER *et al.* 1986). For albumin electrophoresis, we used the technique derived by HAUSSER and ZUBER (1983) from the classical method of ORNSTEIN (1964) and DAVIS (1964). The caracteristic albumin patterns that permit to separate *S. coronatus* and the Vaud race of *S. araneus* are described in detail by NEET and HAUSSER (1989).

For urinary pepsin electrophoresis, we followed the technical indications given by TAGGART *et al.* (1978) and SZYMURA and KLEIN (1981). The urine samples were obtained while shrews were left in clean plastic boxes where each mixion was visible and could immediately be collected using a Gilson 'pipetman'. Sample volumes of about 20 to 30 µl were found to be necessary and were diluted with 15 µl of a 40 % sucrose solution and 15 µl of a 0.01 % bromophenol blue solution. The diluted samples were stored at 4 °C and were used within 24 hours. When stored at –80 °C, the samples should be used within a week or two. Longer storage periods have always failed. A quantity of 40 µl of the diluted sample was used for the electrophoretical analyses.

The bands obtained by pepsin electrophoresis were treated as if they were alleles since several studies have shown allelism in human urinary pepsins (SAMLOFF and TOWNES 1970, HARRIS and HOPKINSON 1976). For allelic frequency calculations and analysis, we followed SEARLE (1985) and PASTEUR *et al.* (1987). Yates's correction for small samples was used in every χ^2 test.

RESULTS

As shown by HAUSSER and ZUBER (1983) and NEET and HAUSSER (1989), *S. coronatus* is caracterized by a slow albumin band (Type C), and the Vaud race of *S. araneus* by a fast band (Type A), which is also the most common in Intermediate and Acrocentric populations, whereas a Type C albumin prevails in Valais race (HAUSSER *et al.* 1991). In this study, 9 individuals belonging to the Acrocentric form and 15 individuals belonging to the Valais race have been analyzed. It appears that in our sample all the Acrocentric individuals possess a Type A albumin, while all the Valais individuals possess a Type C albumin (Table 1).

HAUSSER *et al.* (1991) have found albumine of Type C in two Intermediate (n = 22) and in two Acrocentric individuals, one of them being heterozygote (n = 7). Conversely, they show that Type A albumin is relatively frequent in the western populations of the Valais race (f = 0.25 for Chamonix population). Our data reinforce the contrast between these two taxa, since the prevailing type only was found for each group in our sample. Thus, by pooling our results with them of HAUSSER *et al.* 1991, the frequence of type C is $f_c = 0.09$ (n = 16) in the Acrocentric populations and $f_c = 0.82$ (n = 14) in the Valais populations of the upper Arve valley. With the lack of karyologically attested hybrids (HAUSSER *et al.* 1991), this pattern suggests that hybridization between Valais and Acrocentric forms, although probable, is relatively uncommon.

Table 1.–Complete list of the individuals analyzed, with localities and electrophoretic patterns for serum albumins and urinary pepsins. Symbols: SC = *S. coronatus*, SA = *Sorex araneus*. For *S. araneus*, VD indicates the Vaud race, VS the Valais race, IN the intermediate form and AC the Acrocentric form. Ht: locality in Haslital, Berne; LH: locality at Les Houches, Haute Savoie. Swiss canton or French department is given in brackets for each locality.

Species	No	Locality	Albumin type		Pepsin type			
			A	C	a	b	c	d
SC	1	Born IZEA		+			+	
SC	2	Born IZEA		+			+	
SC	3	Born IZEA		+			+	
SC	4	Born IZEA		+			+	
SC	5	Born IZEA		+			+	
SC	6	Born IZEA		+			+	
SC	7	Born IZEA		+			+	
SC	9	Born IZEA		+			+	
SC	114	La Senoge riv. (VD)		+			+	
SC	113	La Senoge riv. (VD)		+			+	
SC	116	Aclens (VD)		+			+	
SC	111	Echandens (VD)		+			+	
SC	cn5	Bassins (VD)		+			+	
SC	cn4	Bassins (VD)		+			+	
SC	cn2	Bassins (VD)		+			+	
SC	cn3	Bassins (VD)		+			+	
SC	cn7	Bassins (VD)		+			+	
SC	cn9	Bassins (VD)		+			+	
SC	gr 62-1	Le Jorat (VD)		+			+	
SC	gr 62-3	Le Jorat (VD)		+			+	
SC	gr 62-2	Le Jorat (VD)		+			+	
SC	3371	Marais de Crolle (38, F)		+			+	
SC	3372	Belledone (38, F)		+			+	
SC	3381	Vercors (38, F)		+			+	
SC	3378	Vercors (38, F)		+			+	
SC	3705	Lac Vert, Passy (74, F)		+			+	
SA VD	0-27	Champ-Pittet (VD)	+		+	+		
SA VD	0-23	Champ-Pittet (VD)	+			+		
SA VD	cn6	Bassins (VD)	+			+		
SA VD	cn4	Bassins (VD)	+			+		
SA VD	cn10	Bassins (VD)	+			+		
SA VD	cn7	Bassins (VD)	+			+		+
SA VD	gr 58-3	Le Jorat (VD)	+					+
SA VD	gr 58-2	Le Jorat (VD)	+		+			
SA VD	3363	Ht, Urbachtal (BE)	+		+	+		
SA VD	3689	Ht, Urbachtal (BE)	+		+	+		
SA VD	3693	Ht, Urbachtal (BE)				+		
SA VD	3695	Ht, Urbachtal (BE)	+					+
SA VD	3691	Ht, Grund (BE)				+		
SA VD	3694	Ht, Grund (BE)				+		
SA VD	3696	Ht, Grund (BE)	+			+		
SA VD	3738	Ht, Inneri Urweid (BE)				+		

Species	No	Locality	Albumin type		Pepsin type			
			A	C	a	b	c	d
SA IN	1	Flaine (74, F)				+		+
SA IN	2	Flaine (74, F)				+		+
SA AC	3333	LH, R. des Chavants (74, F)	+		+	+		
SA AC	3337	LH, R. des Chavants (74, F)	+		+			+
SA AC	3340	LH, R. des Chavants (74, F)	+		+	+		
SA AC	3341	LH, R. des Chavants (74, F)	+		+	+		
SA AC	3348	LH, R. des Chavants (74, F)	+		+	+		
SA AC	3349	LH, R. des Chavants (74, F)	+		+	+		
SA AC	3335	LH, Maison Neuve (74, F)	+			+		
SA AC	3336	LH, Maison Neuve (74, F)	+					+
SA AC	3342	LH, Renalière (74, F)	+		+	+		
SA AC	3343	LH, Renalière (74, F)			+			
SA AC	3751	LH, Torrent de Griaz (74, F)			+	+		
SA AC	3370	Col du Coq (38,F)			+			
SA VS	1	Ayer (VS)			+	+		
SA VS	2	Mont (VS)			+			
SA VS	3334	LH, le Touchet (74, F)		+		+		
SA VS	3338	LH, le Touchet (74, F)		+	+			
SA VS	3339	LH, le Touchet (74, F)		+		+		
SA VS	hc12	LH, le Touchet (74, F)				+		
SA VS	3698	LH, Torrent de Griaz (74, F)		+		+		
SA VS	3748	LH, Torrent de Griaz (74, F)			+			
SA VS	3729	Ht, Inneri Urweid (BE)			+			
SA VS	3730	Ht, Inneri Urweid (BE)			+			
SA VS	3739	Ht, Inneri Urweid (BE)			+			
SA VS	3690	Ht, Ägerstein (BE)			+			
SA VS	3697	Ht, Ägerstein (BE)		+	+			
SA VS	3704	Ht, Ägerstein (BE)		+	+			
SA VS	3366	Ht, Allmeind (BE)		+		+		
SA VS	3368	Ht, Allmeind (BE)		+	+			
SA VS	3386	Ht, Allmeind (BE)		+	+			
SA VS	ht16	Ht, Allmeind (BE)			+			
SA VS	ht15	Ht, Allmeind (BE)				+		
SA VS	3365	Ht, Sagenwald (BE)		+	+			
SA VS	3367	Ht, Sagenwald (BE)		+		+		
SA VS	3387	Ht, Sagenwald (BE)		+	+			
SA VS	3388	Ht, Sagenwald (BE)		+		+		
SA VS	3364	Ht, Breitwald (BE)		+	+			
SA VS	3369	Ht, Breitwald (BE)		+	+			

With urinary pepsin electrophoresis, four different bands were identified and labeled a to d (Fig. 1). Pepsins a, b and d were found in *S. araneus* while pepsin c is the unique and caracteristic pepsin of *S. coronatus*. As the sample analyzed (81 individuals) is relatively important and covers a fairly wide geographical area, it seems reasonable to conclude that pepsin c is a diagnostic band for *S. coronatus*.

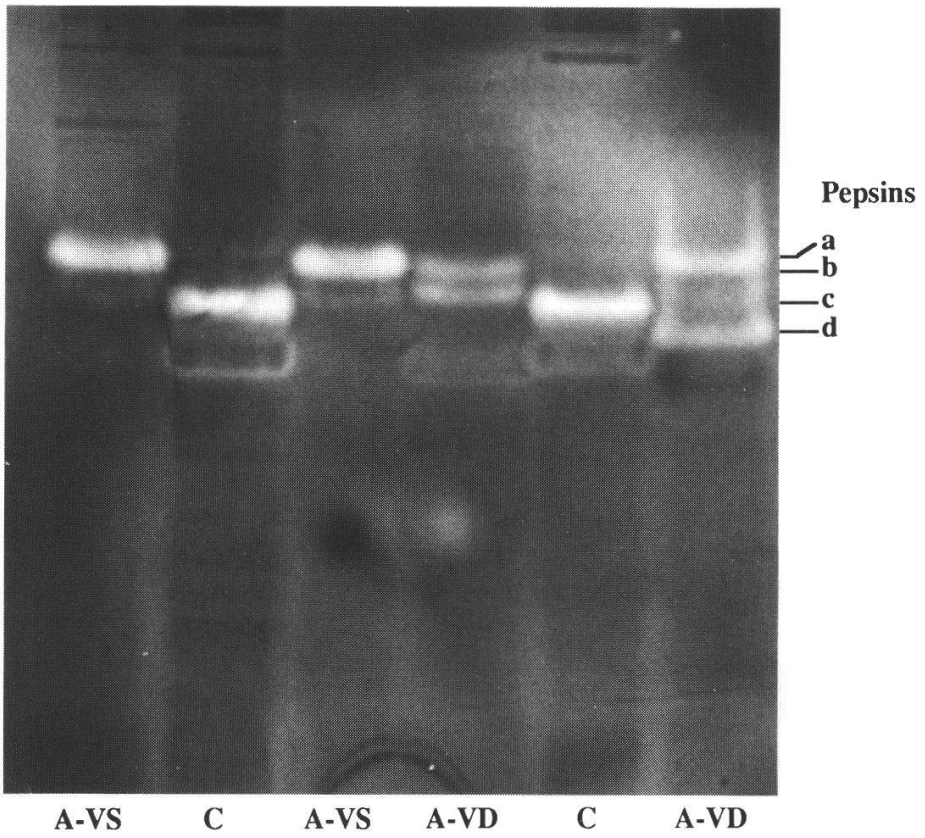

Figure 1.–Example of urinary pepsin electrophoresis of individuals belonging to the *Sorex araneus* group. Four types of pepsin bands have been identified. (A-VS is *araneus* Valais race, A-VD is *araneus* Vaud race and C is *coronatus*). N: number of animals used in each case.

The pepsin d was found only in Vaud, Intermediate Vaud-Acrocentric and Acrocentric samples (VIA group), what confirms their close relationship compared to the Valais race (HAUSSER *et al.* 1991). On the basis of a χ^2 test, the hypothesis of homogeneity between VIA group and the Valais race is clearly rejected: $\chi^2 = 16.51$, $p << 0.001$. The hypothesis of homogeneity between Vaud and Acrocentric could also be rejected by a χ^2 test ($\chi^2 = 6.08$, $p < 0.05$). In this case, nevertheless, not only is our sample small but sampling bias is obvious, because the four heterozygous individuals of Ruisseau des Chavants, which were all juveniles and trapped in two successive nights at the same place, were probably issued from the same litter.

If the overall frequencies of pepsins of our VIA sample does not differ significantly from the expectation of the Hary-Weinberg law ($\chi^2 = 4.755$, $p \approx 0.2$), the lack of heterozygotes in the Valais sample is stricking ($\chi^2 = 17.727$, $p << 0.001$). One heterozygous animal only was observed. The Wahlund effect is probably not sufficient to explain this situation, which needs further sampling of local populations to be clarified.

Although our sample covers a wide area, the Haslital region (Bernese Alps) has received particular attention because of the presence of a contact zone between the Vaud and Valais karyotypic races (HAUSSER *et al.* 1991). All the individuals sampled in the Haslital were distributed along the 15 km of length of the valley, and no intermediate or hybrid individuals were found. As a matter of fact, the contact zone (locality 'Inneri Urweid') is sharp and lies at approximately 900 m of altitude, 3 km above the village of Innertkirchen. Albumin and pepsin frequencies were calculated separately for Haslital (Table 3). These results are similar to those obtained in Table 2, *i.e.* there is a clear difference between the two karyotypic races. This thus confirms, at a local scale, that gene flow between the Vaud and Valais races is likely to be interrupted.

Table 2.–Frequencies of the four pepsin types in the Alpine races and species of the *Sorex araneus* group. Vaud and Acrocentric races of *S. araneus* are shown both separately and pooled with Intermediate form into a unique VIA group, according to their genetic relationships (HAUSSER *et al.* 1991). A detailed list of the individuals is given on Table 1.

Species	Race	N	Frequencies			
			a	**b**	**c**	**d**
S. araneus	Vaud	16	0.156	0.688	-	0.156
S. araneus	Acrocentric	12	0.500	0.375	-	0.125
S. araneus	Valais	25	0.660	0.340	-	-
S. araneus	VIA	30	0.283	0.550	-	0.167
S. coronatus	-	26	-	-	1.00	-

Table 3.–Frequencies of the albumin and pepsin types in the Haslital contact area (Bernese Alps) between the Vaud and Valais races of the *Sorex araneus* group.

Species	Race	N	Albumins		N	Pepsins			
			a	**c**		**a**	**b**	**c**	**d**
S. araneus	Vaud	3	1.0	-	8	0.125	0.750	-	0.125
S. araneus	Valais	12	-	1.0	18	0.720	0.280	-	-

DISCUSSION

An interesting result obtained in this study is that urinary pepsin electrophoresis provides a new determination technique for living individuals of *S. coronatus* and *S. araneus*. However, this technique is somewhat limited by the fact that samples must be analyzed within two weeks or less while serum albumins may be stored for months at –30° C. It is also less practical for field sampling than the electrophoresis of serum albumins (NEET 1989). It has nevertheless a wider range of application for the Alpine taxa since it permits to distinguish *S. coronatus* from the Valais race of *S. araneus*, which is not the case with serum albumin bands. It is not yet clear whether the albumins of these two taxa are really identical or perhaps slightly different. Using increased gel lengths and isoelectric focusing techniques, we have attempted to find slight differences, but without definitive conclusions (NEET 1989). Thus, one should not admit that *S. coronatus* and the Valais race of *S. araneus* share a common albumin allele that may suggest a particular taxonomical relationship. On the contrary, the results obtained by urinary pepsin electrophoresis confirm that *S. coronatus* is indeed well separated from the remaining Alpine taxa of the *S. araneus* group (see also VOLOBOUEV and CATZEFLIS 1989, BENGTSSON and FRYKMAN 1990).

The relative frequencies obtained for the three pepsin types of the *S. araneus* taxa suggest that gene flow must be rather limited between these taxa since the frequencies differ significantly between them. However, this interpretation relies on the assumption that all the taxa have been adequately sampled and that the frequencies are not variable within each taxon. In other contact zones between karyotypic races of the common shrew, allele frequencies have been found to differ appreciably from one race to the other although gene flow crossed the contact zone, *e.g.* BENGTSSON and FRYKMAN (1990). This is in particular the case of the *Mpi* locus in northern Sweden, where a definite amount of gene flow between different karyotypic forms was shown (FRYKMAN 1984, FRYKMAN and BENGTSSON 1984).

In their review of the evidence from the common shrew in favor of karyotype evolution, BENGTSSON and FRYKMAN (1990) underline that direct and indirect evidence tend to support that gene flow penetrates contact zones between all karyotypic races of *S. araneus*, even those that are not closely related. We agree for karyotypic *hybrid* zones. In contrast, the results presented here show that between the Valais and Vaud races of *S. araneus*, gene flow is probably completely interrupted, since the albumins of the two taxa are locally strictly different and the pepsins differ strongly in relative frequencies across a sharp contact zone where no hybrids have yet been found.

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