

## Chromosome localization of microsatellite markers in the shrews of the *Sorex araneus* group

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Received 23 December 2005. Received in revised form and accepted for publication by Pat Heslop-Harrison 3 February 2006

**Key words:** flow-sorting, karyotype, microsatellite mapping, *Sorex araneus*

### Abstract

The extremely high rate of karyotypic evolution that characterizes the shrews of the *Sorex araneus* group makes this group an exceptionally interesting model for population genetics and evolutionary studies. Here, we attempted to map 46 microsatellite markers at the chromosome arm level using flow-sorted chromosomes from three karyotypically different taxa of the *Sorex araneus* group (*S. granarius* and the chromosome races Cordon and Novosibirsk of *S. araneus*). The most likely localizations were provided for 35 markers, among which 25 were each unambiguously mapped to a single locus on the corresponding chromosomes in the three taxa, covering the three sexual chromosomes (XY1Y2) and nine of the 18 autosomal arms of the *S. araneus* group. The results provide further evidence for a high degree of conservation in genome organization in the *S. araneus* group despite the presence of numerous Robertsonian rearrangements. These markers can therefore be used to compare the genetic structure among taxa of the *S. araneus* group at the chromosome level and to study the role of chromosomal rearrangements in the genetic diversification and speciation process of this group.

### Introduction

Comparative gene mapping is of primary interest to understand the evolution of the mammalian karyotype (O'Brien *et al.* 1999). Among mammals, efforts have mostly been concentrated on humans or laboratory and farm animals, and have revealed surprising conservation in mammalian genome organizations. However, to get a satisfactory picture of the genome changes that have occurred during

mammalian radiation, it is important to expand the range of orders examined (O'Brien *et al.* 2001). In this context the former Order Insectivora, defined by morphological characters, is of special interest. Morphologically, its members appear to be the closest to the ancestral eutherian condition (Nowak 1991), but DNA markers suggest their paraphyletic origin (Arnason & Janke 2002, Murphy *et al.* 2004), placing the shrews, hedgehogs and moles in the order Eulipotyphla (i.e., core insectivores, Douady *et al.* 2002).

Among the core insectivores the shrews of the *Sorex araneus* group have been subjected to detailed phylogenetic and population genetic analyses involving chromosome and genetic markers (for reviews see Searle & Wójcik 1998). This monophyletic group consisting of 10 species is characterized by the particular sexual chromosome complex XY1Y2 in males (Sharman 1956) and by a spectacular karyotypic diversification, in spite of their high degree of morphological similarity (Zima *et al.* 1998). In the type species of this group, *Sorex araneus*, Robertsonian polymorphisms are particularly prevalent. The ancestral karyotype of this group is thought to consist of acrocentric chromosomes only. Repeated Robertsonian fusions led to the formation of various metacentric chromosomal complements seen in more than 60 different chromosomal races, each characterized by a particular set of metacentrics and acrocentrics (Wójcik *et al.* 2003). Apart from Robertsonian fusion, high-resolution chromosome analyses (Volobouev 1989, Volobouev & Catzefflis 1989) suggested the occurrence of a few telomere-centromere translocations and centromeric shifts in some species. This outstanding karyotypic variation makes *S. araneus* an exceptionally interesting model species for population genetics and evolutionary studies.

Given its large distribution, its abundance, its ancestral mammalian morphology and its peculiar genome evolution, *S. araneus* represents an obvious 'type' species for the core insectivores. Consequently, a gene mapping project of the common shrew chromosomes was undertaken (Pack *et al.* 1995) and its genome selected as a candidate for complete sequencing (O'Brien *et al.* 2001, Pennisi 2004). The *S. araneus* genetic map currently contains 53 markers (Zhdanova *et al.* 2003) which mark the X (*de*) chromosome and seven out of the nine metacentric chromosomes of the Novosibirsk race. There are, however, some gaps to be filled in order to take full advantage of this map to address many important evolutionary problems. For example, a genetic map of microsatellite loci would provide a powerful tool to unravel the role of chromosomal rearrangements in the speciation process. Indeed, over the entire range of the *S. araneus* group, several populations characterized by different karyotypes come into contact and hybridize. Recently, new models of speciation have been developed to account for the proposed role of chromosomal rearrangements in speciation (Noor *et al.* 2001, Rieseberg 2001, Navarro & Barton

2003); but in the case of the *S. araneus* group it is currently not possible to distinguish between a reproductive barrier caused by genetic effects or by karyotypic differences (Lugon-Moulin *et al.* 1999, Brüner *et al.* 2002). This question could be addressed with a battery of microsatellite markers mapped at the chromosome level. However, only four of these markers have been located so far (Zhdanova *et al.* 2003).

Traditionally, gene mapping in the *S. araneus* group dealt with somatic cell hybrid panels (Pack *et al.* 1995); but, due to numerous hidden chromosome rearrangements in panels, this method proved laborious (Zhdanova *et al.* 2003). Mapping accuracy would be further improved with the use of mostly acrocentric taxa, but only the all-metacentric *S. araneus* Novosibirsk chromosome race has been used so far.

In this study we attempted to map 46 microsatellite loci at the chromosome arm level using flow-sorted chromosomes of three karyotypically different taxa of the *S. araneus* group. The genome-wide chromosomal correspondence among the three taxa studied here is well established (e.g., Volobouev 1989, Volobouev & Catzefflis 1989). The first taxon is *S. granarius*, a species genetically very similar to *S. araneus* (Fumagalli *et al.* 1999), which is believed to have retained the ancestral karyotype of the *S. araneus* group (Wójcik & Searle 1988, Volobouev 1989). Indeed, all its autosomes (except the smallest one *tu*) are in an acrocentric state. The second taxon, *S. araneus* Cordon, is among one of the most acrocentric *S. araneus* chromosome races since most of its autosomes (except for the two large *af* and *bc*, the smallest one *tu* and the polymorphic *j/l*) are acrocentric. The third taxon was the *S. araneus* Novosibirsk race, the traditional 'gene mapping race' for the *S. araneus* group, with all its autosomes being metacentric.

## Material and methods

### *Chromosome isolation*

Chromosomes from fibroblast cell lines of one female of *S. granarius* and *S. araneus* Cordon and one male of *S. araneus* Novosibirsk were sorted on a bivariate fluorescence-activated flow sorter as described previously (Yang *et al.* 1995). Their respective karyo-

types consisted of  $2n = 36$  (*a, b, c, f, g, h, i, j, k, l, m, n, o, p, q, r, tu, X(de)*),  $2n = 30$  (*af, bc, g, h, i, jl, k, m, n, o, p, q, r, tu, X(de)*); Figure 1A) and  $2n = 21$  (*af, bc, go, hn, ik, jl, mp, qr, tu, X(de), Y1(s), Y2(d)*) chromosomes. Note that each of the 21 chromosome arms of the *S. araneus* group is labeled by a letter (*a–u*), with ‘*a*’ being the largest arm and ‘*u*’ the smallest (Searle *et al.* 1991) and that the corresponding chromosomes among the three taxa were each named using the same letter. Chromosome-specific DNA from these individuals was obtained by degenerate oligonucleotide-primed PCR (DOP-PCR) amplification of flow-sorted chromosomes following standard procedures (Telenius *et al.* 1992, Yang *et al.* 1995). The characterization and purity of each chromosome sort was checked by fluorescence *in-situ* hybridization (FISH). Painting probes were hybridized to standard metaphase preparations of their respective species and assigned to specific chromosomes by digital imaging according to the published protocols (Yang *et al.* 1999).

#### *Sorex araneus* group microsatellite loci

Twenty-two markers have already been described in the *S. araneus* group (Wytttenbach *et al.* 1997,

Balloux *et al.* 1998, 2000, Lugon-Moulin *et al.* 2000, Basset *et al.* 2006). Mapping efficiency is sometimes low (Zhdanova *et al.* 2003); therefore this number was not sufficient to ensure that our microsatellite genetic map would mark most of the chromosomes. For this reason we described another 24 loci extracted from four microsatellite-enriched libraries developed by a commercial company (Genetic Identification Services, Inc., Chatsworth, CA). These loci were found to be highly polymorphic and add to the 22 previously characterized loci to give a total of 46 microsatellite markers described for the *S. araneus* group (Table 1).

#### Mapping microsatellite loci to chromosome

Flow-sorted chromosomes were amplified a second time by DOP-PCR (Telenius *et al.* 1992). This second DOP-PCR amplification step was necessary to have enough material and sensitivity to test all loci. Each chromosome-specific DNA was then screened for the presence of each *S. araneus* group microsatellite locus by standard PCR using conditions as described in Table 1.

PCR conditions of formerly described markers are given in Wytttenbach *et al.* (1997), Balloux *et al.*

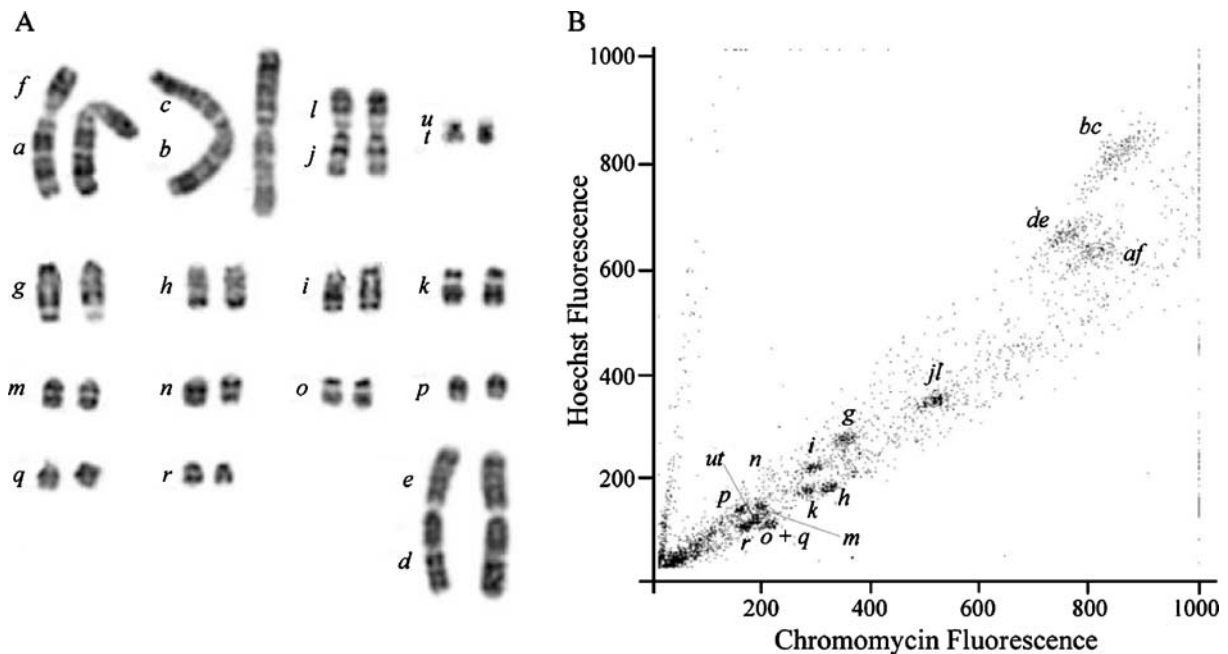


Figure 1. G-banded karyotype (A) and flow karyotype (B) of the female *Sorex araneus* Cordon.

Table 1. PCR primers and amplification conditions used for mapping the *Sorex araneus* group microsatellite loci on *Sorex* chromosomes

MS	Accession number	Primers (5'-3'; for/rev)	Motifs <sup>a</sup>	Annealing (°C)	MgCl <sub>2</sub> (mM)	Refs
L2	U82710	CAAAAACAAAAAGAAGAAAGAAG TCTTATCCTCCATTCCCTC	(GGA)12	55	1.5	1
L9	U82711	TCATGGACTTTTCTGTGCTG CTTTGGCATGAATTTGCC	(AC)29	55	1.0	1
L13	AF175741	TATAACTGTTATCTCACAGCGATTCA ATCCATCTTTATCTTTCCATTGC	(AATT)6	55	1.5	3
L14	AF032911	AGGGAGGGAAACTTGTTAAAGG GTGAGGTCCTGGAATAGTGTCC	(AC)14	55	1.0	2
L16	U82712	TCAGAGTCAGAATTTCTAATTTGGC TTAGTGTATTATGACAGATGCGGG	(AC)16	55	1.5	1
L33	AF032912	TGATTGTATGGAAAAAGCCG ATACTAAGACCACCCTACCAATGC	(AC)19	55	1.5	2
L45	U82713	CTTAAACGTTCTTATCTATTGGTTG GACATATGTGCACTATGAAATTATTG	(AC)10	55	1.5	1
L57	U82714	CTGTTTTTCTGTCCCTCATAGC TGTCTAGTGACATTATCCTATTGG	(AC)10	55	2.0	1
L62	U82715	CAGTCTCTCACTGTGGCACTATG GTCATTCTGGATAAGAACCATATGC	(AC)16	55	1.5	1
L67	U82716	GAAGTGATACATGAGTGCATGAG GTTGTTAACAAGAGAGGTATTACACC	(AC)17	55	1.5	1
L68	AF032913	TCATGGTCATTCATCACATACC GTAGATGTTGCCACTGGTGG	(AC)14	55	1.5	2
L69	U82717	CTTTATGGTAGAAAATGGTG GACCATATACTAAGTTGTTTTG	(AC)17	57	1.5	1
L92	AF032914	ACTGGTGCCCAATCGATAAG GAGAATTGTTGGATGTGCC	(AC)7	55	1.5	2
L97	AF032915	ATTCTCGTGGGTAGACCGTG ATAAATGTGGGAAATGGACAGG	(AC)56	55	1.5	2
L99	AF175744	AAATAATTTCTCCTGGCAAG ATAAATGCAGCAAAGTTATAAACTT	(AC)6	55	1.5	3
L8Y	AF175743	CCTCTTTTGTCTCATCATTTTC AATGATGAAGTGGATGAGTTAGATAACC	(GAA)20	55	1.5	4
A8	DQ247975	CTACGCGCCTTCTTTCAGT GAAGCTGTCCACTGTGTAACG	(CA)26	57	1.5	6
A25	DQ247976	GGCAGTGCTCAGGGATAAC AGTGAGGACAGAATTTCAAGGTG	(CA)25	57	1.0	6
B3	DQ074646	CTTGCCACATTTCCACATC AGCCCCACAGCTTTCTCC	(GA)30	57	1.0	5
B5	DQ074647	ATGTCTTGCTGGCTGAAGG CTGCTGTTCAAACTCCAAG	(CA)12(GA)19	55	1.5	5
B7	DQ247977	AGACGCCCTTGTCTCTCC CCCAGGACTTTCGGTTCTTAC	(GA)29	60	1.5	6
B10	DQ074648	CTCCAAACCCTAACACTCTGTC TTCACGTGTTCTTTGCTTCC	(GA)30	55	1.5	5
B12	DQ247978	GGGGCTTTCTCCACTCTTG TGCTCAGACCTTGATTAGACTC	(GA)34	60	1.5	6
B15	DQ074650	GTAGAGTTGCTGGCTCAAAGG ATGGGAAGACATTGGATTGG	(GA)31	55	1.5	5
B30	DQ247979	TCTCCCTTATCCCCTGTC ACGAAAGGCTGCAACTCAAC	(GA)26	55	1.5	6
C5	DQ074649	TAGATGACTCTGTGTTCAAGC GTTGGGAAGGTAAGATCAGG	(GA)35(CA)12	55	1.5	5
C19	DQ074651	TGCCATAAACACCACTTACC GTGATCAATACCCTGTGGAG	(GA)22	60	1.5	5

Table 1. (continued)

MS	Accession number	Primers (5'-3'; for/rev)	Motifs <sup>a</sup>	Annealing (°C)	MgCl <sub>2</sub> (mM)	Refs
C25	DQ247980	CCCAGGCATAAGTTTCAGG TGTTGAACTGTGGTGGATAGATG	(GA)29	57	1.5	6
C100	DQ247981	CTCGGTGTTTCTACGAT CAGAGATAGAAGAGGCCAAG	(GA)21	55	2.0	6
C117	DQ247982	TAGATGACCAGGATGGAG ACAGAGCTGGGAATCAGT	(GAT)24	55	1.5	6
C119	DQ247983	CCAGCCTTTACTTCTGCTAC TGGGTCTCATTCCTCTGAC	(CAT)29	50	1.5	6
C122	DQ247984	AGTTTTCTTCTCGCCCGTCT CCACTGTGCCAAGGATAGTT	(CT)17	57	1.5	6
C151	DQ247985	CAACGGAGACATTACTGGTG CCAAACTCAAAGGCAGGA	(TGA)30	55	1.5	6
C171	DQ247986	GTGACTGTTCCCATGATGAC ACCAATGTCCCCAGTTTC	(GA)25	55	1.5	6
C240	DQ247987	GGGTTCAATCTCCAACATCC ATCCTGCCCTTCTTTTCCTC	(GA)22	55	1.5	6
D11	DQ247988	GTGTGCGAGAGTCGAAAACC AGCCAGGAACAAGCCCTAC	(TAGA)15	57	1.5	6
D23	DQ247989	ATGGTGGAAAGGCTCAAG AAAAGCAGTATTGGGTCTGG	(TAGA)23	57	1.5	6
D24	DQ247990	CCCAGAGTTACCTTTGAGATATGC TCAATTTTCCCTGGAGGATG	(GA)38	55	1.5	6
D29	DQ247991	GGAAGCAGCGTGAGACTACC AATGGAGACGTGACTGAGACC	(ATC)9	65	1.5	6
D103	DQ247992	TTATGCCACTGATACACCAA ATCCAAAAGGGTTTCCTTAC	(CTAT)12	55	2.0	6
D106	DQ247993	ATTTCTCCCTTCAATCTGGT AGGAGTACCTCTGGGTGTG	(CTAT)10	55	2.0	6
D107	DQ247994	AGGAAGACTGGGGTATGTT TAGGTCTGCTGCCTGCAT	(CTAT)17	55	2.0	6
D109	DQ247995	TGAACTTGGGAGATGCAAT ATAGGAGAGGGCAAGCAG	(CTAT)15CG (CT)15	55	2.0	6
D110	DQ247996	TGTTTTGGTTGAGGTTGG TCACACGCCATCAGTAAGT	(CTAT)36	55	2.0	6
D112	DQ247997	GCAAACCTACCTGTGGCGTATT CCAGCCCTCTTATGAAACTCTT	(CTAT)20	60	2.0	6
D138	DQ247998	ACCTGGAGTGACAGTGAGC GGGTGCTGGAGTGACAGTAT	(CTAT)21	55	1.5	6

References: (1) Wytenbach *et al.* 1997, (2) Balloux *et al.* 1998, (3) Lugon-Moulin *et al.* 2000, (4) Balloux *et al.* 2000, (5) Basset *et al.* 2006; (6) This study.

<sup>a</sup>Represents the size of the original clone.

(1998, 2000), Lugon-Moulin *et al.* (2000) and Basset *et al.* (2006).

PCR conditions of the markers described in this study were as follows: 0.2 mM dNTPs, 0.325 µM of each primer, 1× PCR buffer (Qiagen) and 0.5 U Taq polymerase (Qiagen). MgCl<sub>2</sub> concentration as well as annealing temperature varied (Table 1). For all primers, PCR amplifications were performed on 20–50 ng of DOP-PCR product in a final volume of 20 µl, and cycling was carried out in a PE 9700

thermal cycler (Applied Biosystems) using the cycling profile: 95°C for 5 min; 35 cycles of 30 s at 94°C, 30 s at the annealing temperature, 30 s at 72°C; and a final extension at 72°C for 4 min.

To guarantee correct assignments, each locus was tested against each chromosome sort of the three taxa studied, two to four times. Preliminary analyses showed that unspecific amplifications were sometimes observed in several chromosome sorts. Therefore, we used whole DNA from sorted individuals as

positive controls whose amplification product sizes were used as references. To better estimate the size of the amplification product, one primer of each pair was labeled with a fluorescent dye on the 5' end, and run on an ABI 377XL automated sequencer (Applied Biosystems). Data collection, sizing and analyses of PCR products were done using GENESCAN software (Applied Biosystems).

## Results

### *Chromosome flow-sorting and characterization of flow karyotype*

Bivariate chromosome flow-sorting allowed chromosome-specific sorts for the three taxa to be obtained. Sixteen distinct peaks were identified in the flow karyotype of *S. granarius*, 14 in *S. araneus* Cordon (Figure 1B) and 12 in *S. araneus* Novosibirsk (data not shown). To assign the content of each peak to particular chromosome(s), painting probes from each peak were hybridized to metaphase preparations of each taxon (see Figure 2 for examples in a male of *S. araneus* Cordon). In *S. granarius*, 12 peaks each contained a single type of chromosome, whereas the other four peaks each contained multiple types of chromosomes (i.e.,  $j + k + l$ ,  $r + tu$ ,  $q + r$ ,  $m + o$ ). In addition, chromosomes  $m$  and  $o$  were each represented in a second individual peak. In *S. araneus* Cordon, a single type of chromosome was found in 13 peaks and one peak contained two types of chromosomes ( $o + q$ ). Finally, all 12 peaks of *S. araneus* Novosibirsk each contained a single type of chromosome. Most chromosomes could reliably be assigned to a specific flow peak; therefore, these flow-sorted chromosomes can be used for genetic mapping purposes.

### *Microsatellite mapping*

The mapping results of the 46 microsatellite loci for the three *Sorex* taxa are summarized in Table 2. Among the 41 loci showing positive amplification in *S. granarius*, 26 were each assigned to only one chromosome. For the *S. araneus* Cordon and Novosibirsk, 38 and 40 loci showed positive amplification, respectively, and 23 and 21 loci were each assigned to a single chromosome respectively. These cases clearly provide the best evidence of presence of the

locus on a particular chromosome, particularly when different taxa give the same assignment on their corresponding chromosomes. Such cases where a locus was assigned to multiple chromosomes could be assigned with less confidence.

The most likely localizations have been deduced for 35 microsatellite markers, 25 of which each showed a similar single assignment in different taxa (bold assignments, Table 2) and are therefore considered unambiguously mapped. At least nine of the 18 autosomal chromosome arms found in the *S. araneus* group were represented by these markers: *a* (3 loci), *b* (5), *c* (1), *f* (3), *g* (1), *h* (1), *j* or *l* (2), *n* (1) and *o* (2). We were not able to discriminate between the chromosome arms of the metacentric chromosome *jl* since these arms were part of the same chromosome sort in *S. granarius*. The remaining six loci mapped to the sex chromosomes. The chromosome X (*de*) is the product of a Robertsonian fusion between the 'original' mammalian X (most of arm *e*) and an autosome (all of arm *d* and part of arm *e*; Pack *et al.* 1993). Chromosome Y2 in males corresponds to this autosome and is therefore homologous to chromosome arm *d*. Four loci (L57, C19, C171 and D138) were assigned to chromosomes X and Y2 in *S. araneus* Novosibirsk and probably map to the chromosome arm *d*. One locus (L13) was assigned only to the X chromosome in the same species, suggesting localization on chromosome arm *e*, the 'true' X chromosome. Finally, as expected, the male-specific locus L8Y (Balloux *et al.* 2000) mapped to chromosome Y1, the true Y. The distribution of our mapped loci was not significantly different from the expected null distribution according to chromosome arm size ( $\chi^2 = 0.584$ , d.f. = 17;  $p = \text{NS}$ ). Microsatellite loci thus did not appear to cluster on any particular chromosome. Only one locus assigned to single chromosome sorts in the three taxa showed discordance among the taxa (locus D11 mapped on chromosome *b* in *S. granarius* and *af* in *S. araneus* Cordon and Novosibirsk).

## Discussion

Data concerning genetic exchange between chromosome arms in the *S. araneus* group are scarce. Using high-resolution chromosome analysis, Volobouev & Catzefflis (1989) suggested only a few centromeric shifts between *S. granarius* and *S. araneus*. Our study

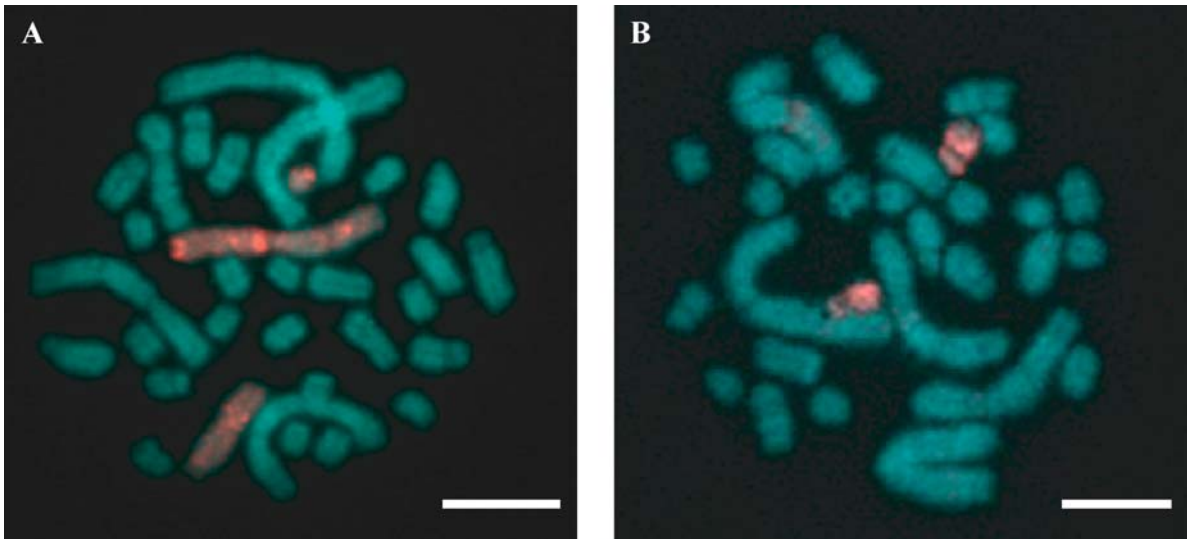


Figure 2. Examples of *Sorex araneus* Cordon male metaphase spreads showing fluorescence of individual chromosome painted by chromosome-specific probes generated from flow-sorted chromosomes: (A) Chr X (*de*); (B) Chr *k*. (Scale bars represent 10  $\mu\text{m}$ .)

corroborates these findings and suggests that, despite the presence of numerous Robertsonian rearrangements, the organization of the genome in the *S. araneus* group is well conserved. Consequently, this high level of conservation suggests that our markers can be used to study and compare the genetic structure within and among the different species and chromosome races of the *S. araneus* group at the level of individual chromosomes.

Due to PCR amplifications in more than one chromosome-specific sort, several loci could not be each unambiguously assigned to a specific chromosome (e.g., question marks in Table 2). Interestingly, these loci were in general ambiguous in all three taxa. Several non-exclusive reasons may explain this pattern: i.e., lack of specificity of markers, contamination between sorts or genomic rearrangements.

First, the competition among binding sites on a single chromosome is probably weaker than over the whole genome. Therefore, the specificity of a primer pair may decrease when tested on isolated chromosomes and allow unspecific amplification. Nevertheless, most of our attempts to increase PCR specificity and design new primer pairs did not improve our capacity to assign loci to a single chromosome sort.

Second, the purity by which individual chromosomes can be sorted can approach but never reach 100% (Ferguson-Smith 1997, Doležel *et al.* 2004;

Ibrahim & van den Engh 2004). Therefore, chromosomes of similar sizes and GC/AT ratios could contaminate the desired chromosome sort. However, some markers were mapped to chromosomes that are quite distant on the flow karyotype. In such cases the flow rate might have been too fast, allowing more than one chromosome to be sorted together. Alternatively, the fragmentation and/or clumping of chromosomes might have altered their size and caused them to be sorted in the wrong sort. No apparent contaminations (except sorting of multiple chromosomes in several sorts) were detected by our painting experiments; but these experiments may fail to detect low-level DNA contamination, which is not the case in PCR.

Finally, sequence duplication is common in the mammalian genome (Samonte & Eichler 2002; Thomas *et al.* 2004). Although the evolution of duplicated non-coding regions (including microsatellites) is still poorly investigated, it is likely that some of our loci belong to such regions. When a locus with high mutation rate (such as microsatellites) is duplicated, it is expected that some individuals bear more than two alleles. The locus C122 probably illustrates this situation since several tri- or tetraploid individuals were detected for this locus. Additionally, this locus was assigned (after numerous attempts to increase PCR specificity) to the same two chromosome arms (*a* and *g*) in the three taxa. Contamination

Table 2. Assignment results of the 46 microsatellite markers in the three karyotypic taxa *Sorex granarius*, *S. araneus* Cordon and *S. araneus* Novosibirsk

Locus	<i>Sorex granarius</i>	<i>S. araneus</i> Cordon	<i>S. araneus</i> Novosibirsk	Most likely localization
L16	<i>a</i>	<i>af</i>	<i>af</i>	<b><i>a</i></b>
D107	<i>a</i>	<i>af</i>	<i>af</i>	<b><i>a</i></b>
D112	<i>a</i>	<i>af</i>	<i>af</i>	<b><i>a</i></b>
L2	<i>b</i>	n.a.	<i>bc</i>	<b><i>b</i></b>
L68	<i>b</i>	<i>bc</i>	<i>bc</i>	<b><i>b</i></b>
B10	<i>b</i>	<i>bc</i>	<i>bc</i>	<b><i>b</i></b>
C100	<i>b</i>	<i>bc</i>	<i>bc</i>	<b><i>b</i></b>
C117	<i>b</i>	<i>bc</i>	<i>bc</i>	<b><i>b</i></b>
L45	<i>b i de</i>	<i>bc af jl de ut</i>	<i>bc af</i>	<i>b</i>
L92	<i>b de</i>	<i>bc de</i>	<i>af bc</i>	<i>b</i>
L97	–	<i>bc i</i>	<i>bc</i>	<i>b</i>
D110	<i>b de c</i>	<i>bc jl ut</i>	<i>bc af qr de d</i>	<i>b</i>
L9	<i>c</i>	<i>bc</i>	–	<b><i>c</i></b>
L57	<i>de</i>	<i>de</i>	<i>de d</i>	<b><i>d</i></b>
C19	<i>de</i>	<i>de</i>	<i>de d</i>	<b><i>d</i></b>
C171	<i>de</i>	n.a.	<i>de d</i>	<b><i>d</i></b>
D138	<i>de</i>	<i>de</i>	<i>de d</i>	<b><i>d</i></b>
L13	<i>de</i>	<i>de</i>	<i>de</i>	<b><i>e</i></b>
L69	<i>f</i>	–	<i>af</i>	<b><i>f</i></b>
B3	<i>f</i>	<i>af</i>	<i>af</i>	<b><i>f</i></b>
B15	<i>f</i>	<i>af</i>	–	<b><i>f</i></b>
C119	<i>blf</i>	n.a.	<i>af ik go</i>	<i>f</i>
A25	<i>blf</i>	<i>af r mp</i>	<i>af mp</i>	<i>f</i>
D23	<i>flj,k,l</i>	–	<i>af go</i>	<i>f</i>
L62	<i>g</i>	<i>g</i>	<i>go</i>	<b><i>g</i></b>
B7	n.a.	<i>g k</i>	<i>af go</i>	<i>g</i>
D106	<i>h</i>	<i>h</i>	–	<b><i>h</i></b>
A8	–	<i>jl</i>	–	<b><i>jl</i></b>
D24	<i>j, k, l</i>	<i>jl</i>	<i>jl</i>	<b><i>jl</i></b>
L33	<i>al,m, o</i>	<i>i/o, q m</i>	<i>mp</i>	<i>m</i>
L99	<i>n</i>	<i>n</i>	<i>hn</i>	<b><i>n</i></b>
B30	<i>o</i>	<i>o, q</i>	<i>go</i>	<b><i>o</i></b>
D109	<i>f</i>	<i>o, q</i>	<i>go</i>	<b><i>o</i></b>
L14	<i>flm o de</i>	<i>g i/o, q</i>	<i>af go</i>	<i>o</i>
L8Y	n.a.	n.a.	<i>s</i>	<b><i>s</i></b>
D11	<i>b</i>	<i>af</i>	<i>af</i>	<i>?</i>
C5	<i>ai de</i>	–	–	<i>?</i>
C25	<i>fli</i>	<i>af i</i>	<i>af mp ik go y1</i>	<i>?</i>
C122	<i>alg</i>	<i>af g</i>	<i>af go</i>	<i>?</i>
L67	<i>b i q, r</i>	<i>af g</i>	<i>bc qr</i>	<i>?</i>
B5	<i>m i</i>	<i>af jl g p</i>	<i>af mp y2</i>	<i>?</i>
B12	n.a.	<i>r m</i>	n.a.	<i>?</i>
C240	<i>i</i>	<i>jl p</i>	<i>af</i>	<i>?</i>
C151	<i>alf</i>	n.a.	<i>af mp</i>	<i>?</i>
D29	<i>b de m, o</i>	<i>ut</i>	<i>af mp</i>	<i>?</i>
D103	<i>i</i>	<i>m ut</i>	<i>mp af</i>	<i>?</i>

Autosomal and sexual chromosome arm names follow the traditional chromosome nomenclature in the *S. araneus* group (Searle *et al.* 1991). Unsorted chromosomes and multiple assignments are indicated by commas and slashes respectively. No amplification of correct size on sorted chromosome (–) nor on both sorted chromosome and positive control (n.a.). Most likely chromosome or chromosome arm assignments are indicated in the last column of the table: in bold, loci showing corresponding single assignment in different taxa; question marks, loci which cannot be assigned with confidence.



between these chromosomes in the three taxa is highly unlikely since these chromosome arms are combined in acrocentric or metacentric chromosomes of different size (i.e., *alg* in *S. granarius*, *afg* in *S. araneus* Cordon and *afgo* in *S. araneus* Novosibirsk).

As previously mentioned, four microsatellite loci have already been mapped by Zhdanova *et al.* (2003) using somatic cell hybrid panels of *S. araneus* Novosibirsk. For two loci the situation was consistent between the two studies: locus L16 was unambiguously mapped to chromosome *af* and the most likely localization for locus L14 is on chromosome arm *o*. The two other loci (L92 and L67), however, give conflicting results. These loci were respectively mapped to chromosome *af* and *jl* by Zhdanova *et al.* (2003) whilst both loci amplified in more than one chromosome sort in our study. Our results for locus L92 point towards chromosome arm *b*, although an amplification product was also detected on chromosome *af* in *S. araneus* Novosibirsk. As for locus L67, the situation is even more complex: our results do not allow for any conclusions but no amplification on chromosome *jl* in any of the three taxa was detected. A possible explanation for these discrepancies could be the high rate of hidden chromosomal rearrangements noticed in the shrew somatic cell hybrid panels (Zhdanova *et al.* 2003). However, as already mentioned, our method suffers from several uncertainties that cannot be ruled out for these loci.

## Conclusions

PCR-based localization of genetic markers on flow-sorted chromosomes has been used in a variety of groups (e.g., Sargan *et al.* 2000, Doležel *et al.* 2004). Although this method suffers from several limitations (e.g., genetic markers are assigned to chromosomes but not positioned on them), we have been able to assign unambiguously 25 microsatellite markers on three karyotypically different taxa of the *S. araneus* group. Also, the use of two particularly acrocentric taxa allowed for most assignments at the chromosome arm level. This last point should not be underestimated since this group shows an extraordinarily large number of Robertsonian metacentrics. Therefore we

provide a microsatellite markers map that includes the Y chromosome (Y1), the two arms *d* and *e* of the X chromosome and at least nine of the 18 autosomal chromosome arms observed in this group.

The role of chromosomal rearrangements in the speciation process is still subject to controversy (for recent review see Coyne & Orr 2004). Recently, several studies used mapped genetic markers to show that some genomic regions experience stronger barriers to gene flow than others and that these regions are linked to rearranged chromosomes (e.g., Rieseberg *et al.* 1999, Panithanarak *et al.* 2004). As previously mentioned, the outstanding karyotypic variation of the *S. araneus* group makes it an interesting model for studying the role of chromosomal rearrangement in the speciation process. Our results show a high level of conservation in the localization of markers among the studied taxa. They will therefore provide useful tools in both comparison of genetic structure among taxa at the individual chromosome level, and evaluation of the role of chromosomal rearrangements in the genetic diversification and speciation process of this group.

## Acknowledgements

We are particularly grateful to Aline Dépraz, Jeremy Searle and an anonymous reviewer for helpful comments on the manuscript. This work was supported by the Swiss NSF (grants no: 3100-05943), a Société Académique Vaudoise (Switzerland) grant to P.B. and a Fondation Agassiz (Switzerland) grant to P.B.

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