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RESTRICTED GENE FLOW AT SPECIFIC PARTS OF THE SHREW GENOME IN CHROMOSOMAL HYBRID ZONES

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Abstract.—The species and races of the shrews of the *Sorex araneus* group exhibit a broad range of chromosomal polymorphisms. European taxa of this group are parapatric and form contact or hybrid zones that span an extraordinary variety of situations, ranging from absolute genetic isolation to almost free gene flow. This variety seems to depend for a large part on the chromosome composition of populations, which are primarily differentiated by various Robertsonian fusions of a subset of acrocentric chromosomes. Previous studies suggested that chromosomal rearrangements play a causative role in the speciation process. In such models, gene flow should be more restricted for markers on chromosomes involved in rearrangements than on chromosomes common in both parent species. In the present study, we address the possibility of such differential gene flow in the context of two genetically very similar but karyotypically different hybrid zones between species of the *S. araneus* group using microsatellite loci mapped to the chromosome arm level. Interspecific genetic structure across rearranged chromosomes was in general larger than across common chromosomes. However, the difference between the two classes of chromosomes was only significant in the hybrid zone where the complexity of hybrids is expected to be larger. These differences did not distinguish populations within species. Therefore, the rearranged chromosomes appear to affect the reproductive barrier between karyotypic species, although the strength of this effect depends on the complexity of the hybrids produced.

Key words.—Chromosomal rearrangements, genetic structure, hybrid zones, reproductive isolation, *Sorex araneus,* speciation.

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Closely related species or even populations within one species are often characterized by differences in karyotype. This observation has prompted several authors to argue that chromosomal rearrangements, such as Robertsonian fusions and fissions, translocations, and inversions, may play a causative role in speciation (e.g., King 1993; Noor et al. 2001; Rieseberg 2001; Navarro and Barton 2003a). Many theoretical models suggest that chromosomal rearrangements facilitate speciation by accelerating genetic differentiation between populations. Traditional models claimed that rearrangements cause meiotic problems for heterozygous individuals and therefore reduce their fertility and reproductive fitness (White 1978; King 1993). In contrast, recent models emphasize a reduction or a suppression of recombination in heterokaryotypes (Noor et al. 2001; Rieseberg 2001; Navarro and Barton 2003a). These models were mainly developed thinking about inversions, which affect recombination especially strongly. However, they can also be applied to other categories of rearrangements (such as Robertsonian fusions), which are also known to affect the frequency and distribution of crossovers (e.g., Bidau et al. 2001; Banaszek et al. 2002; Merico et al. 2003). Although each model has unique features (for recent reviews, see Spirito 2000; Rieseberg 2001; Coyne and Orr 2004; Ayala and Coluzzi 2005; Butlin 2005), all suggest an interesting possibility: gene exchange frequencies should be differentiated according to the chromosome on which they are located. Therefore, chromosomal rearrangements should induce barriers or filters to gene flow that would be specific to some parts of the genome.

The shrews of the *Sorex araneus* group offer an exceptional opportunity to study the impact of chromosomal rearrangements on gene flow. They display one of the most outstanding chromosomal polymorphism rates found among mammals and offer a complete array of every possible level of chromosomal and genetic differentiation. The species of the western clade of this group (*S. araneus*, *S. antinorii*, *S. coronatus* and *S. granarius*) are characterized by homologous chromosome arms, which are labeled from *a* to *u* according to their size (Searle et al. 1991). In the type species, *S. araneus*, Robertsonian polymorphisms are particularly prevalent. The three pairs of metacentric autosomes *af, bc,* and *tu* as well as the sexual chromosomes are invariant, whereas the primitive acrocentric autosomes *g* to *r* may be distributed into various acrocentric and metacentric combinations. Together, these polymorphisms allowed more than 60 chromosome races to be described (Wójcik et al. 2003).

Hybrid zones are often cited as ''natural laboratories for evolutionary studies'' (e.g., Hewitt 1988) and constitute unique opportunities to understand the early processes involved in the establishment of barriers to gene flow and speciation (Barton and Hewitt 1985; Harrison 1990). These systems are therefore among the most interesting for studying the role of chromosomal rearrangements in speciation.

European species and chromosome races of the *S. araneus* group are often parapatric and form hybrid zones of various sizes and shapes (for reviews, see Searle and Wójcik 1998; Wójcik et al. 2002). In such a context, the hybrid zones involving *S. araneus* and *S. antinorii* are of special interest. These species most likely diverged genetically in allopatry

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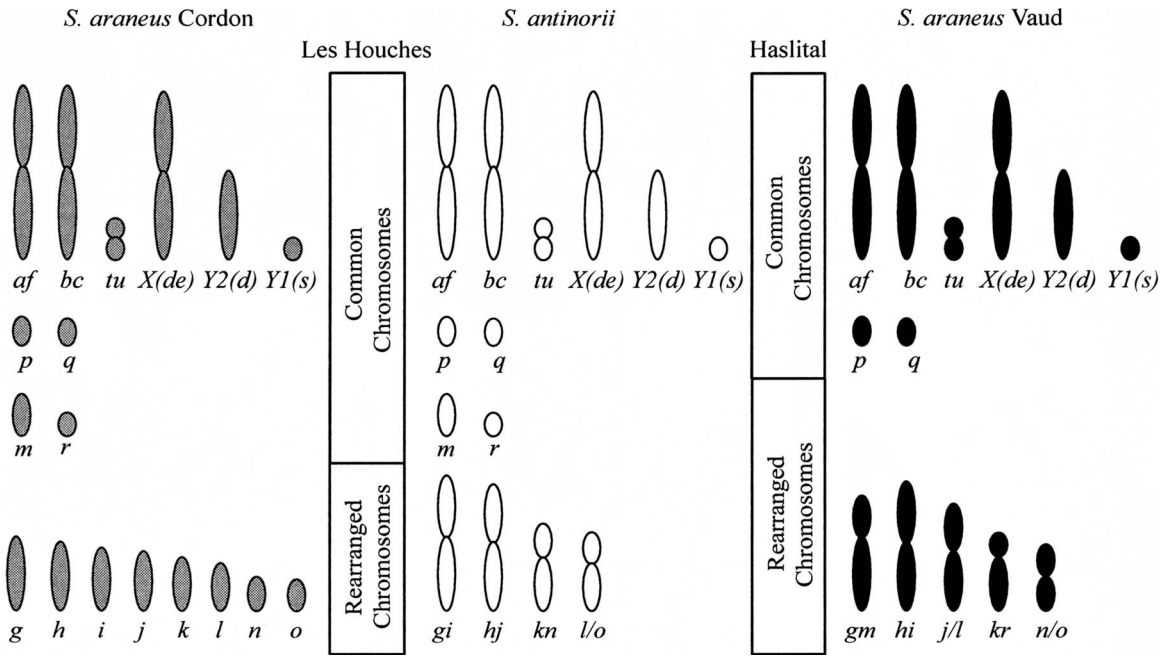

FIG. 1. Karyotypes of the three taxa involved in the two hybrid zones (*Sorex antinorii* and *S. araneus* Cordon in LH, and *S. araneus* Vaud in HT).

during the last Pleistocene glaciations and are likely to have had a long period of independent evolution. The glacial refugia of *S. araneus* were probably situated in southeastern Europe (Taberlet et al. 1994), whereas *S. antinorii* was certainly restricted to refugia situated in the Apennine Peninsula (Brünner et al. 2002a,b; Lugon-Moulin and Hausser 2002). This latter species crossed several lower alpine passes in the Swiss and French Alps after the last Pleistocene glaciations (Lugon-Moulin and Hausser 2002) and came into contact with *S. araneus*.

In this study, we examine the role of karyotypic differences on the genetic structure of two hybrid zones between these species. Both hybrid zones are extremely narrow (<1 km wide) and the observed genetic clines are very steep (Brünner and Hausser 1996; Brünner et al. 2002b). The *S. araneus* chromosome races involved in each hybrid zone are not the same. In the first one, *S. antinorii* (characterized by the metacentrics *gi, hj, kn,* and *lo*; Fig. 1) meets the Cordon race at Les Houches (hereafter LH) in the French Alps (Fig. 2). This chromosome race is one of the most acrocentric of *S. araneus* and it shows clear signs of admixture with *S. antinorii* (Basset et al. 2006a). In the second one, *S. antinorii* meets the Vaud race of *S. araneus* at Haslital (hereafter HT) in the Swiss Alps (Fig. 2). This chromosome race is genetically very similar to the Cordon race (Taberlet et al. 1994; Basset et al. 2006a), but it has a much more metacentric karyotype characterized by *mg, hi, jl, kr,* and *no.*

The status of individuals with hybrid karyotypes is therefore quite different in the two hybrid zones. In LH, all the hybrids sampled were of backcross origin (F_x with $x > 1$; Brünner and Hausser 1996), and F_1 hybrids are expected to carry four trivalents (simple heterozygous, C-III; Fig. 3). This category of hybrids has good survival and does not seem to suffer a reduction in fertility (Narain and Fredga 1997, 1998; Banaszek et al. 2002), and species-specific chromosomes can almost freely introgress into heterospecific populations (Brünner et al. 2002b). In HT, most of the F_1 hybrids with *S. antinorii* present a long chain of 11 elements (complex heterozygous, C-XI; Fig. 3), which should severely impede recombination and fertility of hybrids (Narain and Fredga 1997, 1998; Banaszek et al. 2002). To obtain balanced gametes during meiosis, the species-specific chromosomes have to segregate in the same way as when they formed the F_1 zygote. Therefore, they form a large linkage block. Thus, chromosomal incompatibilities during meiosis are expected to be much larger in HT than in LH hybrid zone. Moreover, when comparing the karyotype of the taxa involved in the two hybrid zones, it is possible to define one group of chromosomes similarly arranged as common acrocentrics or metacentrics and another group of chromosomes rearranged in different acrocentrics or metacentrics (Fig. 1). We will use the nomenclature ''common'' or ''rearranged'' throughout this paper to identify these two groups.

Genetic analyses of both hybrid zones showed that the species-specific status is the main cause of genetic divergence among populations, with the effect of distance or geographic barriers being weak (Lugon-Moulin et al. 1999a; Brünner et al. 2002b). However, comparing these genetically very similar but karyotypically very different hybrid zones, Brünner et al. (2002b) was unable to discriminate between reproductive barriers caused only by genetic factors or in combination with chromosomal differences.

Only by studying gene flow at the chromosome level can the role of these chromosomal rearrangements as a reproductive barrier be addressed. Few studies have addressed the question of differential gene flow in natural hybrid zones (e.g., Rieseberg et al. 1999; Panithanarak et al. 2004), and they examined model species for which genetic maps of

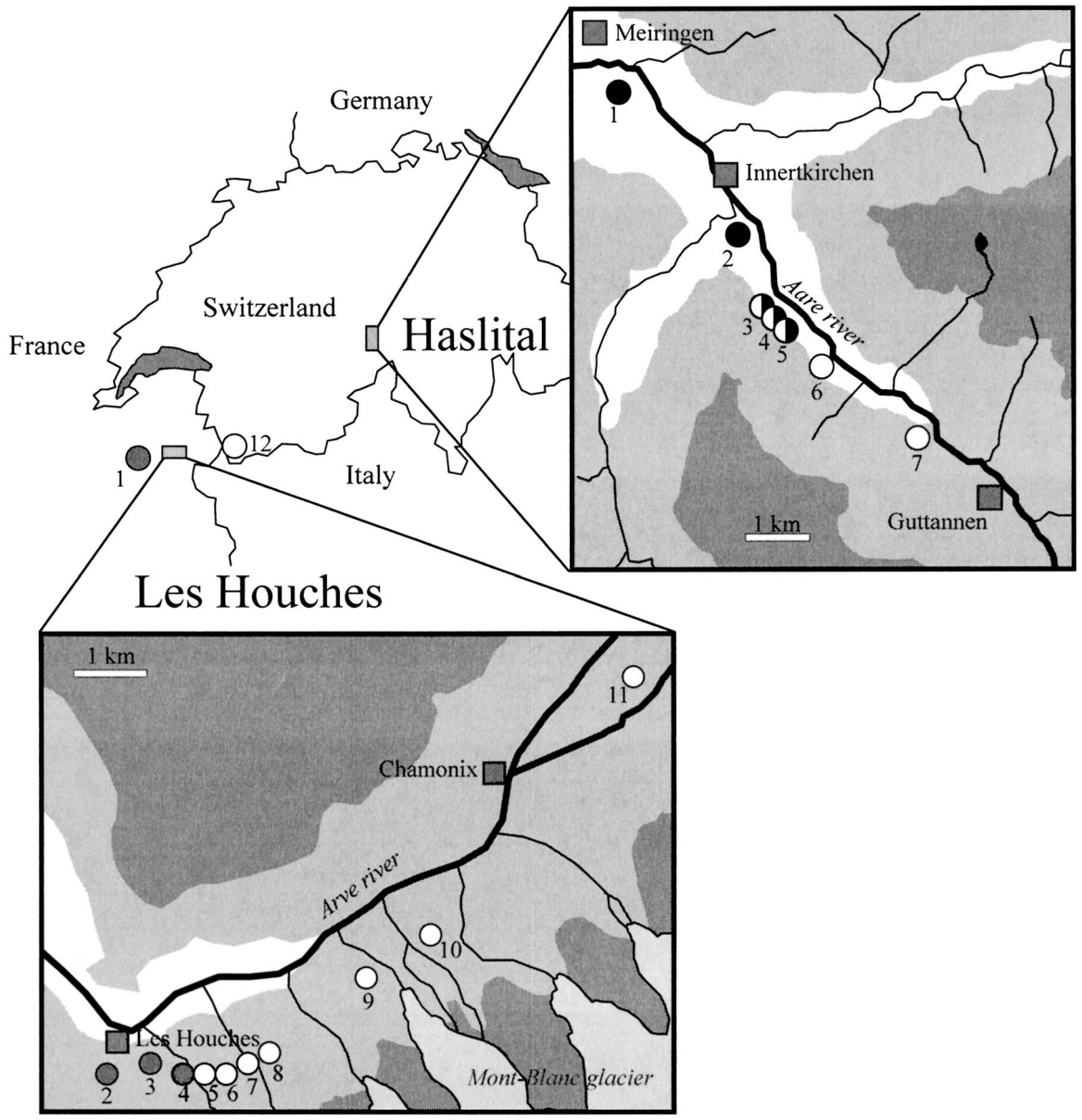

FIG. 2. Study area and sampling localities in the Les Houches and Haslital hybrid zones. Open circles, *Sorex antinorii*; black circles, *S. araneus* Vaud; gray circles, *S. araneus* Cordon. Both species are present in localities 3, 4, and 5 of the Haslital hybrid zone. In hybrid zone insert maps: light gray, area above 1000 m above sea level; dark gray, area above 2000 m above sea level.

high density are available (e.g., sunflowers or house mice). Recently Basset et al. (2006b) mapped more than 20 microsatellite markers to the chromosome arm level in the *S. araneus* group. Several markers are located on chromosome arms belonging to the common group, while others are located on chromosome arms belonging to the rearranged group. Thus, the goals of the present study are to compare the genetic structure measured over the common and rearranged groups of chromosomes and to compare the levels of genetic structure observed in the two *Sorex* hybrid zones. Estimates of population differentiation using microsatellite markers are often subject to heterogeneity, and variation across loci can sometimes be strong (e.g., caused by variation in mutation rate or homoplasy; Balloux and Lugon-Moulin 2002). Moreover, nothing is known about the position of the *Sorex* microsatellites within the chromosomes (Basset et al. 2006b). This factor could add noise to our analyses because differences in recombination rates are expected along the chromosomes (e.g., Kauppi et al. 2004). Nevertheless, if karyotypic differences act as a reproductive barrier, our primary prediction is that genetic structure is higher for rearranged chromosomes than for common chromosomes. Additionally, because the complexity of the hybrids produced in the HT hybrid zone (complex heterozygotes) is larger than in the LH hybrid zone (simple heterozygotes), our second prediction is that the difference between the two groups of chromosomes is larger in the HT than in the LH hybrid zone.

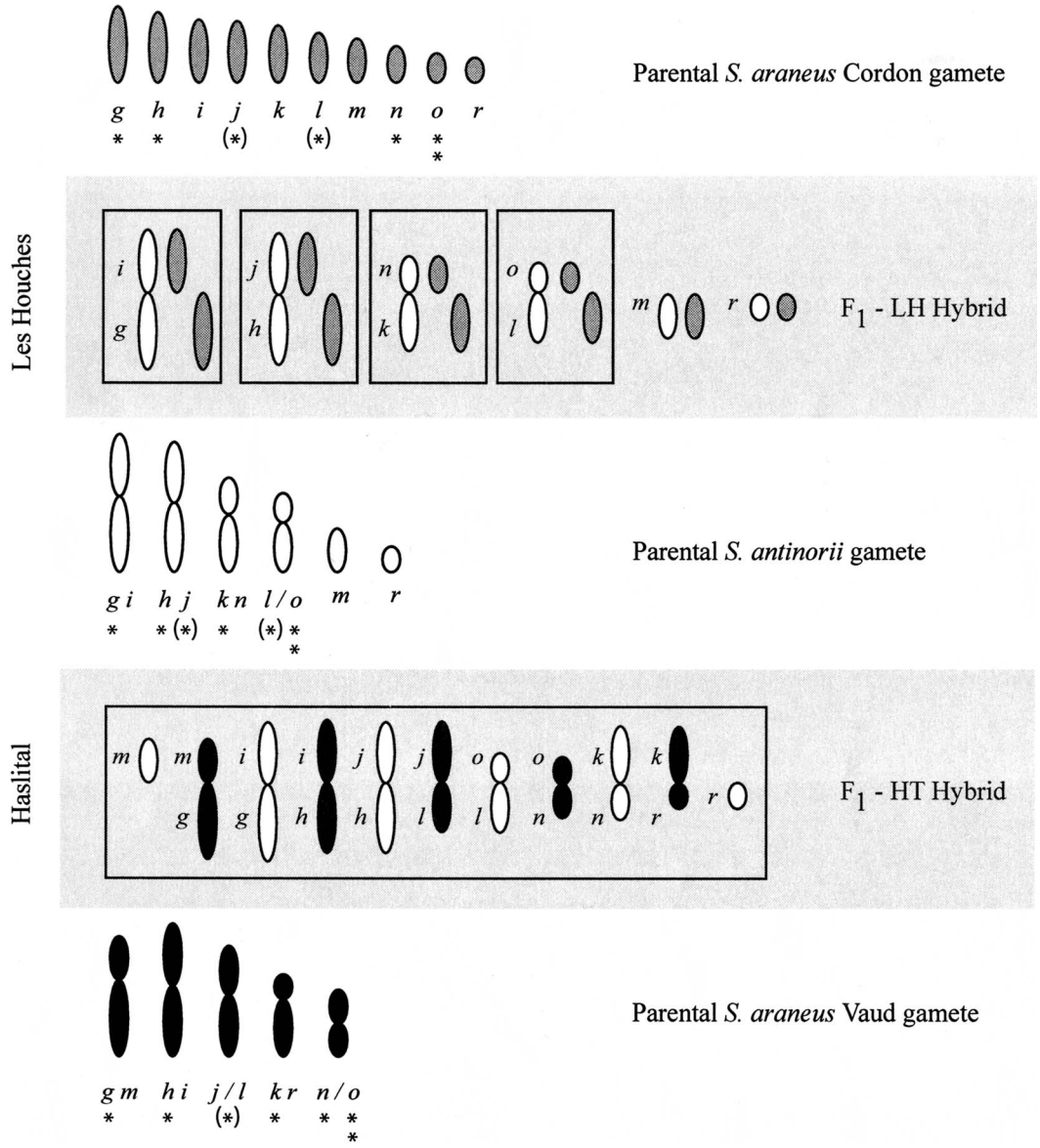

FIG. 3. Rearranged chromosomes in the three parent taxa and their chromosomal combinations in the F_1 hybrids of Les Houches and Haslital hybrid zones (i.e., simple vs. complex heterozygotes). Black rectangles indicate the heterozygote configurations in F_1 hybrids. The chromosome arm localization of the markers of the rearranged group is indicated by asterisks. The position of locus D24 is indicated by parentheses because its position is not clear (*j* or *l* chromosome arm; Basset et al. 2006b).

MATERIALS AND METHODS

Specimens from the Hybrid Zones

Most of the specimens analyzed in the LH hybrid zone were collected in 1992–1995 (except localities 1 and 12 sampled in 2003) as part of earlier studies (Brünner and Hausser 1996; Lugon-Moulin et al. 1996). These shrews were trapped in 12 localities, and the mean sample size for each locality was 15 (range 4–29; Table 1). In the HT hybrid zone, all specimens analyzed were collected in 1992–1995 as part of an earlier study (Brünner et al. 2002b). These shrews were trapped in seven localities and the mean sample size for each locality was 16 (range 6–33; Table 1).

According to karyotype analysis (Brünner and Hausser 1996; Brünner et al. 2002b), all localities could be classified as containing one or both species (Table 1). Note that the two species are only found in sympatry in the three central localities of the HT hybrid zone. Because the goal of this study was to compare levels of genetic structure between species, these three localities (nos. 3, 4, 5) were split according to karyotype into monospecific subsamples. For this reason, no individuals with hybrid karyotypes have been analyzed in this study. Trapping and karyotype preparation conditions were described in Brünner and Hausser (1996) and Brünner et al. (2002b).

DNA Extraction and Microsatellite Typing

Tissue samples (phalanges) were stored in alcohol (70%) at 4°C and total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA).

TABLE 1. Number of *Sorex* individuals analyzed of each taxon per sample sites (see maps in Figure 1 for trapping localities) in both hybrid zones. Number of karyotyped individuals is given in parentheses.

Les Houches			Haslital		
Locality	*S. araneus* Cordon	*S. antinorii*	Locality	*S. araneus* Vaud	*S. antinorii*
1	29 (4)	0	1	6 (6)	0
2	4 (1)	0	2	7 (6)	0
3	5 (4)	0	3	8 (8)	5 (5)
4	21 (21)	0	4	12 (12)	21 (21)
5	0	17 (12)	5	7 (7)	25 (25)
6	0	20 (14)	6	0	11 (5)
7	0	18 (13)	7	0	13 (11)
8	0	14 (13)	Total	40 (39)	75 (67)
9	0	8 (6)			
10	0	8 (7)			
11	0	9 (8)			
12	0	22 (4)			
Total	59 (30)	116 (77)			

Seventeen microsatellite loci were chosen from the loci unambiguously mapped to the chromosome arm level in Basset et al. (2006b), and the letters in parentheses indicate their chromosome localization: L16 (*a*), L69 (*f*), B3 (*f*), D107 (*a*), D112 (*a*), L9 (*c*), L68 (*b*), C117 (*b*), L13 (*de*), C171 (*de*), L57 (*de*), L62 (*g*), D24 (*jl*), D106 (*h*), L99 (*n*), B30 (*o*), D109 (*o*). The first 11 loci belong to the common group and the last six to the rearranged group (Fig. 1).

Polymerase chain reaction (PCR) conditions are described elsewhere (Wyttenbach et al. 1997; Balloux et al. 1998; Lugon-Moulin et al. 2000; Basset et al. 2006a,b), except that all PCR amplifications were performed in a 20-μl total volume. Cycling was carried out in a PE9700 (Applied Biosystems, Foster City, CA) using the following profile: 95°C for 5 min; 35 cycles of 30 s at 94°C, 30 s at annealing temperature (Basset et al. 2006b), 30 s at 72°C; and a final extension at 72°C for 4 min. One primer of each pair was labeled with a fluorescent dye (HEX, FAM, or NED) on the 5′end, which allowed analyses on an ABI 377XL sequencer (Applied Biosystems). Data collection, sizing of the bands, and analysis were done using the GeneScan software (Applied Biosystems). The individuals already genotyped at some loci (L9, L16, L57, L62, and L69) in other studies (Lugon-Moulin et al. 1999a; Brünner et al. 2002b) were not analyzed again for this study.

Statistical Analyses

The software package FSTAT version 2.9.3 (updated from Goudet 1995; http://www2.unil.ch/popgen/softwares/fstat.htm) was used to calculate allele frequencies, allele numbers, observed heterozygosities (H_O), and expected heterozygosities within (H_S) and between (H_T) samples, following Nei (1987). Both *F*-statistics (i.e., deviation from Hardy-Weinberg assuming that mutations follow the infinite allele model [IAM]; Wright 1965) and *R*-statistics (i.e., an analogue of *F*-statistics assuming a stepwise mutation model [SMM]; Slatkin 1995) have their strength and drawbacks in inferring genetic structure from microsatellite data (for a review, see Balloux and Lugon-Moulin 2002). However, it is not our aim in this study to compare the relative efficiency of these statistics. Therefore, we decided to analyze genetic structure using estimates derived from *F*-statistics according to Weir and Cockerham (1984), using FSTAT version 2.9.3. Allele frequencies were weighted according to sample size. Heterozygote deficit within populations ($F_{IS} > 0$) was tested using a permutation procedure (10,000 randomizations) to infer random mating. The exact *G*-test (Goudet et al. 1996), as implemented in FSTAT 2.9.3, was used to assess the significance of genetic differentiation. To assess population structure within and between the different taxa, only monospecific populations were analyzed (i.e., central populations in HT were split into monospecific subpopulations). Hierarchical estimates of *F*-statistics, that is, deviations from Hardy-Weinberg equilibrium within a population (F_{IS}) and within the entire zone (F_{IT}) and differentiation of populations within species (intraspecific, F_{SR}) and between populations of the two species (interspecific, F_{RT}; Weir 1996) were obtained using the software package Arlequin version 2.000 (Schneider et al. 2000; http://anthro.unige.ch/arlequin). The genetic variance at different hierarchical levels (within and between species) was estimated using AMOVA (Michalakis and Excoffier 1996) in Arlequin 2.000.

When heterozygote deficit was found for a population, the software MicroChecker version 2.2.3 was used to look for genotyping errors (e.g., null alleles; Van Oosterhout et al. 2004, http://www.microchecker.hull.ac.uk). In cases where null alleles were found, adjusted genotype frequencies (Chakraborty et al. 1992; Brookfield 1996; Van Oosterhout et al. 2004) were used to insure that this did not influence the genetic structure results.

Differences between the two groups of chromosomes (common and rearranged) were tested by permutation tests. A distribution of differences between groups was generated by doing 10,000 permutations of microsatellite loci between the two groups and the observed difference was compared to this distribution.

Preliminary results and the large genetic variance observed among microsatellite loci estimations seemed to indicate that historical factors related to markers (such as homoplasy) could mask a part of the difference observed between the two classes of chromosomes. Therefore, we reanalyzed the center of each hybrid zone independently because the impact of chromosomal rearrangements on gene exchange should be the stronger in these regions. The center of both zones was defined by the localities where karyotypic hybrids had been found (Brünner et al. 1996; Brünner et al. 2002b), which correspond to populations 3, 4, 5, 6, 7, and 8 in LH and 3, 4, and 5 in HT (Fig. 2).

RESULTS

Polymorphism, Genetic Variability of Loci, and Heterozygote Deficit within Population

The number of total alleles and species-specific alleles as well as the observed and expected heterozygosities from the LH and HT hybrid zones are detailed in Supplementary Tables 1 and 2, respectively (available online only at http://dx.doi.org/10.1554/06-181.1.s1). In the two hybrid zones, the number of total and species-specific alleles did not signifi-

TABLE 2. Values of F_{IS} estimated from the loci located on common or rearranged chromosomes and across all loci for the *Sorex araneus* side, the *S. antinorii* side, and the whole Les Houches and Haslital hybrid zones. ''Diff.'' indicates if the values given by the common and rearranged loci are different. Asterisks indicate significant values for the estimators: ***$P < 0.001$; NS, not significant.

	Les Houches					
	S. araneus side		*S. antinorii* side		Overall	
	$F_{IS\text{-}Cordon}$	Diff.	$F_{IS\text{-}ant}$	Diff.	F_{IS}	Diff.
Common loci	0.089***	NS	0.062***	NS	0.071***	NS
Rearranged loci	0.161***		0.155***		0.152***	
All loci	0.115***		0.090***		0.100***	
	Haslital					
	S. araneus side		*S. antinorii* side		Overall	
	$F_{IS\text{-}Vaud}$	Diff.	$F_{IS\text{-}ant}$	Diff.	F_{IS}	Diff.
Common loci	0.139***	NS	0.094***	NS	0.109***	NS
Rearranged loci	0.183***		0.183***		0.183***	
All loci	0.152***		0.123***		0.133***	

cantly differ between loci located across common or rearranged chromosomes (*t*-test, $P \geq 0.645$). In both hybrid zones and across all loci, within-population heterozygote deficit was highly significantly different from zero ($F_{IS\text{-}LH} = 0.100$, $F_{IS\text{-}HT} = 0.133$; Table 2). Although, heterozygote deficit was slightly higher in the rearranged than in the common group, no significant difference was observed between these two groups (permutation test: $P = 0.301$ for LH and 0.289 for HT; Table 2). At least a part of this deficit could be explained by the presence of genotyping errors (Van Oosterhout et al. 2004). In both hybrid zones, null alleles were detected in 12 of the 17 loci in at least one population. Using adjusted frequencies (Van Oosterhout et al. 2004) for these loci, among-population genetic structures across common, rearranged, and all loci were in the same order of magnitude as unadjusted frequencies. Therefore, only the genetic structure results based on observed frequencies are presented in the following sections.

Genetic Structuring

The genetic structure parameters according to Weir and Cockerham (1984) of both hybrid zones are summarized in Figure 4.

LH hybrid zone.—Across all loci, the highly significant F_{ST} (0.082, $P < 0.001$) suggests a moderate genetic structuring. The genetic structure estimated across loci located on rearranged chromosomes is slightly higher than across loci located on common chromosomes ($F_{ST\text{-}rearranged} = 0.109$ vs. $F_{ST\text{-}common} = 0.067$), but this difference is not significant (permutation test: $P = 0.159$). As two species are involved, *F*-statistics were also estimated for *S. araneus* Cordon and *S. antinorii* samples independently (Fig. 4A). In both species, we found highly significant ($P < 0.001$) over all loci F_{SR}-values (0.065 for *S. araneus* Cordon and 0.032 for *S. antinorii*) but we did not find any significant difference between the two groups of loci (Fig. 4A; $F_{SR\text{-}Cordon} = 0.060$ and 0.073, $F_{SR\text{-}antinorii} = 0.029$ and 0.037 for common and rearranged loci, respectively; permutation tests: $P = 0.736$ and 0.403).

HT hybrid zone.—Here also, the highly significant across all loci F_{ST} (0.104, $P < 0.001$) suggests a moderate genetic structuring of this zone. The genetic structure estimated across the rearranged loci was significantly higher than across the common loci ($F_{ST\text{-}rearranged} = 0.181$ vs. $F_{ST\text{-}common} = 0.063$; permutation test: $P = 0.023$). This suggests that chromosomal rearrangements have an effect on gene exchange in this hybrid zone. Although highly significant genetic structures were observed across all loci in both species ($F_{SR} = 0.029$ for *S. araneus* Vaud and $F_{SR} = 0.025$ for *S. antinorii*), we did not find any significant difference between the common and rearranged loci within each species (Fig. 4B; $F_{SR\text{-}Vaud} = 0.024$ and 0.040, $F_{SR\text{-}antinorii} = 0.016$ and 0.043 for common and rearranged loci, respectively; permutation tests: $P = 0.398$ and 0.191).

Hierarchical F-Statistics

In a second step, hierarchical *F*-statistics were estimated for both hybrid zones. Values per locus, across common, across rearranged, and across all loci of both hybrid zones are presented in Tables 3 and 4, and a summary of the genetic structure is given in Figure 4.

LH hybrid zone.—Differentiation of populations within each species (F_{SR}) was highly significant across common (0.037, $P < 0.001$), rearranged (0.048, $P < 0.001$) and all loci (0.041, $P < 0.001$). The values across common and rearranged loci were not significantly different (permutation test: $P = 0.487$). Differentiation of populations between species (F_{RT}) strongly varied across loci (Table 3). Across all loci, this last parameter was highly significant (0.082, $P < 0.001$) and higher than within species. Moreover, this genetic differentiation was higher across rearranged ($F_{RT\text{-}LH\text{-}specific} = 0.122$, $P < 0.001$) than across common loci ($F_{RT\text{-}LH\text{-}common} = 0.059$, $P < 0.001$) but the difference between these two groups was not significant (permutation test: $P = 0.182$).

HT hybrid zone.—Differentiation of populations within each species (F_{SR}) was highly significant across common (0.019, $P < 0.001$), rearranged (0.041, $P < 0.001$), and all loci (0.026, $P < 0.001$). The values of common and rearranged loci were not significantly different (permutation test: $P = 0.222$). Again, differentiation of populations between species (F_{RT}) strongly varied across loci (Table 4), was highly significant across all loci (0.143, $P < 0.001$), and was much higher than within species. This genetic differentiation was

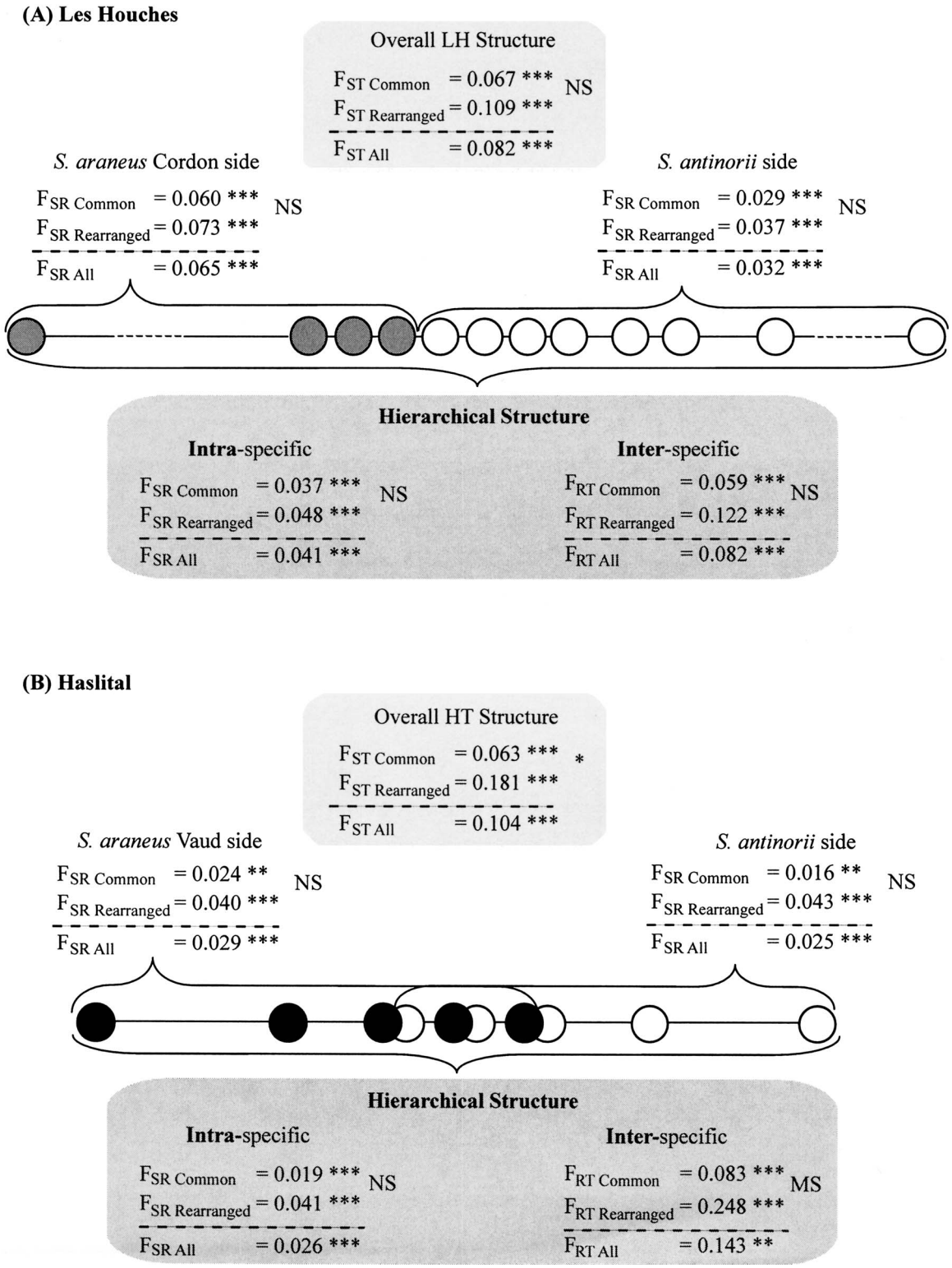

FIG. 4. Summary of the genetic structure observed in the Les Houches and Haslital hybrid zones over common, rearranged, and all loci. Open, gray, and black circles represent the *Sorex antinorii*, *S. araneus* Cordon, and *S. araneus* Vaud populations, respectively.

TABLE 3. Hierarchical *F*-statistics per locus, across common (C), rearranged (R), and all loci in the Les Houches hybrid zone. Subscripts I, S, R, and T stand for individuals, samples, species, and total, respectively. ''Diff.'' indicates the values given by the common and rearranged loci are different. Asterisks indicate significant values for the estimators: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; NS, not significant.

Les Houches hybrid zone								
Chromosome class	Locus	Hierarchical *F*-statistics						
		F_{IS}		F_{SR}		F_{RT}		F_{IT}	
C	L16	0.064 NS		0.149***		0.148*		0.322***	
C	L69	0.114**		0.028***		0.025**		0.160***	
C	B3	0.123***		0.035***		0.012**		0.164***	
C	D107	−0.023 NS		0.032***		0.029**		0.038 NS	
C	D112	0.069**		0.046***		0.011 NS		0.122***	
C	L9	0.019 NS		0.009 NS		0.053**		0.078*	
C	L68	0.012 NS		0.026**		0.104**		0.138**	
C	C117	0.135**		0.017 NS		0.081**		0.218***	
C	L13	0.470***		0.328***		0.021 NS		0.651***	
C	C171	0.085*		0.036**		0.019 NS		0.134**	
C	L57	0.087**		0.003 NS		0.139**		0.215***	
R	L62	−0.033 NS		0.062***		0.089**		0.117**	
R	D24	0.475***		0.032**		0.012 NS		0.497***	
R	D106	0.044 NS		0.071***		0.127**		0.225***	
R	L99	0.065 NS		0.072**		0.375**		0.458***	
R	B30	0.251***		0.037**		0.181**		0.409***	
R	D109	0.023 NS		0.033***		0.008 NS		0.062**	
			Diff.		Diff.		Diff.		Diff.
Common		0.072***	NS	0.037***	NS	0.059***	NS	0.159***	NS
Rearranged		0.152***		0.048***		0.122***		0.291***	
All loci		0.100***		0.041***		0.082***		0.208***	

marginally but significantly higher across the rearranged chromosomes ($F_{RT\text{-}HT\text{-}rearranged} = 0.248$, $P < 0.001$) than across the common chromosomes ($F_{RT\text{-}HT\text{-}common} = 0.083$, $P < 0.001$; permutation test: $P = 0.063$).

Center of Hybrid Zones

Because the center of hybrid zones might provide higher resolution, we estimated hierarchical *F*-statistics in the center of both zones (Table 5). In both zones, heterozygote deficit was highly significant ($F_{IS\text{-}center\text{-}LH} = 0.103$, $P < 0.001$; $F_{IS\text{-}center\text{-}HT} = 0.148$, $P < 0.001$). However, no significant differences were observed between the values estimated across common or rearranged loci (permutation tests: $P = 0.220$ for LH and 0.381 for HT).

Within species.—Population differentiation within species across all loci was highly significant in the center of the LH hybrid zone ($F_{SR\text{-}LH} = 0.024$, $P < 0.001$ but not significant in the center of the HT hybrid zone ($F_{SR\text{-}HT} = 0.006$, $P = 0.305$). Again, in both zones, no significant differences were observed between the values estimated across common or rearranged loci (permutation tests: $P = 0.677$ for LH and 0.694 for HT).

Between species.—Population differentiation between species in the center of the LH hybrid zone across all loci gave a slightly lower value ($F_{RT\text{-}center\text{-}LH} = 0.071$, $P < 0.001$) than across the whole hybrid zone, and no difference was observed between the common and rearranged loci ($F_{RT\text{-}center\text{-}LH\text{-}common} = 0.062$, $P < 0.001$; $F_{RT\text{-}center\text{-}LH\text{-}rearranged} = 0.085$, $P < 0.001$; permutation test: $P = 0.398$). In the center of the HT hybrid zone, population differentiation between species gave a slightly higher value ($F_{RT\text{-}center\text{-}HT} = 0.163$, $P < 0.001$) than over the whole hybrid zone. In this case, the loci located on rearranged chromosomes ($F_{RT\text{-}center\text{-}HT\text{-}rearranged} = 0.276$, $P < 0.001$) were significantly more structured than the loci located on common chromosomes ($F_{RT\text{-}center\text{-}HT\text{-}common} = 0.098$, $P < 0.001$; permutation test: $P = 0.036$). This last result clearly supports the hypothesis that in the center of this hybrid zone, chromosomal rearrangements significantly act as a barrier to gene flow for only some parts of the genome.

DISCUSSION

Overall Genetic Structure in the LH and HT Hybrid Zones

Our results using 17 microsatellite loci indicate a relatively high level of genetic differentiation in both hybrid zones ($F_{ST} \approx 0.10$; Fig. 4) compared to other hybrid zones between karyotypic taxa of the *S. araneus* group (e.g., $F_{ST} \approx 0.02$; Wyttenbach et al. 1999; Andersson et al. 2004). These differences are probably explained by the occurrence in the two alpine hybrid zones of the genetically differentiated *S. antinorii* (Brünner et al. 2002a; Basset et al. 2006a), as suggested by the larger inter- than intraspecific structure observed in both hybrid zones (Fig. 4). The genetic structure values measured in this study are globally similar to what Lugon-Moulin et al. (1999a) and Brünner et al. (2002b) observed in the same hybrid zones using only seven microsatellite loci. This implies that the set of 10 loci added in this study carry similar information to the first seven loci.

Genetic structure over all populations is slightly larger in HT than LH ($F_{ST\text{-}All\text{-}HT} = 0.104$ vs. $F_{ST\text{-}All\text{-}LH} = 0.082$). The only probable explanation for this difference relates to differences between the taxa in contact (*S. antinorii* vs. *S. araneus* Cordon in LH and *S. araneus* Vaud in HT). This is

TABLE 4. Hierarchical *F*-statistics per locus, across common (C), rearranged (R), and all loci in the Haslital hybrid zone. Subscripts I, S, R, and T stand for individuals, samples, species, and total, respectively. ''Diff.'' indicates the values given by the common and rearranged loci are different. Asterisks indicate significant values for the estimators: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; MS, marginally significant ($0.05 \leq P \leq 0.10$); NS, not significant.

Haslital hybrid zone								
Chromosome class	Locus	Hierarchical *F*-statistics						
		F_{IS}		F_{SR}		F_{RT}		F_{IT}	
C	L16	0.098 NS		0.051 NS		0.328*		0.424**	
C	L69	0.032 NS		−0.006 NS		0.073**		0.097*	
C	B3	0.204***		0.010 NS		0.065**		0.263***	
C	D107	0.045 NS		0.003 NS		0.002 NS		0.050 NS	
C	D112	0.045 NS		0.020*		0.043**		0.104**	
C	L9	−0.006 NS		0.000 NS		0.089*		0.084 NS	
C	L68	0.076*		0.024*		0.043**		0.138**	
C	C117	0.068*		0.038**		0.079*		0.174***	
C	L13	0.542***		−0.019 NS		0.319**		0.683***	
C	C171	0.197***		0.062***		0.022 NS		0.263***	
C	L57	0.110**		0.033**		0.026 NS		0.161***	
R	L62	0.076*		0.022*		0.036*		0.128***	
R	D24	0.382***		0.037**		0.049*		0.434***	
R	D106	0.308***		0.000 NS		0.530**		0.675***	
R	L99	0.322***		0.009 NS		0.638*		0.757***	
R	B30	0.060 NS		0.147***		0.203*		0.361***	
R	D109	0.065*		0.017*		0.066**		0.142***	
			Diff.		Diff.		Diff.		Diff.
Common		0.109***	NS	0.019***	NS	0.083***	MS	0.198***	MS
Rearranged		0.183***		0.041***		0.248***		0.411***	
All loci		0.133***		0.026***		0.143**		0.276***	

further supported by the much larger interspecific structure detected in HT compared to LH ($F_{RT\text{-}HT} = 0.143$ and $F_{RT\text{-}LH} = 0.082$). Moreover, larger levels of introgression between *S. araneus* Cordon and *S. antinorii* than between *S. araneus* Vaud and *S. antinorii* were noted by Basset et al. (2006a) at a larger geographical scale. Genetic incompatibilities are predicted to be similar in both hybrid zones because genetic differentiation between the Cordon and Vaud chromosome races is extremely low (Taberlet et al. 1994; Basset et al. 2006a), but as previously mentioned, chromosomal incompatibilities are expected to be stronger in HT. The presence of numerous rivers in the LH hybrid zone could also influence the overall genetic structure observed in this zone (Brünner et al. 2002b). However, rivers have been shown not to constitute a strong barrier to gene flow for these shrews (Lugon-Moulin et al. 1999b). Furthermore, these geographical factors should increase the genetic structure in LH instead of decreasing it. These observations therefore support our hypothesis of an impact of chromosomal rearrangements on the genetic structure of these hybrid zones. Nevertheless, only by separately studying each category of chromosomes (common and rearranged) can other historical factors be ruled out.

Differences between Common and Rearranged Chromosomes

As expected from our primary prediction, in the HT hybrid zone we detected significantly higher levels of genetic structure and interspecific structure (although only marginally significant for the latter) across loci located on the rearranged chromosomes than across loci located on common chromo-

TABLE 5. Hierarchical *F*-statistics over common, rearranged, and all loci in the center of the Les Houches and Haslital hybrid zones. Subscripts I, S, R, and T stand for individuals, samples, species, and total, respectively. ''Diff.'' indicates if the values given by the common and rearranged loci are different. Asterisks indicate significant values for the estimators: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; MS, marginally significant ($0.05 \leq P \leq 0.10$); NS, not significant.

	Hierarchical *F*-statistics							
	F_{IS}	Diff.	F_{SR}	Diff.	F_{RT}	Diff.	F_{IT}	Diff.
Center Les Houches hybrid zone								
Common	0.066***	NS	0.022***	NS	0.062***	NS	0.143***	NS
Rearranged	0.169***		0.029***		0.085***		0.262***	
All loci	0.103***		0.024***		0.071***		0.186***	
Center Haslital hybrid zone								
Common	0.127***	NS	0.005 NS	NS	0.098***	*	0.216***	MS
Rearranged	0.193***		0.008 NS		0.276***		0.420***	
All loci	0.148***		0.006 NS		0.163***		0.291***	

somes (Table 4). Intraspecific structure could be used as a control for the real significance of observed differences because there are no karyotypic differences within species. Not surprisingly, no significant difference was observed between the two classes of chromosomes within species. Therefore, our hypothesis of chromosomal rearrangements playing a role in the reproductive barrier of this hybrid zone is strongly supported.

As previously mentioned, *S. antinorii* probably diverged genetically in allopatry during the last glaciation period (Brünner et al. 2002a). After recolonization, this species made contact with *S. araneus* in several valleys. Through this contact, gene flow between *S. antinorii* and *S. araneus* reduced interspecific differences for most regions of the genome. However, in the HT hybrid zone, hybrids produce a chain of rearranged chromosomes (i.e., complex heterozygotes), which compose a large linkage block (Fig. 3). Introgression of alleles into the heterospecific background should be strongly impeded by this block. Therefore, loci located on this block would have been protected from gene flow and have remained strongly differentiated between *S. araneus* Vaud and *S. antinorii*.

In the LH hybrid zone, hybrids mostly produce trivalents (i.e., simple heterozygotes; Fig. 3). This category of hybrids has been shown to be as fit as homozygotes in these shrews (Narain and Fredga 1997, 1998; Banaszek et al. 2002) and several backcross hybrids have been detected in this zone (Brünner and Hausser 1996). In this situation, rearranged chromosomes can recombine independently and should form a much weaker linkage block than in HT. Therefore, rearranged chromosomes in the LH hybrid zone should introgress much more easily than in the HT zone. Our results support this second prediction because the difference in the genetic structure of common and rearranged chromosomes in this zone is of lesser magnitude than in HT hybrid zone and is not significant (Table 3). However, it should be noted that, even in this hybrid zone, the markers located on the rearranged chromosomes show larger interspecific F_{RT} than the markers located across common chromosomes, suggesting that karyotypic differences influence the genetic structure of this zone as well.

Interestingly, most of the discrepancies observed between LH and HT are carried by the rearranged chromosomes ($F_{RT\text{-rearranged-LH}} = 0.122$, $F_{RT\text{-rearranged-HT}} = 0.248$). This difference probably reflects the larger impact of chromosomal rearrangements on the genetic structure in the HT hybrid zone. Finally, the genetic differentiation observed across common chromosomes in both zones ($F_{RT\text{-common-LH}} = 0.059$, $F_{RT\text{-common-HT}} = 0.083$) certainly reflects the genetic differences accumulated between the two species involved in these zones.

Variation across Loci

The loci within each chromosome class are not equally informative, and the variance observed across loci is large (Tables 3, 4). For example, interspecific structure (F_{RT}) across rearranged chromosomes varies from 0.008 (locus D109) to 0.375 (L99) in LH and 0.036 (L62) to 0.638 (L99) in HT. Several nonexclusive factors may explain this strong variance: intrinsic characteristics of the loci (e.g., number of alleles, evolution patterns), which chromosome a locus is located on, or the position within the chromosome.

First, we observed large variation in the allele number per locus, and this may bias the estimated population differentiation. It was shown that when calculated from microsatellites characterized by high mutation rates, F_{ST} is deflated (and, consequently, gene flow is overestimated) when migration is reduced, as is the case in these hybrid zones (Balloux and Lugon-Moulin 2002). In our analyses, we compared two groups of microsatellites. The number of alleles observed in both hybrid zones is similar for the common and rearranged categories (see Supplementary Tables 1 and 2 available online). Therefore, this parameter should not influence our conclusions. However, analysis of groups of microsatellites with low variation in allele number in future studies of differential gene flow would be of primary interest to control for this parameter.

Second, in our analyses we pooled loci located on different chromosomes (e.g., the six loci that compose the rearranged group are located on five different chromosomes). For example, loci contributing to reproductive isolation in animals are disproportionately found on particular chromosomes (e.g., chromosome 17 in mice; Yeom et al. 1992) and in this context, the X chromosome plays an important role (Coyne and Orr 1989). Moreover, X-linked markers show reduced introgression across a number of mouse hybrid zones (Tucker et al. 1992; Dod et al. 1993; Payseur et al. 2004; Payseur and Nachman 2005). In the *S. araneus* group, the sex chromosome system in males is unusual (i.e., XY1Y2), and only the smallest arm *e* can be considered as the real X chromosome (Zima et al. 1998). In our study, this arm has been treated identically to the other chromosomes of the common group, but more extensive studies of its role as a reproductive barrier between *Sorex* species are necessary to confirm the appropriateness of this decision.

Third, genetic differentiation estimates strongly differ among loci situated over the same chromosome arm. For example, loci L16, D107, and D112 all map to chromosome arm *a* but show F_{RT} values in HT ranging from 0.002 to 0.328. Several genetic factors are suspected to play important roles in gene flow among populations. One is the rate of recombination along the chromosome (e.g., Ortíz-Barrientos et al. 2002; Butlin 2005; Stump et al. 2005). Studies in numerous organisms demonstrate that recombination is not uniformly distributed along the genome and that most recombination events occur at highly localized hot spots (e.g., Kauppi et al. 2004).

In general, lower recombination rates are observed near the centromere of metacentric chromosomes (Nachman 2001). The potential impact of the variation of recombination rate along chromosomes was described by Panithanarak et al. (2004). These authors showed in a mouse hybrid zone that loci near the centromere (i.e., experiencing low levels of recombination) of rearranged chromosomes were protected from gene flow, which was not the case for loci located close to the telomeres. Unfortunately, data about the localization of loci within chromosomes in the *S. araneus* group are lacking, and fine-scale localizations are necessary to test the possible impact of recombination.

Finally, low genetic differentiation for several loci located on rearranged chromosomes could reflect the retention of same ancestral polymorphism or homoplasy. The study of the center of these hybrid zones brings an important perspective to this question. It is indeed in localities where hybrids were identified that effective gene flow occurs and that differences in chromosomal rearrangements will have the strongest impact. Interspecific structure (F_{RT}) increased in HT in the localities where the two species occur in sympatry (Table 5). Moreover, the difference between common and rearranged interspecific structure increased over the same localities and was significant ($P = 0.038$). This highlights again the role of chromosomal rearrangements in the reproductive barrier between *S. antinorii* and *S. araneus* Vaud and suggests an impact stronger and/or less masked in the center than over the whole hybrid zone. Therefore, when studying similar hybrid zones, examining loci with comparable intrinsic characteristics or increasing the number of markers used in each chromosomal category are recommended.

Hybrid Dysfunction Versus Suppressed Recombination?

Models proposing that chromosomal rearrangements facilitate speciation fall into two main categories: the hybrid dysfunction and the suppressed recombination models (Ayala and Coluzzi 2005).

Hybrid dysfunction models claim that rearrangements reduce the fertility and the reproductive fitness of heterozygous hybrids (White 1978; King 1993), but these models suffer from several empirical and theoretical difficulties (e.g., Rieseberg 2001; Navarro and Barton 2003b; Coyne and Orr 2004). Data from the *S. araneus* group suggest that Robertsonian heterozygotes do not suffer from infertility as substantially as other taxa (Searle 1993; Narain and Fredga 1997, 1998; Banaszek 2000). Nevertheless, Banaszek et al. (2002) observed levels of nondisjunction high enough to affect fertility of complex heterozygotes. These authors highlighted the difference between simple and complex heterozygotes because they did not find any evidence that simple heterozygotes are less fit than homozygotes. Furthermore, the absence of male-mediated gene flow detected in the LH hybrid zone (Balloux et al. 2000) suggests that male F_1 hybrids could be sterile in this zone.

Suppressed recombination models claim that suppression of recombination by chromosomal rearrangements could be more important than their effect on fitness (Rieseberg 2001). Data concerning reduction of recombination in the *S. araneus* group are scarce, but suppression of recombination in heterozygous Robertsonian individuals was reported for mice (Davisson and Akeson 1993; Haigis and Dove 2003; Merico et al. 2003).

With our data it is not possible to tease apart the two categories of models. As previously mentioned, the high variance observed across the loci of the rearranged group is concordant with variation of recombination along chromosomes and therefore concordant with the suppressed recombination model. However, if recombination only affects the differential genetic structure of these hybrid zones, we expect structure to be similar for common chromosomes in both hybrid zones. Interspecific structure of common chromosomes was larger in HT than in LH ($F_{RT\text{-common}} = 0.083$ in HT and 0.059 in LH), suggesting that hybrid dysfunction also acts in these hybrid zones. Thus, both hybrid dysfunction and reduced recombination likely contribute to the genetic structure of these hybrid zones.

Conclusions

Using mapped genetic markers we have revealed differences in the porosity of gene flow for some regions of the genome. Furthermore, our data add to recent studies (e.g., Rieseberg et al. 1999; Noor et al. 2001; Delneri et al. 2003; Navarro and Barton 2003b; Panithanarak et al. 2004) supporting the role of chromosomal rearrangements as a reproductive barrier between species. It is likely that other factors, such as genetic incompatibilities accumulated in allopatry, also affect gene flow between taxa. Moreover, the reduction of gene flow for some parts of the genome does not necessarily imply speciation. However, our results strongly suggest that chromosomal rearrangements, if linked to ''isolation'' genes (e.g., Rieseberg 2001), could facilitate the genetic diversification of the *S. araneus* group, ultimately promoting speciation events.

Comparing two hybrid zones with different characteristics allowed us to highlight the importance of the chromosomal composition of hybrids. Although the genetic incompatibilities were essentially the same for both zones, an effect of chromosomal rearrangements was only detected in the zone with more chromosomal incompatibilities. The variation observed across loci probably prevents detection of low differences between the two groups of chromosomes. Moreover, in our study, we analyzed only one hybrid zone of each category. Additional studies of *Sorex* hybrid zones with diverse chromosomal (e.g., complex heterozygotes forming rings or shorter chains of chromosome) and genetic (e.g., within *S. araneus*) characteristics would allow us to address further important issues about the relative roles of genes and chromosomes in the evolution of reproductive barriers between chromosomal variants.

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