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Evolution of the common shrew *Sorex araneus*: chromosomal and molecular aspects

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We review data on the chromosomal variation in the common shrew *Sorex araneus* Linnaeus, 1758 in the context of recent molecular findings. The article considers all aspects of chromosomal variation in this species: within-population polymorphism, karyotypic races, hybrid zones between karyotypic races, chromosomal evolution, and speciation. The recent molecular data provide vital information on different evolutionary processes such as inbreeding, genetic drift, population expansion, and selective forces. In particular, the molecular data challenge traditional models for the fixation of chromosomal variants, provide new insights into the manner of spread of such variants once they are formed and allow in-depth analysis of gene exchange between karyotypic races.

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Introduction

Intraspecific chromosome variation due to the presence or absence of Robertsonian (Rb) rearrangements is frequently observed in mammals. Such variation may involve either Rb fusions or Rb fissions. Rb fusion entails the joining together of two single-armed chromosomes ("acrocentrics") at their centromeres to form a single bi-armed chromosome ("metacentric"), while Rb fission is the reverse process. Robertsonian rearrangements change the numbers of chromosomes in the karyotype, but not the number of chromosome arms.

Robertsonian variation has been observed in several species of shrews (Zima *et al.* 1998). The common shrew *Sorex araneus* Linnaeus, 1758 displays phenomenal chromosomal variability of the Robertsonian type, usually assumed to result from Rb fusions (eg Searle 1984, Wójcik and Searle 1988, Volobouev and Catzeflis 1989, Zima 1991, Searle and Wójcik 1998, Polyakov *et al.* 2001). The diploid number

varies within the species from 2n = 20 to 2n = 33, while fundamental number remains constant at FN = 40. There is increasing evidence that whole-arm reciprocal translocations (WARTs) also play an important role in karyotype evolution in the common shrew (see Halkka *et al.* 1987, Searle *et al.* 1990, Searle 1993, Polyakov *et al.* 2001).

In the description of the karyotype of the common shrew, each substantial chromosome arm is denoted by an italicised lower-case letter of the alphabet with *a* the largest (Searle *et al.* 1991). The sex chromosome system in *S. araneus* is unusual: XY_1Y_2 in males and XX in females, as shared with seven related species (see Zima *et al.* 1998). However, it is the autosomal complement that shows Robertsonian variation within the common shrew. Three pairs of bi-armed autosomes *af*, *bc* and *tu* are invariant while other autosomal arms *g–r* may occur as acrocentrics and/or combined together as different metacentrics (Fig. 1).

Studies on chromosome variation in the common shrew have been focused on topics such as the role of Rb chromosomes in speciation, the evolutionary consequences of Rb variation (eg Searle and Wójcik 1998), the evolutionary history of Rb races (eg Ratkiewicz *et al*. 2002), the fitness of hybrids (eg Narain and Fredga 1997, 1998), and the properties of hybrid zones (eg Hatfield *et al*. 1992, Lugon-Moulin *et al*. 1996). In this paper, we call attention to recent studies that are trying to solve these problems using chromosomal and molecular markers. This paper is partly based on the earlier review by Searle and Wójcik (1998) but is updated and modified. New parts of the present review are those concerning recent molecular studies of *S. araneus*.

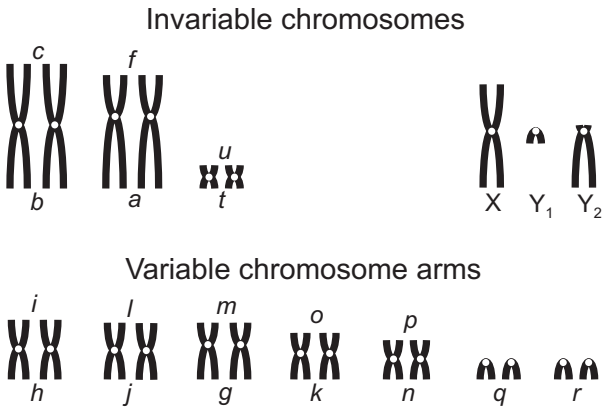


Fig. 1. Diagram showing the different chromosomes in the karyotype of *Sorex araneus*. Three pairs of bi-armed autosomes *af*, *bc* and *tu* are invariant, while other autosomal arms *g–r* may occur as acrocentrics and/or combined together as different metacentrics forming different karyotypic races (see text and Fig. 2). Each substantial chromosome arm is denoted by an italicised lower-case letter of the alphabet with *a* the largest (see Searle *et al.* 1991).

Karyotypic races

The chromosomal variation of the common shrew has been the subject of detailed study by many workers in Eurasia (see Searle and Wójcik 1998). On the basis of comparisons with other species of *Sorex*, it is reasonable to conclude that the ancestral karyotype of *S. araneus* consisted of acrocentric chromosomes (Meylan and Hausser 1973, Wójcik and Searle 1988, Volobouev and Catzeflis 1989, Volobouev 1989). From this ancestral karyotype, numerous karyotypic races have evolved by the occurrence of multiple Rb fusions and WARTs. In total, 62 different karyotypic races have so far been described in the common shrew, using the standard definition of "race" provided by Hausser *et al.* (1994; see also Zima *et al.* 1996): "a group of geographically contiguous or recently separated populations which share the same set of metacentrics and acrocentrics by descent". The list of 50 races of this species was published by Zima *et al*. (1996) and Searle and Wójcik (1998) and since then 14 new races have been described by Bulatova *et al.* (2000, 2002), Kozlovsky *et al*. (2000), Mishta *et al*. (2000), and Polyakov *et al.* (2000a, b). In karyotypes of different races of the common shrew, the chromosome arms *g–r* combine together to form 41 different metacentrics overall (Fig. 2).

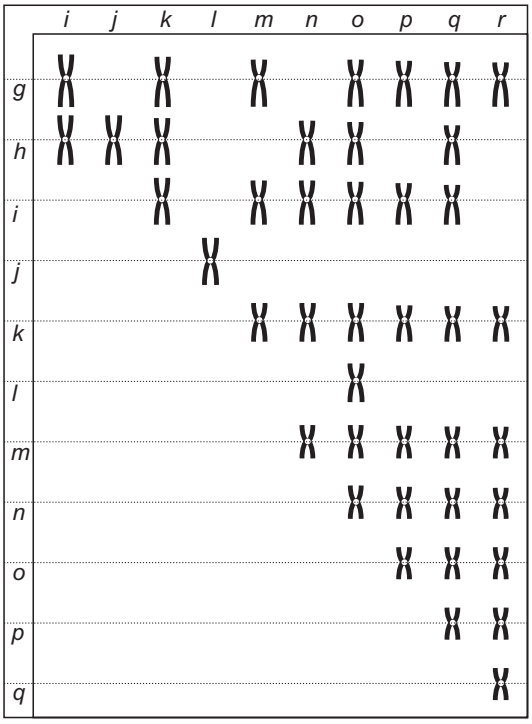


Fig. 2. Metacentrics formed from the variable chromosome arms *g–r* that have been described in *Sorex araneus*.

The first cladistic phylogeny of karyotypic races in *S. araneus* was conducted by Searle (1984) using the method of Camin and Sokal (1965). This analysis involved the 12 races known at that time. The four main clades identified by Searle were geographically discrete and defined the Valais race, and the West European, East European and Siberian karyotypic groups.

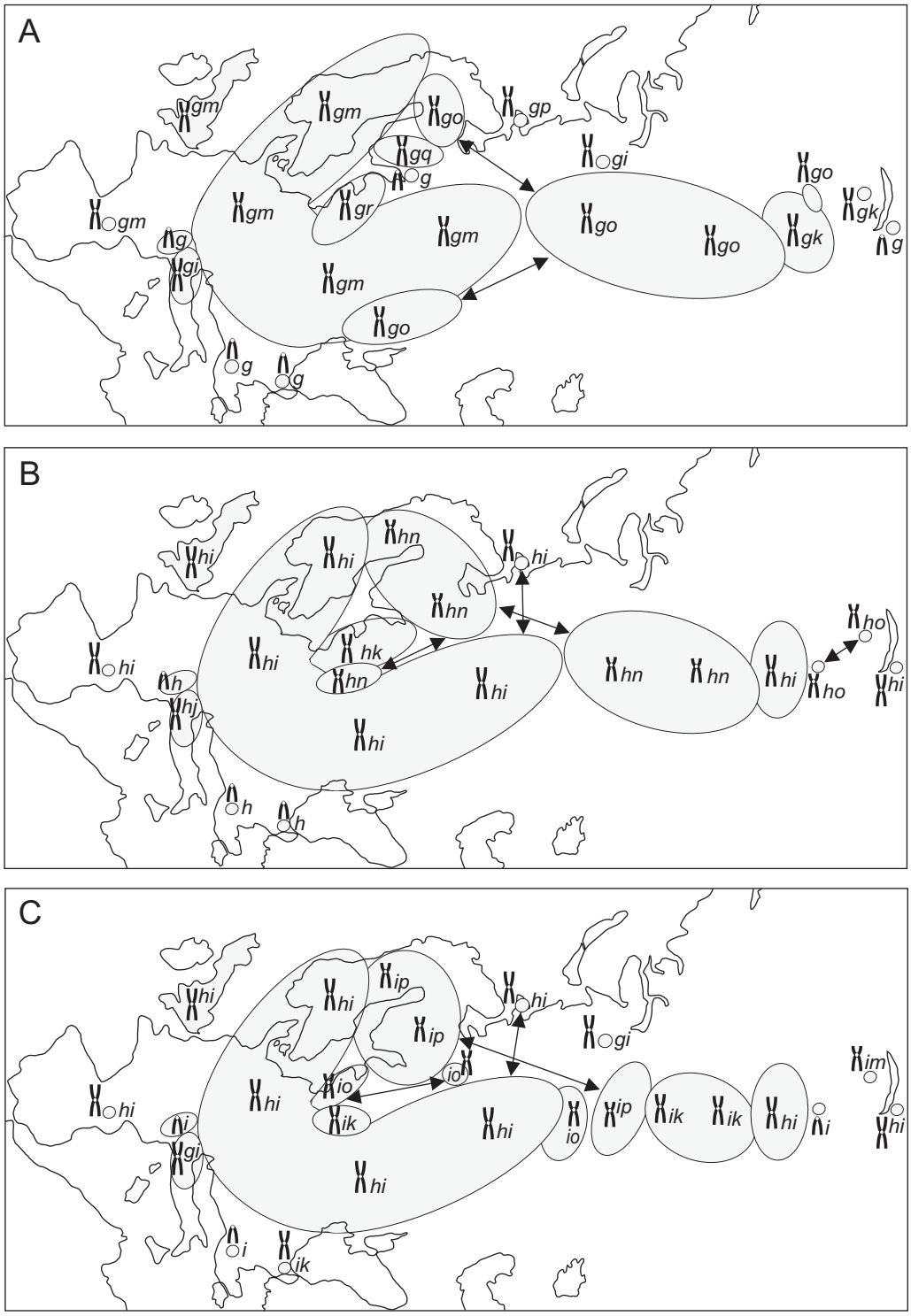
There were further cladistic studies, incorporating races described since 1984. Wójcik (1993) examined the relationship of 24 European races of *S. araneus* using PAUP (Phylogenetic Analysis Using Parsimony; Swofford 1985). He found five karyotypic groups of *S. araneus* in Europe, two of which only consisted of one race. As with Searle's (1984) analysis, the clades showed geographic coherence. Ivanitskaya (1994) published a cladogram reflecting phylogenetic relationships of 24 races of the common shrew constructed by the methods of Farris *et al.* (1970). Her results concerning European races were in general agreement with those of Wójcik (1993).

A phylogenetic analysis of 50 karyotypic races of the common shrew was performed by Searle and Wójcik (1998). A strict consensus tree based on a parsimony algorithm (Swofford 1985) was presented by Searle and Wójcik (1998). Their results were also in agreement with those of Wójcik (1993), and they identified five karyotypic groups: the Valais race, and the West European, North European, East European and Siberian karyotypic groups.

It is important to stress that the Valais race, characterized by the metacentrics *gi*, *hj*, *kn* and *lo*, forms the sister group to all other karyotypic races of *S. araneus*. It currently occurs in southern Switzerland and Italy, and almost certainly survived on the Italian peninsula during the last glaciation (Hausser *et al.* 1986, 1991). Given the distinctiveness of this race on the basis of karyotype, proteins and mitochondrial DNA (Neet and Hausser 1991, Taberlet *et al.* 1994, Hausser *et al.* 1998), it has presumably had a long period of independent evolution. Recently, Brünner *et al*. 2002 have presented strong evidence to show that the Valais race is karyologically, morphologically, biochemically, and genetically clearly distinct from all other chromosome races of *S. araneus*. They have reconsidered the taxonomic status of this karyotypic race and have proposed to promote it to species rank. In this situation the Valais race is a new sibling species of *S. araneus* named *Sorex antinorii* (Brünner *et al*. 2002). In this review we will use both names: the Valais race and the new species name.

All remaining karyotypic races of *S. araneus* are characterized by the metacentric *jl*. We wish to emphasise the particular importance of chromosome arms *g*, *h* and *i* in defining karyotypic groups of the common shrew. The metacentrics *gk*,

Fig. 3. Approximate ranges of particular metacentrics found in *Sorex araneus*: (A) metacentrics possessing the arm *g*, (B) metacentrics possessing the arm *h*, (C) metacentrics possessing the arm *i*. Arrows indicate areas, where continuous populations with certain metacentrics could occur but have yet to be proven. The distributions of the homologous acrocentrics (*g*, *h*, *i*) are also included. After Searle and Wójcik (1998), updated and modified.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gm, *go*, *gr*, *hi*, *hk*, *hn, ik*, *io* and *ip*, that involve these chromosome arms are particularly widespread (Fig. 3A–C). It is intriguing that these chromosome arms are the largest among those to show Robertsonian variation. It would appear that in the karyotypic evolution leading from a *Sorex granarius*-like karyotype in present-day *S. araneus*, the first autosomal metacentrics to be formed by Rb fusion were *af* and *bc* (see also Zima *et al.* 1998). Then, the medium-sized metacentric *jl* became fixed, followed by various metacentrics involving *g*, *h* and *i*. Metacentrics formed from at least one large or medium-sized autosomal acrocentric appear to be much more likely to arise and/or become fixed than fusion products only involving the small acrocentrics *m*, *n*, *o*, *p*, *q,* and *r* (Searle 1983, Zima *et al.* 1988).

Recently, Polyakov *et al.* (2001) have identified two independent karyotypic groups of the common shrew in the Urals and Siberia. One of these groups includes 4 races occurring in the area between the Southern Urals and the eastern borders of the Western Siberian Plain. Polyakov *et al.* (2001) found that these races consecutively replace one another, and each of them differs from its neighbour by one WART. The other karyotypic group (the Eastern Siberian group) includes several races from East Siberia and Altai that appear to differ mainly in the number and composition of metacentrics that have arisen by Rb fusions. The results obtained by Polyakov *et al.* (2001) provide a new perspective on the chromosomal variation and karyotype evolution of the common shrew in the Asian part of the species range.

Robertsonian polymorphism

The common shrew is subdivided into geographic forms ("karyotypic races") that differ in their complement of metacentrics and acrocentrics, as we describe above. Another feature of this species is that Robertsonian polymorphism occurs within populations. Such polymorphisms have been found in many populations of *S. araneus*, often at the contact between karyotypic races. While most well--described Robertsonian polymorphisms in the common shrew refer to populations at the contact of clearly-defined karyotypic races, there are some that appear not to be in current hybrid zones.

Five examples of polymorphisms have been described in populations that are apparently well-distant from hybrid zones: (1) the low-level polymorphism for *jl* found sporadically in different karyotypic races over a huge range from England to Siberia (Searle and Wilkinson 1987, Zima *et al.* 1994), (2) the polymorphism for *pr* in the Oxford race on the island of Islay, United Kingdom (Ford and Graham 1964, Searle and Wójcik 1998), (3) the polymorphism for *ko* in the Öland race on the island of Öland in the Baltic off the south-east coast of Sweden (Fredga 1996), (4) the polymorphism for *mp* in the Białowieża race in Poland (Fedyk 1980, 1982,

Wójcik 1991, Wójcik *et al.* 1996), and (5) the polymorphism for *go* and *mp* in the Novosibirsk race in Siberia (Král and Radjabli 1974, Polyakov *et al.* 1997a).

In all cases of Robertsonian polymorphisms listed above, the externality from hybrid zones is rather difficult to prove. Firstly, geographical sampling is usually not at sufficient intensity to rule out the possibility of an undetected interracial contact nearby. Secondly, one of the alternative morphs (twin acrocentric/metacentric) is rare in all the polymorphisms, as expected for populations well-distant from the centre of a hybrid zone. There is an expectation that hybrid zones in the common shrew involving simple Rb heterozygotes will tend to be wide, with low-level polymorphism extending for tens of kilometres (because the selection against simple Rb heterozygotes is very small). Such long-distance "introgression" has been recorded in other species; the clines that make up hybrid zones are sigmoid, very steep at around the 50% point, but much more shallow at very low and very high frequencies (Barton and Hewitt 1985, Harrison 1990, Barton and Gale 1993).

Searle and Wójcik (1998) have discussed the contention that the polymorphisms listed above may be external to current hybrid zones in the common shrew. The strongest case is the low-level polymorphism for *jl* found sporadically over a huge area. It is absolutely inconceivable that the broad low-level polymorphism for *jl* relates to a current hybrid zone. It also appears certain that the polymorphisms for *pr* on the island of Islay and for *ko* on the island of Öland are external to all current hybrid zones. Islay is situated midway along the west coast of Scotland and has been isolated from the mainland since the rise of sea level at the end of the last glaciation (9000 years ago). There is no known hybrid zone involving *pr* on the nearby mainland (J. B. Searle, unpubl.). Öland has never been connected with the mainland of Sweden before the bridge was completed in 1972. Thus, the island has been isolated for about 10 000 years. There is no evidence that Öland was ever connected to northern Poland (Fredga 1996).

Searle and Wójcik (1998) have presented different hypotheses that can explain these polymorphisms in the common shrew. One possibility is that the polymorphisms represent the "ghost of hybridization past" (Polyakov *et al.* 1997b). There are a number of ways in which past hybridization events may generate long-lasting polymorphisms. Searle and Wójcik (1998) have suggested that such a polymorphism may be generated following a colonization process similar to that envisaged for Scandinavian house mice (Gyllensten and Wilson 1987, Prager *et al.* 1993). For instance, the shrews that initially colonized the bulk of the current range of the common shrew may have ultimately derived from a hybrid zone between a race characterized by acrocentrics *j* and *l* and a race characterized by metacentric *jl*, then the Robertsonian polymorphism for *jl* may have become very widely spread. Likewise, the shrews that colonized Islay may have been from a hybrid zone between a race with metacentric *pr* and a race with acrocentrics *p* and *r*.

A second possible explanation for polymorphisms external to current hybrid zones is that they occur in populations where there has been a *de novo* chromosome mutation (Searle and Wójcik 1998). Under that second model, the fact that such polymorphisms are, in general, rare would imply that chromosome mutations tend to occur at low frequency in the common shrew. However, in the case of the very widespread polymorphism for *jl*, an unusually high mutation rate for this particular metacentric may be suggested (Searle and Wójcik 1998). Thus, the *jl* polymorphism would be viewed as in a mutation-selection balance on this model, as used to explain Robertsonian polymorphisms in humans (Bengtsson and Bodmer 1976). Although the predominant morph is the metacentric *jl*, the acrocentrics *j* and *l* may arise regularly due to a high mutation frequency (Robertsonian fission) but also tend to be lost regularly because of selection against the single simple heterozygotes.

Searle and Wójcik (1998) in their third model suggested that a particular polymorphism came into being either through past hybridization events or *de novo* mutations, but that the particular polymorphism is maintained by natural selection. In this case, it is most reasonable to consider that natural selection operates via genes linked to the polymorphic chromosome. There needs to be consistent allelic differences between the twin acrocentric morph of the polymorphic chromosome and the metacentric morph. Any such allelic differences are likely to reside in the centromeric regions, as these are expected to display cross-over suppression in Rb heterozygotes. In this way, any differentiation between the twin acrocentric and metacentric morphs of a particular chromosome may be maintained in populations of the common shrew (see Searle 1988). There is the possibility of heterozygous advantage of the sort demonstrated for inversion heterozygotes in *Drosophila* (Kreitman 1991).

Wójcik (1991) has put forward a different selective hypothesis for the Robertsonian polymorphism involving arm combination *mp* at Białowieża in Poland. He suggested that the polymorphism relates to environmental heterogeneity on the basis of finding a significantly higher frequency of the twin acrocentric morph in wet habitats than in dry habitats. It is worth noting that the frequency of the twin acrocentric morph was very low (6%) even in the wet habitats, so it does not appear to be simply a matter of shrews occupying two habitats with one morph being favoured in one habitat and the other morph in the other. Thus, if Wójcik (1991) is right that the Robertsonian polymorphism in Białowieża has an adaptive significance relating to environmental heterogeneity, it may require a rather complex model to explain the polymorphism.

There is no definitive explanation for Robertsonian polymorphisms in the common shrew that are external to hybrid zones. The same explanation may not apply to all polymorphisms and models presented by Searle and Wójcik (1998) may be too simplistic. Those models not only have relevance to our understanding of Robertsonian polymorphisms in the common shrew, they also reflect a wide range of possibilities with regards to the colonization history of the species and have implications for the initial fixation of Rb metacentrics.

Genetic variation within karyotypic races

The common shrew has been extensively studied by protein electrophoresis (Heikkilä 1989, Wójcik and Wójcik 1994, Banaszek *et al.* 1996, Brünner and Hausser 1996) and microsatellites (Lugon-Moulin *et al.* 1999, Wyttenbach *et al.* 1999a, b, Balloux *et al.* 2000). More than 40 enzyme loci and 7 microsatellite markers have been used to assess the level of variability in different parts of the species' range. About 12 enzyme loci have been found to be polymorphic (*adenylate kinase*, *alcohol dehydrogenase*, *aminoacylase*, *phosphoglucomutase-1*, *-2, -3*, *isocitrate dehydrogenase-2*, *lactate dehydrogenase-2*, *esterase-1*, *-2*, *esterase-D*, *mannose phosphate isomerase*), at least in some populations. The average expected heterozygosity (H_e) calculated over more than 30 enzyme loci is relatively low and ranges from 0.03 to 0.07 (Frykman *et al.* 1983, Wójcik and Wójcik 1994, Ratkiewicz *et al.* 2002). On the other hand, the analysis of shrew populations using 6 microsatellite markers show a 10- to 20-fold increase in the average H_e (0.69–0.71) in the Bretolet, Uppsala, and Hällefors races (Wyttenbach *et al.* 1999a, b). Hardy-Weinberg proportions have generally been found for all enzyme loci and microsatellite markers. Only the L16 microsatellite locus has shown significant heterozygote deficit (Wyttenbach and Hausser 1996, Wyttenbach *et al.* 1999a), probably indicating the presence of null alleles or selective constraints at this locus. Furthermore, RFLP analyses of mtDNA by H. Tegelström (1987, and unpubl.) has shown that shrews are highly variable in their mtDNA, both with respect to the number of clones and the degree of differentiation between the clones. Thus, the data available indicate that the common shrew is not a particularly inbred animal and that inbreeding cannot explain the extensive chromosome variation in the species (Bengtsson and Frykman 1990).

Geographical barriers may affect the genetic structure of shrew populations by reducing gene exchange among them. Using microsatellites, Lugon-Moulin *et al.* (2000) showed significant genetic structuring between several populations from different valleys in Swiss Alps (overall $F_{ST} = 0.054$, $R_{ST} = 0.114$). The authors suggested that dispersal may be reduced at altitudes > 2400 m, affecting population structure. Such a situation, however, seems to be rather exceptional for the common shrew.

Gene diversity within shrew populations is generally high and differentiation among populations within a given race is usually low (Banaszek *et al.* 1996, Wójcik *et al.* 1996, Wyttenbach *et al.* 1999a). Thus, the common shrew seems to consist of continuous populations showing little phylogeographic structure, with individuals dispersing fairly large distances (Wyttenbach *et al.* 1999a). This fact is important with regard to our understanding of the mechanisms that contribute to the rapid spread and fixation of metacentric chromosomes. Wyttenbach *et al.* (1999a) suggested that the high migration rate of the shrews might enable a newly arisen Rb mutation to spread quickly through a population. This will be discussed further in the section entitled "Local fixation of chromosomal variants".

Genetic differentiation among different karyotypic races and groups

Allozyme and DNA studies of the common shrew have been initiated in response to the extensive chromosomal variation found in this species (see Searle and Wójcik 1998). In general, the extraordinary chromosomal subdivision is not matched by comparable differentiation on the basis of allozymes and mtDNA sequences (Frykman *et al.* 1983, Wójcik and Wójcik 1994, Ratkiewicz *et al.* 2002). Clinal variation in enzyme allele frequencies has been found in Sweden (Frykman and Bengtsson 1984) and Great Britain (Searle 1985). These clines extend from north to south, according to the distribution of races in Sweden and Great Britain. In Poland, where most of the hybrid zones between races are orientated East West, no clinal variation has been demonstrated for allozymes (Wójcik and Wójcik 1994, Ratkiewicz 1997). The only example of clear differentiation occurs in Switzerland (Hausser *et al.* 1991, Neet and Hausser 1991, Lugon-Moulin *et al.* 2000), where two enzyme loci (albumin and urinary pepsinogen) and one microsatellite locus (L99) distinguish the Valais race (*S. antinorii*) from the Vaud and Cordon races. Additionally, the Valais race (*S. antinorii*) differs from the other shrew races in the Alps with respect to uniparently inherited markers: mitochondrial cytochrome *b* (Taberlet *et al.* 1994) and the Y-chromosome microsatellite L8Y (Balloux *et al.* 2000). This is a rare example of interracial differentiation that suggests reduced gene flow between the different shrew races in the Alps (Brünner and Hausser 1996, Lugon-Moulin *et al.* 1999, Balloux *et al.* 2000). *R*-statistics for the Y-chromosome microsatellite show that differentiation is nearly complete (R_{ST} = 0.98) between the Valais race (*S. antinorii*) and Cordon race. Thus, the races may be considered two incipient species (Taberlet *et al.* 1994, Brünner and Hausser 1996, Balloux *et al.* 2000, Brünner *et al.* 2002). It is very probable that the Valais (*S. antinorii*) and the other shrew races in Alps have become genetically differentiated in allopatry, in different glacial refugia on either side of the Alps (Hausser *et al.* 1991, Bilton *et al.* 1998, Taberlet *et al.* 1998). The Cordon and Vaud races are, however, poorly differentiated. The only recorded difference between them is that the L8Y locus was not successfully amplified in the shrews from Vaud race. This means a mutation could have occurred within the sequence homologous to the primer sequence, resulting in no PCR product (F. Balloux, pers. comm.).

Despite no clear geographical trends of allozyme variation for the common shrew in Europe (Heikkilä 1989), there might be considerable differences among regions as shown, for example, by Catzeflis (1984) between populations from Finland/Denmark and Italy. In this case, Nei's *D* was equal to 0.210, while, for example, Ratkiewicz *et al.* (2002) found that the average *D* among different chromosome races from the East and West European karyotypic groups in Poland was 0.003 (0.000–0.006). Another estimate of *D* was 0.039 between the Drnholec and Białowieża races (Wójcik and Wójcik 1994). Thus, there is some discrepancy in the level of allozyme differentiation assessed among distinct populations and races by different authors. This discrepancy may be due to different numbers and types

of loci studied. Certainly, the result obtained for two loci will greatly differ from those obtained for tens of loci. Another difficulty comes from the lack of standardised nomenclature of the loci and alleles studied in the common shrew. This makes any comparisons among different geographic regions and races surveyed very difficult, as it can be based on the comparisons of genetic distances only. Ratkiewicz *et al.* (1996) made a partial effort to standardise loci and alleles, but they were unable to standardise all the loci studied so far due to lack of samples from many parts of *S. araneus* range. However, this effort is quite promising, as it resulted in the standardised description of alleles and loci according to Searle (1985). In effect, all papers published in Poland and Czech Republic, dealing with allozymes in shrews are fully compatible to the data from Britain (Searle 1985, 1986c), and partly comparable to the data from Sweden (Frykman *et al.* 1983).

Glacial refugia of *Sorex araneus*

One of the major issues that needs to be addressed in the common shrew is the location of glacial refugia from which the species expanded at the end of the last glaciation. This will be addressed further in detail in a forthcoming article (J. B. Searle *et al.* in prep.). Here we briefly discuss the published data on mtDNA and chromosomal variation that address this issue.

Hewitt (1996) suggested that populations of temperate species currently occupying central and northern Europe derive from "refugial" populations in the Mediterranean peninsulae of Iberia, Italy, and the Balkans. The populations of the common shrew in the Italian peninsula may have been geographically isolated for a long period of time, permitting accumulation of new mutations, which includes substantial divergence in mtDNA sequence and karyotype (Taberlet *et al.* 1994). According to Taberlet *et al.* (1998) the northern part of Europe has been colonized primarily from the Iberian and the Balkan refugia. On the other hand, shrew populations of the Valais race (*S. antinorii*) evolving in Italy were not able to spread northwards due to the Alpine barrier (Taberlet *et al.* 1994, 1998). The authors noted, however, that due to very few data available for eastern Europe and Fennoscandia, the contribution of potential easternmost refugia localized in Europe and/or Asia may have been underestimated. Indeed, research on small-mammal mtDNA has questioned the universality of Mediterranean refugia as the areas from which different temperate taxa colonized northern and central Europe at the end of the last glacial period. Bilton *et al.* (1998) in their study postulated that Mediterranean Europe was an area of endemism for small mammals, such as *S. araneus*, *Sorex minutus* and *Clethrionomys glareolus*, rather than a source for northwards postglacial colonization. According to these authors more northerly areas of Europe become occupied by range expansion from one or more refugia in central Europe – western Siberia. Data from the moor frog *Rana arvalis* (Rafiński and Babik 2000) and the common beech *Fagus sylvaticus* (Demesure *et al.* 1996)

support this model. The existence of central European refugia has been further promoted by Stewart and Lister (2001).

What is also noteworthy from the study of Bilton *et al.* (1998) is that all *S. araneus* haplotypes deriving from throughout central and northern Europe and Siberia are grouped together on the same branch of the neighbour-joining tree for the mtDNA sequence, suggesting a single glacial refugium. Chromosomal data suggest multiple refugia (see Searle and Wójcik 1998). The variation observed for the cytochrome *b* gene may not be high enough to discriminate different glacial refugia for *S. araneus*. On the other hand, chromosomal data alone may help infer more precisely the postglacial colonization of northern Europe and Asia. For instance, karyotypic races of the common shrew from Finland and western Siberia might have derived from a glacial refugium in the Southern Urals (Halkka *et al.* 1994, Polyakov *et al.* 1997a, 2001).

Signatures of population expansion

What then can explain the lack of differentiation in mtDNA haplotypes or allozyme frequencies over huge areas of the common shrew range? It is very probable that a rapid radiation from a single or several poorly differentiated source population(s) into the geographic regions examined could result in this pattern. Such population expansion has recently been documented for *S. araneus* in Poland (Ratkiewicz *et al.* 2002). The starlike genealogy of *S. araneus* haplotypes (Fig. 4), unimodal mismatch distribution (ie distribution of pairwise sequence differences among shrews studied), and excess of low frequency mutations indicate population expansion in this species occupying Poland (Ratkiewicz *et al.* 2002). This, rather than widespread gene flow, best explains the low within species F_{ST} values demonstrated in allozyme studies (F_{ST} in many cases was much below 0.05; Banaszek *et al.* 1996, Wójcik *et al.* 1996). Further evidence of rapid population expansion from the mtDNA study of Ratkiewicz *et al.* (2002) is that most of the individuals sampled had private haplotypes that were not observed elsewhere, and the distribution of the most common haplotype was limited to 3 out of 10 populations only. This observation may indicate that present-day gene flow among the common shrew races could be limited.

Hybrid zones

Chromosomal characteristics

Hybrid zones are narrow regions of phenotypic or genotypic change, which separate otherwise more or less homogenous taxa (Jiggins and Mallet 2000). In the case of the common shrew a hybrid zone between karyotypic races is specifically defined as the area of occurrence of Rb heterozygotes involving chromosomes which differ between the two races (see Searle and Wójcik 1998).

Metacentric clines are formed in hybrid zones where two different races come into contact. In the simplest case, where one of the races is characterized by a single metacentric not found in the other, there will be a single cline formed on hybridization. If one race has several metacentrics not found in the other, the hybrid zone will consist of multiple concordant metacentric clines. Opposing metacentric clines are expected when a race with a metacentric comes into contact with a race with a different metacentric. If there are multiple differences of this sort, there will be multiple opposing metacentric clines.

There is a qualified presumption that heterozygous individuals of the common shrew are less fit than homozygous ones (Searle 1986a, 1988, 1993, Garagna *et al*. 1989, Wallace and Searle 1990, 1994, Mercer *et al*. 1991), and that hybrid zones between karyotypic races in this species are tension zones (see review in Searle and Wójcik 1998). Tension zones are a particular category of hybrid zone, where the

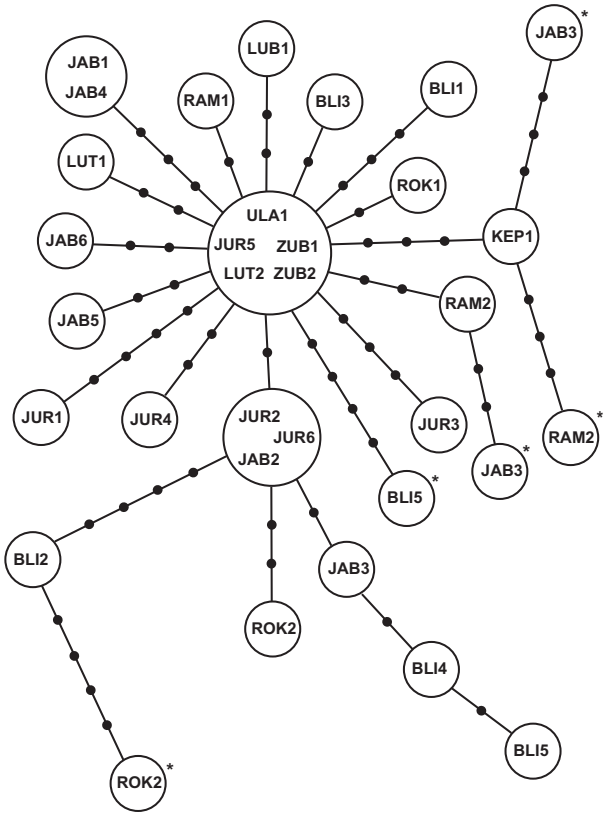

Fig. 4. Minimum spanning network obtained for 9 populations of *Sorex araneus* in Poland. Population names and their abbreviations are given in Ratkiewicz *et al*. (2002). The small black nodes refer to one substitution. The alternative links between haplotypes are indicated with an asterisk (*). After Ratkiewicz *et al*. (2002).

hybrids are unfit relative to both the pure races (Barton and Hewitt 1985). Consistent with this contention, Rb heterozygotes in *S. araneus* tend to be limited to a narrow area (kilometers or tens of kilometers wide) at the contact between races.

There is an extraordinary variety in the hybrid zones of the common shrew (see review in Searle and Wójcik 1998). All the basic structures that might reasonably be expected on secondary contact of either a metacentric race and an acrocentric race (single cline or multiple concordant clines) or two metacentric races (opposing clines or multiple opposing clines) have been observed. There are also features in the common shrew zones, acrocentric and recombinant peaks (Searle 1993), that would not automatically be expected on secondary contact of karyotypic races.

The simplest situation of a hybrid zone consisting of a single cline certainly occurs in various parts of the range of the common shrew. Two such hybrid zones have been studied in detail in southern Britain (Mercer 1991, J. B. Searle, unpubl.). The Oxford and Wrentham races and Chysauster and Hermitage races, respectively, each differ by presence of a single metacentric in one race (Oxford, Chysauster) and the homologous acrocentrics in the other (Wrentham, Hermitage). In both cases there is a smooth gradient in the frequency of these single metacentrics going from one pure race to the other. The areas of polymorphism are about 100 km wide both for *pr* (the Oxford–Wrentham zone) and *np* (the Chysauster–Hermitage zone). In terms of standardized width, the values for these clines are in the order of 30–40 km, compatible with very small selection against simple Rb heterozygotes (Mercer 1991, J. B. Searle, unpubl.).

A hybrid zone with multiple concordant clines was described for the Drnholec––Ulm contact in the Czech Republic (Lukáčová *et al.* 1994). The area of polymorphism and the standardized cline widths are similar to those recorded for the single clines.

There are several hybrid zones in the common shrew involving multiple opposing clines that cross at a frequency of 0.5. For instance, in the Uppsala–Hällefors hybrid zone in Sweden (see Table 1), there are two metacentric clines involved (Narain and Fredga 1996). The clines that make up this hybrid zone are much narrower than those found in zones comprising single clines or multiple concordant clines. This presumably partly reflects somewhat greater selection against the complex heterozygotes than single or double simple heterozygotes. However, the dispersal of shrews across this hybrid zone may also be reduced because of the presence of a river at the contact between the two races. The importance of environmental barriers in attracting and narrowing hybrid zones has been emphasised before (Barton and Hewitt 1985, Barton and Gale 1993). Another zone dominated by complex heterozygotes is that between the Łęgucki Młyn and Drużno races in Poland (Banaszek 1994, Fedyk 1995). As for the Uppsala–Hällefors contact, this is a narrow zone, with standardized cline widths much less than those recorded for single clines and multiple concordant clines (Table 1). Other examples of hybrid zones with the opposing clines crossing at a frequency of approximately

0.5 are characterized by hybrids that form long meiotic chain-configurations and very narrow chromosome clines (1 km standardized width or less): the Valais–Vaud contacts in Switzerland (Hausser *et al.* 1991, Brünner *et al.* 1994), the Drnholec––Białowieża zone (Szałaj *et al.* 1996), and that between the Novosibirsk and Tomsk races in Siberia (Volobouev 1983, Aniskin and Lukianova 1989, V. T. Volobouev, unpubl.). These zones show decreasing metacentric cline widths with decreasing fitness of the hybrids, in the order long chain-forming complex heterozygotes more unfit than ring-of-four forming complex heterozygotes more unfit than single simple heterozygotes (Searle 1993).

There are two interesting situations in the common shrew where opposing clines within hybrid zones are staggered from each other and are not centred at the same position: (1) the "acrocentric peak", where the opposing metacentric clines cross at a frequency of less than 0.5 because in the centre of the hybrid zone there is a high frequency of acrocentrics (homologous to the metacentrics that comprise the opposing clines), and (2) the "recombinant peak", where the opposing metacentric clines cross at a frequency of greater than 0.5 because of a high frequency of individuals at the centre of the hybrid zone that are homozygous for metacentrics that define opposing clines.

The Oxford–Hermitage hybrid zone in England and the Drnholec–Łęgucki Młyn hybrid zone in Poland are the best examples of these types of structure (Searle 1986b, Fedyk *et al.* 1991, 2000, Hatfield *et al.* 1992, Fedyk 1995). It might be expected that natural selection would favour the evolution of zones with acrocentric and recombinant peaks from those with opposing or multiple opposing clines crossing at a frequency of 0.5. This is because the presence of such peaks reduces the frequency of Rb heterozygotes which are expected to have a particularly low fitness. In the case of an acrocentric peak, a hybrid zone which used to be dominated by complex heterozygotes will become occupied by acrocentric homozygotes and simple heterozygotes. In the case of a recombinant peak, the frequency of heterozygotes expected to form two heterozygous configurations at meiosis I is reduced in favour of individuals homozygous for metacentrics deriving from both hybridizing races ("recombinant homozygotes") and individuals expected to form only one heterozygous configuration at meiosis I (see Searle and Wójcik 1998). There is an expectation that complex heterozygotes will be considerably more unfit than individuals that are simple heterozygotes for one or a few metacentrics. Although there are fewer data it would also be expected that heterozygotes with two rings/long chains will be considerably more unfit than those which produce one such ring/long chain (Searle 1993). Under these conditions, natural selection would be expected to favour the separation of metacentric clines, to generate the staggered clines associated with acrocentric and recombinant peaks (see Barton and Bengtsson 1986).

Six examples of acrocentric peaks have been demonstrated in hybrid zones of the common shrew (see review in Searle and Wójcik 1998). An acrocentric peak associated with opposing clines is that found on contact of the Wirral race

Table 1. Chromosomal and molecular characteristics of 3 hybrid zones between different karyotypic races in *Sorex araneus*. N_m – effective number of migrants. [1] the Valais race is now considered a new sibling species of *S. araneus* (Brünner *et al.* 2002); [2] calculated from the private allele frequency in the populations throughout Sweden.

Races making contact	Chromosomal characteristics						Source
	Geographic area	Variable chromosomes	Basic structure	Special features	Absolute width (km)	Approximate standardized cline width (km)	
Uppsala Hällefors	Central Sweden	*kp, oq* *ko, pq*	Multiple opposing clines	Contact at river	> 20	2	Narain and Fredga 1996
Drnholec Łęgucki Młyn	Northeast Poland	*gm, hi, ko, nr* *gr, hk, io, mn*	Multiple opposing clines	Recombinant peak, acrocentric peak	> 10	5	Fedyk *et al.* 1991, 2000, Fedyk 1995
Valais (*Sorex antinorii*)[1] Cordon	Switzerland	*gi, hj, kn, lo* *g, h, i, jl, k, n, o*	Multiple opposing clines	Contact at river	about 1	< 1	Brünner and Hausser 1996

Races making contact	Molecular characteristics												Source
	Allozymes		Microsatellites				mtDNA		Y-chromosome microsatellite				
	F_{ST}	N_m	F_{ST}	N_m	R_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	R_{ST}	N_m	
Uppsala Hällefors	–	4[2]	0.026	9.200	0	infinite	–	–	–	–	–	–	Bengtsson and Frykman 1990, Wyttenbach *et al.* 1999b
Drnholec Łęgucki Młyn	0.015	16	–	–	–	–	0	infinite	–	–	–	–	Ratkiewicz *et al.* 2000, Ratkiewicz *et al.* 2002
Valais (*S. antinorii*) Cordon	0.618	0.150	0.103	2.180	0.199	0.560	0.030	8.100	0.190	1.100	0.980	0.005	Brünner and Hausser 1996, Lugon–Moulin *et al.* 1999, Balloux *et al.* 2000

(characterized by chromosomes *pr*, *n*) and the Chysauster race (characterized by *np*, *r*) in SW Wales (J. B. Searle, unpubl.). This is the simplest situation of origin of the acrocentric peak: in response to the unfitness of complex heterozygotes. Although only very few sites have been examined, it is clear that there is a high frequency of the acrocentric *p* at the contact of these races. In this case the acrocentric *p* could have penetrated the hybrid zone from the south where it characterizes the Hermitage race. Five other examples of acrocentric peaks have been demonstrated in hybrid zones where there are multiple opposing clines (the Oxford–Hermitage, Oxford–Wirral, Abisko–Sidensjö, Drnholec–Łęgucki Młyn, and Białowieża–Popielno hybrid zones; for references see Searle and Wójcik 1998). In all cases, the frequency of complex heterozygotes is low. Also, in all cases except the Drnholec–Łęgucki Młyn zone, the widths of the metacentric clines are similar to single clines. The acrocentric peak is equivalent to an acrocentric race making contact with one metacentric race on one side and another on the other side.

The Drnholec–Łęgucki Młyn zone in northeastern Poland is of particular interest because of the presence of both an acrocentric peak and a recombinant peak (Table 1; Fedyk *et al.* 1991, 2000, Fedyk 1995). Shrews of the Drnholec race with the *gm*, *hi*, *ko, nr* metacentrics, and those of the Łęgucki Młyn race with the *gr*, *hk*, *io*, *mn* metacentrics come into contact, and different complex heterozygotes are expected in this zone. There is a part of the zone where ring-of-four (RIV: *io*/*ok*/*kh*/*hi*) complex heterozygotes are expected and a part of the zone where RIV (*rn*/*nm*/*mg*/*gr*) complex heterozygotes are expected, but RIV + RIV (*io*/*ok*/*kh*/*hi* + *rn*/*nm*/*mg*/*gr*) heterozygotes should be rare. The area of occurrence of RIV (*io*/*ok*/*kh*/*hi*) heterozygotes is indeed observed and as expected the clines are relatively narrow (ca 5 km standardized width). However, in the area where RIV (*rn*/*nm*/*mg*/*gr*) complex heterozygotes are expected, usually chain-of-five (CV) or chain-of-four (CIV) complex heterozygotes occur (Fedyk *et al.* 1991, 2000). This is because all the chromosome pairs forming this complex meiotic configuration are polymorphic with moderate frequencies of acrocentrics. An acrocentric peak also occur there (with a high frequency of *n*,*n*, *m*,*m*, *g*,*g*, *r*,*r* homozygotes). On the basis of other acrocentric peaks in the common shrew, there is an expectation that the associated metacentric clines should be wide (10–40 km standardized width). For the Drnholec–Łęgucki Młyn zone, however, the clines are only about 5 km wide; this may be a consequence of an environmental barrier to the south of the zone (hills) blocking the widening of clines (see Fedyk *et al.* 1991).

Given the selective forces that apparently should favour development of acrocentric peaks, it may seem surprising that they have not developed in some situations. In the Hällefors–Uppsala hybrid zone, for instance, there is no acrocentric peak despite the presence of complex heterozygous hybrids (Narain and Fredga 1997). In this case the hybrids are RIV heterozygotes and if acrocentrics were to penetrate the hybrid zone they may be selected *against* because the presence of such acrocentrics would lead to formation of CV complex heterozygotes, which would be expected to have lower fitness than the RIV heterozygotes.

Hybrid zones with long-chain-forming complex heterozygotes are expected to form at contacts between races that are chromosomally highly divergent, ie races that most likely belong to distinct karyotypic groups. Such chromosomal divergence will have taken a long time to evolve, over which time genic differences between the races are also likely to have accumulated. Therefore, in these zones the hybrids may be unfit due to genic factors as well the chromosomal difference.

In the case of the Valais–Vaud hybrid zone in Switzerland, it is known that the races making contact differ genically, on the basis of protein, morphological and DNA studies (Hausser *et al.* 1991, Neet and Hausser 1991, Taberlet *et al.* 1994, Brünner *et al.* 2002). The Valais race (*S. antinorii*) is the most chromosomally distinctive of all *Sorex araneus* races, and is likely to have had a long period of independent evolution. The Valais race (*S. antinorii*) also forms a very narrow hybrid zone with the Cordon race (Table 1). In this case, due to the highly acrocentric karyotype of the Cordon race, the hybrids are not anticipated to suffer extreme infertility on chromosomal grounds. In that zone, simple heterozygotes are expected to predominate rather than complex heterozygotes because of the polymorphism of arm combinations *jl* and *lo* and the possibility of independent segregration of the other Valais metacentrics. By applying Mantel tests to microsatellite data, Lougon-Moulin *et al.* (1996) were able to show that racial differentiation is a more important barrier to gene flow in the region of contact of the races Cordon and Valais (*S. antinorii*) than isolation by distance or presence of a river separating the two races. The extreme narrowness of the Cordon–Valais hybrid zone, with a very low incidence of Rb heterozygotes of any sort, surely primarily reflects genic differences between these races.

The only modification of common shrew hybrid zones discussed so far is the generation of recombinant and acrocentric peaks; these are changes that should increase gene flow across zones. However, another possible modification that may occur in hybrid zones characterized by unfit hybrids is a reduction in the production of hybrids, by development of assortative mating, ie the reinforcement process (Dobzhansky 1940, Howard 1993). Such a development will, of course, reduce gene flow across the hybrid zone. For reasons that have been expanded upon elsewhere (Hauffe and Searle 1993, Searle 1993), karyotypic hybrid zones where only a single type of highly unfit hybrid is produced may be rather likely sites for reinforcement. Thus, zones such as that between the Novosibirsk and Tomsk races, the Białowieża and Drnholec races, and the Valais (*S. antinorii*) and Vaud races are prime candidates for reinforcement (see Searle and Wójcik 1998).

Gene flow across hybrid zones

Gene flow across chromosomal hybrid zones in common shrews has been analysed with a variety of genetic markers including those that are co-dominant such as allozymes and microsatellite DNA (Table 1; Frykman and Bengtsson 1984, Searle 1985, Brünner and Hausser 1996, Lugon-Moulin *et al.* 1996, 1999, Ratkiewicz *et al.* 2000) and those that are uniparently inherited such as the mitochondrial

cytochrome *b* gene and the L8Y microsatellite locus on the Y chromosome (Balloux *et al.* 2000).

Analyses of gene flow in hybrid zones based on enzyme electrophoresis have been difficult because there is usually a lack of diagnostic loci. Gene flow between the races Cordon and Valais (*S. antinorii*), which are distinguishable with allozymes, seems to be seriously limited (Brünner and Hausser 1996), while in other hybrid zones interracial gene flow appears to occur even when the zones are narrow (Frykman and Bengtsson 1984, Bengtsson and Frykman 1990, Wyttenbach *et al.* 1999b). Recent analysis of the Cordon–Valais hybrid zone with a Y-linked locus (Table 1), revealed a complete absence of Y-chromosome exchange between the races (Balloux *et al.* 2000). According to these authors, the lack of introgression at the Y microsatellite locus and some limited mtDNA interracial exchange implied either extremely strong interracial assortative mating with choosy females and/or male hybrid sterility. Balloux *et al.* (2000) suggested that the most likely explanation is male hybrid sterility in accordance with Haldane's rule (Haldane 1922, Coyne and Orr 1998).

In contrast to this discussion of chromosomal hybrid zones as strong barriers, Bengtsson and Frykman (1990) suggested that evolutionary processes in the common shrew should decrease isolation rather than strengthen it. This could particularly have happened in the zones with secondary modifications such as an acrocentric peak, or a recombinant peak, both increasing the fertility of hybrid populations (Searle 1986a, Fedyk *et al.* 1991). Indeed, it was found that the level of gene flow in a hybrid zone with a recombinant peak (Fedyk *et al.* 1991) did not seem to be seriously restricted on the basis of the 31 enzyme loci studied (Ratkiewicz *et al.* 2000). However, demographic explanations (eg an expansion scenario) could also account for the lack of divergence between the Drnholec and Białowieża races (Ratkiewicz *et al.* 2000), particularly given the results from a subsequent cytochrome *b* sequence analysis (Ratkiewicz *et al*. 2002). Therefore, if there are no diagnostic loci between the races under study, the analyses of hybrid zones may be difficult to make.

Moreover, Balloux *et al.* (2000) stressed that particular caution should be taken when estimating gene flow from different measures of divergence such as F_{ST} and R_{ST}. For the Y microsatellite locus, F_{ST} between the Valais race (*S. antinorii*) and Cordon race was 0.19, while R_{ST} was considerably higher at 0.98 (Table 1; Balloux *et al.* 2000). Furthermore, if only very polymorphic autosomal microsatellite loci are used, this will often lead to an overestimate of the extent of the gene flow between races. This is very likely when mutation rate is high and the mutation process deviates from a strict stepwise mutation model.

Despite many difficulties and limitations, there is still much to do in the survey of *S. araneus* hybrid zones. For example, the presence of rare electrophoretic variants in hybrid zones (Barton *et al.* 1983) could be carefully checked in the common shrew, as there are some preliminary indications of this phenomenon (Ratkiewicz 1997). Second, linkage disequilibria are commonly generated within hybrid populations, by assortative mating and/or selection against hybrids (Barton

and Gale 1993). Significant linkage disequilibria were found in the Cordon–Valais hybrid zone (Brünner and Hausser 1996), but not found in the Drnholec–Łęgucki Młyn zone (Ratkiewicz *et al.* 2000). Third, almost 40 genes have been mapped on the common shrew chromosomes (Larkin *et al.* 2000), which makes more precise analyses of gene flow in hybrid zones possible. If gene flow is restricted due to chromosomal incompatibilities, the loci located on the chromosome involved in meiotic complexes should display greater genetic differentiation than those located on selectively neutral chromosomes. Also, in different hybrid zones, where short or long meiotic complexes are produced in hybrids, one should expect different levels of genetic differentiation.

Models of chromosomal evolution

According to Searle and Wójcik (1998), three facts of chromosomal evolution in the common shrew seem to be certain: (1) the presence of chromosomal mutations, (2) local fixation of chromosomal variants, and (3) in some cases, spread of the variants more widely. The alternative models for each of these processes are discussed with respect to the results obtained using molecular markers such as the mitochondrial cytochrome *b* gene.

The ancestral karyotype for *S. araneus* was most probably acrocentric (see Wójcik and Searle 1988), thus Rb (centric) fusions leading to a metacentric state must have occurred in this species. There is also the possibility of whole-arm reciprocal translocations (WARTs) generating new metacentrics (Halkka *et al.* 1987, Polyakov *et al.* 2001). The distribution and chromosomal relationships of some races in Finland, Sweden and west Siberia appears to be most easily explained using stepwise WARTs along a geographical sequence of races (Halkka *et al.* 1987, Fredga 1996, Polyakov *et al.* 2000b, 2001).

Local fixation of chromosomal variants

New underdominant mutations (such as those of the Robertsonian type) are generally expected to be lost from populations. Theoretical studies that were carried out by Lande (1979) and Hedrick (1981) suggested that such mutations could be fixed either by strong deterministic processes (selection or meiotic drive) or as a result of small population size (due to genetic drift and/or inbreeding in small, isolated populations). Each chromosomal variant, which at present characterize a karyotypic race of the common shrew had to become fixed in a local population, that is, attain a frequency of 100%. It is well known that a high mutation rate and low heterozygous disadvantage favour fixation of new chromosome variants (Searle and Wójcik 1998). The simplest model for the local fixation of chromosomal variants in *S. araneus* assumes genetic drift. In situations where chromosomal variants are generated at a reasonable rate in populations that are reasonably small (Lande 1979), fixation by genetic drift seems to be inevitable. However, ecological data show that in favourable habitats *S. araneus* exists in

large, continuous populations (Croin Michielsen 1966). Also, allozyme, mtDNA and microsatellite DNA studies in the common shrew suggest no clear subdivision into small populations (Bengtsson and Frykman 1990, Wyttenbach and Hausser 1996, Ratkiewicz *et al*. 2002).

It seems that conditions are not suitable for genetic drift in many localities now. However, the typical population structure of *S. araneus* in the past (during race formation) may have been different from that observed now (Searle and Wójcik 1998). An attempt to determine past population size, making use of molecular data collected from present-day populations (Ratkiewicz *et al*. 2002) has revealed a very high long-term effective female population size N_e. Out of 28 shrew samples 21 distinct haplotypes were found, the majority of which were unique. In one of the populations studied, all of 6 shrews possessed different haplotypes (Ratkiewicz *et al*. 2002). Moreover, as the mismatch distribution was not strongly L-shaped (left truncated), as is normal for strongly bottlenecked populations (Marjoram and Donnely 1994), no severe population reduction appears to have occurred in the *S. araneus* populations studied since the last glaciation, ie over the period when chromosomal variants presumably became fixed (Fig. 5; Ratkiewicz *et al*. 2002). This conclusion, however, should be viewed as tentative, as it may hold true only for chromosome races in Poland. Thus, no final conclusion excluding the role of genetic drift in the fixation of chromosomal variants should be reached before studying other chromosome races (in Britain, Scandinavia and Siberia, for example).

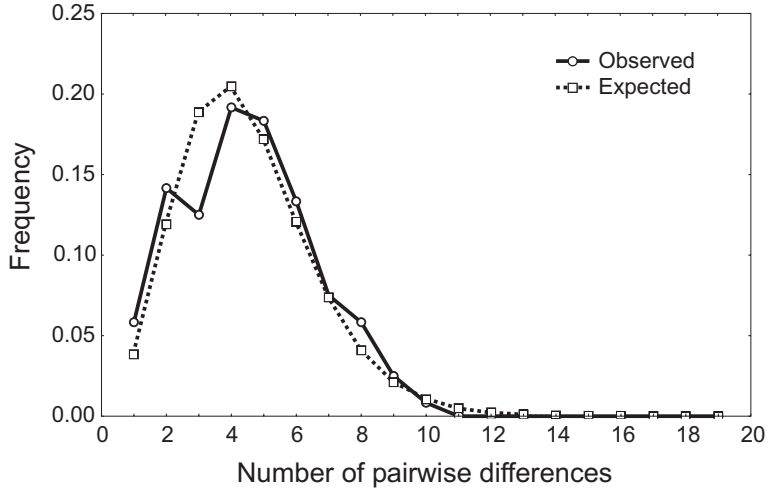

Fig. 5. The observed and expected distribution of pairwise differences under a model of population expansion (Rogers and Harpending 1992). The parameters used were for the East European Karyotypic Group of *Sorex araneus*: = 3.856, $_0$ = 0.126 and $_1$ = 16.977. After Ratkiewicz *et al*. (2002).

Considering the above molecular results and the large number of metacentrics that are found (Searle and Wójcik 1998), there is certainly some doubt that genetic drift is sufficient to generate so many fixations. Therefore, it is probable that chromosomal variants in *S. araneus* have some sort of selective advantage, which would greatly increase the chance that a variant becomes fixed (Searle and Wójcik 1998). A very attractive possibility is that Rb fusions of *S. araneus* could display meiotic drive. This would permit a rapid increase in frequency and local fixation (Searle and Wójcik 1998). Wyttenbach *et al*. (1998) showed meiotic drive in favour of some metacentrics in the male common shrews, giving credence to this model of local fixation of Rb fusions in *S. araneus*.

Spread of chromosomal variants

To explain the accumulation and geographical spread of Rb fusions for house mice White (1978) proposed the chain processes variant of stasipatric model. The distribution of *S. araneus* metacentrics in Poland fits the chain processes model remarkably well, with different metacentrics (assumed to be *de novo* Rb fusions) having spread different distances into an ancestral acrocentric distribution (Wójcik 1993).

Current molecular data appears to support this scenario. Taberlet *et al.* (1994), in their study of cytochrome *b* gene in shrews from western Europe, proposed the spread of metacentrics from one race to another. Second, the "sudden expansion" model suggested in the study of cytochrome *b* gene of the common shrew in Poland (Ratkiewicz *et al*. 2002) is in good agreement with the chain processes variant of the stasipatric model of chromosome evolution (White 1978) proposed for chromosome races in Poland (Wójcik 1993). It is worth mentioning that this model does not require a population bottleneck.

While metacentrics may have spread through populations as inferred above, another way they could have expanded their range was by the common shrew itself enlarging its distribution. This could of course have happened at the end of the last glaciation, as first suggested by Searle (1984). It is a challenge in the study of the common shrew to untangle the relative importance of these two modes by which metacentrics could attain their current distribution.

Range changes

Some of *S. araneus* races that are very similar in terms of karyotype occur geographically distant and disjunct from one another (see Searle and Wójcik 1998 for examples). This could be caused by two completely different processes: (1) independent evolution or (2) common ancestry. The second alternative would have profound implications for the historical biogeography of the common shrew. Unfortunately, no molecular studies have been performed so far to establish the identity (or otherwise) of *S. araneus* metacentrics with widely disjunct distributions. This type of analysis was, however, carried out for the house mice Rb fusions, using microsatellite DNA as genetic markers (Riginos and Nachman 1999). There is an

urgent need now for such molecular studies in the common shrew to confirm (or reject) the common ancestry of similar races with disjunct distribution.

The recentness of the Rb mutations in the common shrew

Searle (1984) suggested that chromosomal races of the common shrew could have evolved during the last glacial period and spread in the process of post--Pleistocene recolonization. Thus, chromosomal evolution appears to have been extremely rapid. It is not, however, possible to determine directly at what point in the past chromosomal rearrangements occurred and became fixed (Baker *et al.* 1987). Late changes are thus as likely as early changes. Therefore, molecular markers are needed to provide arguments for or against the recentness of Rb polymorphism. For the house mouse, chromosomal and molecular data support the idea that the massive accumulation of Rb chromosomes has occurred within the past 10 000 years (Nachman *et al*. 1994).

Molecular data of Taberlet *et al.* (1994) based on the cytochrome *b* gene of shrews in western Europe support the hypothesis of the migration of metacentrics through acrocentric populations. The authors postulated a recent origin of chromosomal variability in the *Sorex araneus* group. Furthermore, the time since the beginning of the expansion, estimated for the shrew races in Poland, using the mismatch distribution, coincides with the last glacial period (37 400–62 400 years ago; Ratkiewicz *et al*. 2002). The lack of geographic structure and the starlike phylogeny of the shrew haplotypes (Ratkiewicz *et al*. 2002) give another argument for a recent origin of chromosomal variability in the common shrew in Poland.

Chromosomal speciation

The study of Rb variation in the common shrew can contribute to a basic understanding of chromosomal evolution. Because the Rb races of the common shrew are most probably of recent origin, it is possible to make comparisons between shrews that differ in karyotype but that are otherwise very similar. In fact, genetic and morphological differences between shrews with different karyotypes are almost always small or non-existent.

Searle and Wójcik (1998) pointed out that the concept of chromosomal speciation would be difficult to sustain if chromosomal differences does not promote speciation in a species as chromosomally variable as *S. araneus*. It seems that the existence of unimodal clines, through flat and bimodal hybrid zones to sympatric overlap without hybridization in *S. araneus*, demonstrates a continuum of stable intermediates between clinal differentiation and good species. In particular, bimodality within a local hybrid populations indicates that speciation of parental forms is nearly complete. This seems to be the case for the Valais race (*S. antinorii*) and Cordon race in the Alps (Balloux *et al*. 2000, Brünner *et al*. 2002) and could be for some other races in Eurasia. Brünner *et al*. 2002 have stated that the Valais race (*S. antinorii*) represents a "good" sibling species of *S. araneus*, and this is new

indirect evidence for the process of chromosomal speciation in shrews of the *Sorex araneus* group.

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References

Aniskin V. M. and Lukianova I. V. 1989. [A new chromosome race and the analysis of hybridization zone of two karyoforms of *Sorex araneus* (Insectivora, Soricidae)]. Doklady Akademii Nauk SSSR 309: 1260–1262. [In Russian]

Baker R. J., Qumsiyeh M. B. and Hoop C. S. 1987. Role of chromosome banding patterns in understanding mammalian evolution. [In: Current mammalogy. Vol. 1. H. Genoways, ed]. Plenum, New York: 67–96.

Balloux F., Brünner H., Lugon-Moulin N., Hausser J. and Goudet J. 2000. Microsatellites can be misleading: an empirical and simulation study. Evolution 54: 1414–1422.

Banaszek A. 1994. The structure of the contact zone between the chromosomal races Drużno and Łęgucki Młyn in the common shrew (*Sorex araneus*) in north-eastern Poland: preliminary results. Folia Zoologica 43, Suppl. 1: 11–19.

Banaszek A., Ratkiewicz M., Fedyk S., Szałaj K. A. and Chętnicki W. 1996. The chromosomes and isoenzymes in marginal populations of the common shrew (*Sorex araneus*) in the Vistula delta. Zeitschrift für Säugetierkunde 61: 65–72.

Barton N. H. and Bengtsson B. O. 1986. The barrier to genetic exchange between hybridizing populations. Heredity 56: 357–376.

Barton N. H., Halliday R. B. and Hewitt G. M. 1983. Rare electrophoretic variants in a hybrid zone. Heredity 50: 139–146.

Barton N. H. and Gale K. S. 1993. Genetic analysis of hybrid zones. [In: Hybrid zones and evolutionary process. R. G. Harrison, ed]. Oxford University Press, New York: 13–45.

Barton N. H. and Hewitt G. M. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16: 113–148.

Bengtsson B. O. and Bodmer W. F. 1976. The fitness of human translocation carriers. Annals of Human Genetics 40: 253–257.

Bengtsson B. O. and Frykman I. 1990. Karyotype evolution: evidence from the common shrew (*Sorex araneus* L.). Journal of Evolutionary Biology 3: 85–101.

Bilton D. T., Mirol P. M., Mascheretti S., Fredga K., Zima J. and Searle J. B. 1998. Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization. Proceedings of the Royal Society of London, B 265: 1219–1226.

Brünner H., Fumagalli L. and Hausser J. 1994. A comparison of two contact zones between chromosomal races of *Sorex araneus* in the western Alps: karyology and genetics. Folia Zoologica 43, Suppl. 1: 113.

Brünner H. and Hausser J. 1996. Genetic and karyotypic structure of a hybrid zone between the chromosomal races Cordon and Valais in the common shrew, *Sorex araneus*. Hereditas 125: 147–158.

Brünner H., Lugon-Moulin N., Balloux F., Fumagalli L. and Hausser J. 2002. A taxonomical revaluation of the Valais chromosome race of the common shrew *Sorex araneus* (Insectivora: Soricidae). Acta Theriologica 47 (in press).

Bulatova N. Sh., Nadjafova R. S. and Krapivko T. P. 2002. Intraspecific phylogenetic relationships in *Sorex araneus* L.: The southern Baltic subgroup of chromosome races. Russian Journal of Genetics. 38: 64–69

Bulatova N., Searle J. B., Bystrakova N., Nadjafova R., Shchipanov N. and Orlov V. 2000. The diversity of chromosome races in *Sorex araneus* from European Russia. Acta Theriologica 45, Suppl. 1: 33–46.

Camin J. H. and Sokal R. R. 1965. A method for deducing branching sequences in phylogeny. Evolution 19: 311–326.

Catzeflis F. 1984. Systématique biochemique, taxonomie et phylogénie des Musaraignes d'Europe (*Soricidae*, *Mammalia*). PhD thesis, Universite de Lausanne, Lausanne: 1–164.

Coyone J. A. and Orr H. A. 1998. The evolutionary genetics of speciation. Philosophical Transactions: Biological Sciences, Royal Society, London B 353: 287–305.

Croin-Michielsen N. 1966. Intraspecific and interspecific competition in the shrews *Sorex araneus* L. and *Sorex minutus* L. Archives Néerlandaises de Zoologie 17: 73–174.

Demesure B., Comps B. and Petit R. J. 1996. Choloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. Evolution 50: 2515–2520.

Dobzhansky T. 1940. Speciation as a stage in evolutionary divergence. The American Naturalist 74: 312–321.

Farris J. S., Kluge A. G. and Eckardt M. J. 1970. A numerical approach to phylogenetic systematics. Systematic Zoology 19: 172–191.

Fedyk S. 1980. Chromosome polymorphism in a population of *Sorex araneus* L. at Białowieża. Folia Biologica (Kraków) 28: 83–120.

Fedyk S. 1982. Further studies on chromosome polymorphism of the common shrew. Acta Theriologica 27: 149–153.

Fedyk S. 1995. [Geographic chromosomal differentiation and hybrid zones between chromosome races of *Sorex araneus* in north-eastern Poland]. Dissertationes Universitatis Varsoviensis 439, Białystok: 1–125. [In Polish]

Fedyk S., Banaszek A., Chętnicki W., Cichomska A. and Szałaj K. A. 2000. Reassessment of the range of the Drnholec race: studies on meiosis in *Sorex araneus* hybrids. Acta Theriologica 45, Suppl. 1: 59–67.

Fedyk S., Chętnicki W. and Banaszek A. 1991. Genetic differentiation of Polish populations of *Sorex araneus* L. III. Interchromosomal recombination in a hybrid zone. Evolution 45: 1384–1392.

Ford P. J. and Graham C. F. 1964. The chromosome number in the common shrew, *Sorex araneus*. L. Bulletin of the Mammal Society of the British Isles 22: 10–11.

Fredga K. 1996. The chromosome races of *Sorex araneus* in Scandinavia. Hereditas 125: 123–135.

Frykman I. and Bengtsson B. O. 1984. Genetic differentiation in *Sorex*. III. Electrophoretic analysis of a hybrid zone between two karyotypic races in *Sorex araneus*. Hereditas 100: 259– 270.

Frykman I., Simonsen V. and Bengtsson B. O. 1983. Genetic differentiation in *Sorex*. I. Electrophoretic analysis of the karyotypic races of *Sorex araneus* in Sweden. Hereditas 99: 279–292.

Garagna S., Zuccoti M., Searle J. B., Redi C. A. and Wilkinson P. J. 1989. Spermatogenesis in heterozygotes for Robertsonian chromosomal rearrangements from natural populations of the common shrew, *Sorex araneus*. Journal of Reproduction and Fertility 87: 431–438.

Gyllensten U. and Wilson A. C. 1987. Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. Genetical Research 49: 25–29.

Haldane J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics 12: 101–109.

Halkka L., Söderlund V., Skarén U. and Heikkilä J. 1987. Chromosomal polymorphism and racial evolution of *Sorex araneus* L. in Finland. Hereditas 106: 257–275.

Halkka L., Kaikusalo A. and Vakula N. 1994. Revision of *Sorex araneus* L. chromosome nomenclature, and rece N new to Finland. Annales Zoologici Fennici 31: 283–288.

Harrison R. G. 1990. Hybrid zones: windows on the evolutionary process. Oxford Surveys of Evolutionary Biology 7: 69–128.

Hatfield T., Barton N. and Searle J. B. 1992. A model of a hybrid zone between two chromosomal races of the common shrew (*Sorex araneus*). Evolution 46: 1129–1145.

Hauffe H. C. and Searle J. B. 1993. Extreme karyotypic variation in a *Mus musculus domesticus* hybrid zone: the tobacco mouse story revisited. Evolution 47: 1374–1395.

Hausser J., Bosshard F., Taberlet P. and Wójcik J. M. 1991. Relationships between chromosome races and species of *Sorex* of the *araneus* group in the western Alps. Mémoires de la Société Vaudoise des Sciences Naturelles 19: 79–95.

Hausser J., Dannelid E. and Catzeflis F. 1986. Distribution of two karyotypic races of *Sorex araneus* (Insectivora, Soricidae) in Switzerland and the post-glacial recolonization of the Valais: First results. Zeitschrift für zoologische Systematik und Evolutionsforschung 24: 307–314.

Hausser J., Fedyk S., Fredga K., Searle J. B., Volobouev V., Wójcik J. M. and Zima J. 1994. Definition and nomenclature of chromosome races of *Sorex araneus*. Folia Zoologica 43, Suppl. 1: 1–9.

Hausser J., Fumagalli L. and Taberlet P. 1998. Mitochondrial DNA evolution in shrews. [In: Evolution of shrews. J. M. Wójcik and M. Wolsan, eds]. Mammal Research Institute PAS, Białowieża: 295–308.

Hedrick P. W. 1981. The establishment of chromosomal variants. Evolution 35: 322–332.

Heikkilä J. 1989. Population genetics of north temperate shrews (Soricidae). A review. Annales Zoologici Fennici 26: 459–467.

Hewitt G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58: 247–276.

Howard D. J. 1993. Reinforcement: The origin, dynamics, and fate of an evolutionary hypothesis. [In: Hybrid zones and the evolutionary process. R. G. Harrison, ed]. Oxford University Press, New York: 46–69.

Ivanitskaya E. Yu. 1994. Comparative cytogenetics and systematics of *Sorex*: a cladistic approach. [In: Advances in the Biology of Shrews. J. F. Merritt, G. L. Kirkland Jr and R. K. Rose, eds]. Special Publication of Carnegie Museum of Natural History No. 18: 313–323.

Jiggins C. D. and Mallet J. 2000. Bimodal hybrid zones and speciation. Trends in Ecology and Evolution 15: 250–255.

Kozlovsky A., Orlov V., Okulova N., Kovalskaya J. and Searle J. B. 2000. Chromosome studies on common shrews from northern and central parts of European Russia. Acta Theriologica 45, Suppl. 1: 27–31.

Král B. and Radjabli S. I. 1974. Banding patterns and Robertsonian fusion in the western Siberian population of *Sorex araneus* (Insectivora, Soricidae). Zoologické Listy 23: 217–227.

Kreitman M. 1991. Detecting section at the level of DNA. [In: Evolution at the molecular level. R. K. Selander, A. G. Clark and T. S. Whittam, eds]. Sinauer, Sunderland, Massachusetts: 204–221.

Lande R. 1979. Effective deme sizes during long-term evolution estimated from rates of chromosomal rearrangement. Evolution 33: 234–251.

Larkin D. M., Serov O. L., Borodin P. M., Zhdanova N. S. and Searle J. B. 2000. Comparative genome mapping in mammals: the shrew map. Acta Theriologica 45, Suppl. 1: 131–141.

Lugon-Moulin N., Balloux F. and Hausser J. 2000. Genetic differentiation of common shrew *Sorex araneus* populations among different alpine valleys revealed by microsatellites. Acta Theriologica 45, Suppl. 1: 103–117.

Lugon-Moulin N., Brünner H., Balloux F., Hausser J. and Goudet J. 1999. Do riverine barriers, history or introgression shape the genetic structuring of a common shrew (*Sorex araneus*) population? Heredity 83: 155–161.

Lugon-Moulin N., Wyttenbach A., Brünner H., Goudet J. and Hausser J. 1996. Study of gene flow through a hybrid zone in the common shrew (*Sorex araneus*) using microsatellites. Hereditas 125: 159–168.

Lukáčová L., Pialek J. and Zima J. 1994. A hybrid zone between the Ulm and Drnholec karyotypic races of *Sorex araneus* in the Czech Republic. Folia Zoologica 43, Suppl. 1: 37–42.

Marjoram P. and Donnelly P. 1994. Pairwise comparisons of mitochondrial DNA sequences in subdivided populations and implications for early human evolution. Genetics 136: 673–683.

Mercer S. J. 1991. Chromosomal variation of the common shrew *Sorex araneus* L. in Britain. PhD thesis, University of Oxford, Oxford.

Mercer S. J., Searle J. B. and Wallace B. M. N. 1991. Meiotic studies of karyotypically homozygous and heterozygous male common shrews. Mémoires de la Société Vaudoise des Sciences Naturelles 19: 33–43.

Meylan A. and Hausser J. 1973. Les chromosomes des *Sorex* du groupe *araneus-arcticus* (Mammalia, Insectivora). Zeitschrift für Säugetierkunde 38: 143–158.

Mishta A. V., Searle J. B. and Wójcik J. M. 2000. Karyotypic variation of the common shrew *Sorex araneus* in Belarus, Estonia, Latvia, Lithuania, and Ukraine. Acta Theriologica 45, Suppl. 1: 47–58.

Nachman M. W., Boyer S. R., Searle J. B. and Aquadro C. F. 1994. Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. Genetics 136: 1105–1120.

Narain Y. and Fredga K. 1996. A hybrid zone between the Hällefors and Uppsala chromosome races of *Sorex araneus* in central Sweden. Hereditas 125: 137–145.

Narain Y. and Fredga K. 1997. Meiosis and fertility in common shrews, *Sorex araneus*, from a chromosomal hybrid zone in central Sweden. Cytogenetics and Cell Genetics 78: 253–259.

Narain Y. and Fredga K. 1998. Spermatogenesis in common shrews, *Sorex araneus*, from a hybrid with extensive Robertsonian polymorphism. Cytogenetics and Cell Genetics 80: 158–164.

Neet C. R. and Hausser J. 1991. Biochemical analysis and determination of living individuals of the Alpine chromosome races and species of the *Sorex araneus* group. Mémoires de la Société Vaudoise des Sciences Naturelles 19: 97–107.

Polyakov A. V., Borodin P. M., Lukáčová L., Searle J. B. and Zima J. 1997a. The hypothetical Old-Northern chromosome race of *Sorex araneus* found in the Ural Mts. Annales Zoologici Fennici 34: 139–142.

Polyakov A. V., Chadova N. B., Rodionova M. I., Panov V. V., Dobrotvorsky A. K., Searle J. B. and Borodin P. M. 1997b. Novosibirsk revisited 24 years on: chromosome polymorphism in the Novosibirsk population of the common shrew *Sorex araneus* L. Heredity 78: 172–177.

Polyakov A. V., Panov V. V., Ladygina T. Yu., Bochkarev M. N., Rodionova M. I. and Borodin P. M. 2001. Chromosomal evolution of the common shrew *Sorex araneus* L. from the Southern Urals and Siberia in the postglacial period. Russian Journal of Genetics 37: 351–357.

Polyakov A. V., Zima J., Banaszek A., Searle J. B. and Borodin P. M. 2000a. New chromosome races of the common shrew *Sorex araneus* from Eastern Siberia. Acta Theriologica 45, Suppl. 1: 11–18.

Polyakov A. V., Zima J., Searle J. B., Borodin P. M. and Ladygina T. 2000b. Chromosome races of the common shrew *Sorex araneus* in the Ural Mts: a link between Siberia and Scandinavia? Acta Theriologica 45, Suppl. 1: 19–26.

Prager E. M., Sage R. D., Gyllensten U., Thomas W. K., Hübner R., Jones C. S., Noble L., Searle J. B. and Wilson A. C. 1993. Mitochondrial DNA sequence diversity and the colonization of Scandinavia by house mice from East Holstein. Biological Journal of the Linnean Society 50: 85–122.

Rafiński J. and Babik W. 2000. Genetic differentiation among northern and southern populations of the moor frog *Rana arvalis* Nilsson in central Europe. Heredity 84: 610–618.

Ratkiewicz M. 1997. [Genetic consequences of karyotype divergence in *Sorex araneus* Linnaeus, 1758, revealed by isoenzymes and mitochondrial DNA]. PhD thesis, Warsaw University, Białystok Branch, Białystok: 1–85. [In Polish]

Ratkiewicz M., Fedyk S., Banaszek A., Chętnicki W., Szałaj K., Gielly L. and Taberlet P. 2002. The evolutionary history of the two karyotypic groups of the common shrew, *Sorex araneus*, in Poland. Heredity 88: 235–242.

Ratkiewicz M., Supruniuk J., Fedyk S., Banaszek A., Chętnicki W. and Szałaj K. A. 2000. Genetic differentiation and gene flow between the Drnholec and Łęgucki Młyn chromosome races of the common shrew *Sorex araneus* in northern Poland. Acta Theriologica 45, Suppl. 1: 79–91.

Ratkiewicz M., Wójcik J. M., Lukáčová L. and Macholán M. 1996. Nomenclature of electrophoretically studied loci and allozymes in the common shrew (*Sorex araneus* L.). [In: Abstract of the ISAAC Fourth International Meeting "Evolution in the *Sorex araneus* group. Cytogenetic and molecular aspects". Norr Malma and Uppsala, Sweden, August 22–27, 1996]. Uppsala University, Uppsala.

Riginos C. and Nachman M. W. 1999. The origin of a Robertsonian chromosomal translocation in house mice inferred from linked microsatellite markers. Molecular Biology and Evolution 16: 1763–1773.

Rogers A. R. and Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9: 552–569.

Searle J. B. 1983. Robertsonian chromosomal variation in the common shrew *Sorex araneus* L. PhD thesis, University of Aberdeen, Aberdeen.

Searle J. B. 1984. Three new karyotypic races of the common shrew *Sorex araneus* (Mammalia: Insectivora) and a phylogeny. Systematic Zoology 33: 184–194.

Searle J. B. 1985. Isoenzyme variation in the common shrew (*Sorex araneus*) in Britain, in relation to karyotype. Heredity 55: 175–180.

Searle J. B. 1986a. Meiotic studies of Robertsonian heterozygotes form natural populations of the common shrew, *Sorex araneus* L. Cytogenetics and Cell Genetics 41: 154–162.

Searle J. B. 1986b. Factors responsible for a karyotypic polymorphism in the common shrew, *Sorex araneus*. Proceedings of the Royal Society London, B 229: 277–298.

Searle J. B. 1986c. A trimeric esterase in the common shrew (*Sorex araneus*). Journal of Heredity 77: 121–122.

Searle J. B. 1988 Selection and Robertsonian variation in nature: The case of the common shrew. [In: The cytogenetics of mammalian autosomal rearrangements. A. Daniel, ed]. Alan R. Liss Inc., New York: 507–531.

Searle J. B. 1993. Chromosomal hybrid zones in eutherian mammals. [In: Hybrid zones and evolutionary process. R. G. Harrison, ed]. Oxford University Press, New York: 309–353.

Searle J. B., Fedyk S., Fredga K., Hausser J. and Volobouev V. T. 1991. Nomenclature for the chromosomes of the common shrew (*Sorex araneus*). Mémoires de la Société Vaudoise des Sciences Naturelles 19: 13–22.

Searle J. B., Hübner R., Wallace B. M. N. and Garagna S. 1990. Robertsonian variation in wild mice and shrews. Chromosomes Today 10: 253–263.

Searle J. B. and Wilkinson P. J. 1987. Karyotypic variation in the common shrew (*Sorex araneus*) in Britain – a "Celtic Fringe". Heredity 59: 345–351.

Searle J. B. and Wójcik J. M. 1998. Chromosomal evolution: the case of *Sorex araneus*. [In: Evolution of shrews. J. M. Wójcik and M. Wolsan, eds]. Mammal Research Institute PAS, Białowieża: 219–268.

Stewart J. R. and Lister A. M. 2001. Cryptic northern refugia and the origins of the modern biota. Trends in Ecology and Evolution 16: 608–613.

Swofford D. L. 1985. PAUP: Phylogenetic Analysis Using Parsimony, Version 2.4. Illinois Natural History Survey, Champaign.

Szałaj K. A., Fedyk S., Banaszek A., Chętnicki W. and Ratkiewicz M. 1996. A hybrid zone between two chromosome races of the common shrew, *Sorex araneus* L., in eastern Poland: preliminary results. Hereditas 125: 169–176.

Taberlet P., Fumagalli L. and Hausser J. 1994. Chromosomal versus mitochondrial DNA evolution: Tracking the evolutionary history of the southwestern European populations of the *Sorex araneus* group (Mammalia, Insectivora). Evolution 48: 623–636.

Taberlet P., Fumagalli L., Wust-Saucy A. and Cosson J. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 8: 1923–1934.

Tegelström H. 1987. Genetic variability in mitochondrial DNA in a regional population of the great tit (*Parus major*). Biochemical Genetics 25: 95–110.

Volobouev V. T. 1983. Les types du polymorphisme chromosomique et leur rôle evolutif chez les mammiferes (Insectivora, Rodentia et Carnivora). PhD thesis, University of Pierre and Marie Curie, Paris.

Volobouev V. T. 1989. Phylogenetic relationships of the *Sorex araneus-arcticus* species complex (Insectivora, Soricidae) based on high-resolution chromosome analysis. Journal of Heredity 80: 284–290.

Volobouev V. T. and Catzeflis F. 1989. Mechanisms of chromosomal evolution in three European species of the *Sorex araneus-arcticus* group (Insectivora: Soricidae). Zeitschrift für Zoologische Systematik und Evolutionsforschung 27: 252–262.

Wallace B. M. N. and Searle J. B. 1990. Synaptonemal complex studies of the common shrew (*Sorex araneus*). Comparison of Robertsonian heterozygotes and homozygotes by light microscopy. Heredity 65: 359–367.

Wallace B. M. N. and Searle J. B. 1994. Oogenesis in homozygotes and heterozygotes for Robertsonian chromosomal rearrangements from natural populations of the common shrew, *Sorex araneus*. Journal of Reproduction and Fertility 100: 231–237.

White M. J. D. 1978. Modes of speciation. Freeman, San Francisco: 1–455.

Wójcik J. M. 1991. Chromosomal polymorphism in the common shrew *Sorex araneus* and its adaptive singnificance. Mémoires de la Société Vaudoise des Sciences Naturelles 19: 51–62.

Wójcik J. M. 1993. Chromosome races of the common shrew *Sorex araneus* in Poland: a model of karyotype evolution. Acta Theriologica 38: 315–338.

Wójcik J. M. and Searle J. B. 1988. The chromosome complement of *Sorex granarius* – the ancestral karyotype of the common shrew (*Sorex araneus*)? Heredity 61: 225–229.

Wójcik J. M. and Wójcik A. M. 1994. Protein variation in the common shrew (*Sorex araneus* L.) in Poland, in relation to karyotype. Folia Zoologica 43, Suppl. 1: 53–61.

Wójcik J. M., Wójcik A. M. and Zalewska H. 1996. Chromosome and allozyme variation of the common shrew, *Sorex araneus*, in different habitats. Hereditas 125: 183–189.

Wyttenbach A., Borodin P. and Hausser J. 1998. Meiotic drive favours Robertsonian metacentric chromosomes in the common shrew (*Sorex araneus*, Insectivora, Mammalia). Cytogenetics and Cell Genetics 83: 199–206.

Wyttenbach A., Goudet J., Cornuet J.-M. and Hausser J. 1999a. Microsatellite variation reveals low genetic subdivision in a chromosome race of *Sorex araneus* (Mammalia, Insectivora). Journal of Heredity 90: 323–327.

Wyttenbach A. and Hausser J. 1996. The fixation of metacentric chromosomes during the chromosomal evolution in the common shrew (*Sorex araneus*, Insectivora). Hereditas 125: 209–217.

Wyttenbach A., Narain Y. and Fredga K. 1999b. Genetic structuring and gene flow in a hybrid zone between two chromosome races of the common shrew (*Sorex araneus*, Insectivora) revealed by microsatellites. Heredity 82: 79–88.

Zima J. 1991. Is the trend toward low 2Na numbers inescapable for *Sorex araneus* populations? Mémoires de la Société Vaudoise des Sciences Naturelles 19: 63–71.

Zima J., Fedyk S., Fredga K., Hausser J., Mishta A., Searle J. B., Volobouev V. T. and Wójcik J. M. 1996. The list of the chromosome races of the common shrew (*Sorex araneus*). Hereditas 125: 97–107.

Zima J., Lukáčová L. and Macholán M. 1998. Chromosomal evolution in shrews. [In: Evolution of shrews. J. M. Wójcik and M. Wolsan, eds]. Mammal Research Institute PAS, Białowieża: 175–218.

Zima J., Macholán M., Filippucci M. G., Reiter A., Andreas M., Lípa M. and Kryštufek B. 1994. Karyotypic and biochemical status of certain marginal populations of *Sorex araneus*. Folia Zoologica 43, Suppl. 1: 43–51.

Zima J., Wójcik J. M. and Horáková M. 1988. The number of karyotypic variants in the common shrew (*Sorex araneus*). Acta Theriologica 33: 467–475.

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