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Phylogeographic differentiation in *Sorex araneus*: morphology in relation to geography and karyotype

P. David Polly

ABSTRACT. Findings of gene flow across hybrid zones between karyotypic races of the common shrew, *Sorex araneus* Linnaeus, 1758, have contributed to the debate over the role of chromosomal changes in speciation. The correlation between chromosomal and morphological evolution was examined here across the full geographic range of the species. Previous studies on selected karyotypic races yielded ambiguous results: some found significant differences between races, but others concluded that local variation was more important than karyotype for morphological structuring. Forty three samples, representing 24 karyotypic races and three species were studied here. Geometric morphometrics were used to determine whether karyotypic, geographic or population-level structuring was present in the size and shape of molars, skulls and mandibles. Significant structuring was found in all traits among populations, among karyotypic races, among phylogenetic groups of karyotypic races and among species, greatest among populations (F_{ST} ranged from 0.08 to 0.11) and groups (F_{ST} 0.04 to 0.15). Within *S. araneus* structuring was greater in skull centroid size and molar shape than in skull or mandible shape. Large-scale east-to-west clines were found in molar and skull shape. The skull cline is probably associated with changes in the frequency of the upper fifth antemolar. Mandible shape was not highly differentiated between karyotypic races compared to molar shape, but it was better at discriminating among karyotypic groups and species. It is likely that fossil specimens can be determined to the level of karyotypic group, but not to a specific karyotypic race.

KEY WORDS: F_{ST}, geometric morphometrics, karyotypic race, phenotypic evolution, *Sorex araneus*.

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Филогеографическая дифференциация у *Sorex araneus*: морфология в связи с географией и кариотипом

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РЕЗЮМЕ. Недавнее открытие значительного потока генов через гибридные зоны между хромосомными расами обыкновенной бурозубки *Sorex araneus* послужило новым импульсом для дискуссии о роли хромосомных перестроек в видообразовании. В настоящей работе оценивается корреляция между эволюцией хромосом и морфологических признаков на всем ареале этого вида. Результаты предшествующих исследований отдельных хромосомных рас оказались неоднозначными: в то время как в одних случаях была обнаружена достоверная разница между расами, в других морфологическая подразделенность популяций определялась в большей степени локальными различиями, нежели кариотипом. В настоящей работе методом геометрической морфометрии размеров тела, формы моляра, черепа и нижней челюсти была произведена оценка морфологической структурированности в 43 выборках, представляющих 25 хромосомных рас и три вида землероек. Структурированность между популяциями, расами, кариотипическими группами и видами была достоверно подтверждена для всех исследованных признаков. Наиболее отчетливая подразделенность наблюдалась между популяциями (F_{ST} от 0.08 до 0.11) и кариотипическими группами (F_{ST} от 0.04 до 0.15). Внутри вида *S. araneus* подразделенность по центроиду черепа и по форме моляра была выражена существеннее, чем по форме черепа и нижней челюсти. В результате работы была выявлена клинальная изменчивость (с востока на запад) формы черепа и моляра. Изменение формы черепа связано, по-видимому, с изменением частоты верхнего пятого антемоляра. Форма нижней челюсти, которая прежде служила стандартным объектом морфометрических исследований у *S. araneus*, оказалась более слабым дискриминатором между хромосомными расами по сравнению с формой моляра, однако лучше остальных признаков дискриминировала виды. По-видимому, палеонтологические материалы вида *Sorex araneus* могут быть идентифицированы до уровня кариотипических групп, но не хромосомных рас.

КЛЮЧЕВЫЕ СЛОВА: F_{ST}, геометрическая морфометрия, хромосомные расы, эволюция фенотипа, *Sorex araneus*.

Introduction

Do chromosomal mutations lead to speciation? Rearrangements of chromosome arms can cause incompatibilities that inhibit gene flow and allow genetic differences to accumulate. With the observation that more than 90 percent of speciation events have associated chromosomal rearrangements, karyotypic change is arguably a major factor in speciation (White, 1978). But the discovery that gene flow can cross karyotype boundaries has led some to conclude that chromosomal variation is unconnected to speciation and long-term genetic differentiation. Bengtsson & Frykman (1990) went so far as to argue that karyotypically fragmented species like *Sorex araneus* are in the process of "despeciation", bleeding through secondary hybrid zones the genetic structure that built up during past periods of glacial isolation. Several examples of gene flow across *S. araneus* racial hybrid zones now support this hypothesis (Frykman *et al.*, 1983; Frykman & Bengtsson, 1984; Searle, 1985; Wójcik & Wójcik, 1994; Ratkiewicz *et al.,* 2002; Balloux *et al.*, 2003; Fivaz *et al.*, 2003; Andersson, 2004), as do examples of morphometric differentiation that are unassociated with racial boundaries (Searle & Thorpe, 1987; Wójcik *et al.*, 2000; Banaszek *et al.*, 2003). Nevertheless, Hausser (1994) considered that chromosomal rearrangements do lead to genetic differentiation. He pointed out that gene flow between parapatric races depends on the size of their metacentrics and on the way in which they partition local habitats. Further he argued that even though gene flow may occur in some situations, these are the exception rather than the rule: normally gene flow does not occur between karyotypically distinct groups; chromosome rearrangements should on the whole lead to genetic differentiation and speciation. Thus, the issue of how karyotypic variation relates to other types of differentiation remains of interest. As emphasized by Wójcik *et al.* (2000), genetic differentiation at broad spatial scales needs more study.

In this paper, variation among karyotypic groups of *S. araneus* was studied across its entire geographic range. The effect of karyotype on morphology was tested at four hierarchical levels: within races, between races, between phylogenetic groups of karyotypic races and between sibling species. Robertsonian variation divides the species into nearly 70 races (Wójcik *et al*., 2003) that are grouped into four putative phylogenetic groups: the western European karyotypic group with races from Britain to the Ukraine that share the *hi* and, usually, *gm* metacentrics; the eastern European karyotypic group with five races in Poland and adjacent areas sharing the metacentric *gr*; the northern European group with races from northern Scandinavia through the Urals to western Siberia that share the metacentric *ip*; and the Siberian group with races in south-central Siberia that share metacentrics *gk* and *hi* (Searle, 1984; Wójcik, 1993; Ivanitskaya, 1994; Polyakov *et al.*, 2001). The Valais race, now elevated to species rank as *S. antinorii* Bonaparte, 1840, is the sister-group to the rest, and the Cordon race of eastern France and Switzerland and Pelister race of the Balkans have predominantly acrocentric karyotypes that are thought to represent the ancestral chromosome condition before the origin of the metacentric groups. The races are characterized by different combinations of metacentric chromosomes which arose through Robertsonian fusions and whole-arm reciprocal translocations (WARTs; Halkka *et al.*, 1987; Searle *et al.*, 1990; Hausser, 1994; Fredga, 1996; Searle & Wójcik, 1998). In this study, the karyotypic diversity of *Sorex araneus* was represented by 24 races (Fig. 1). Two of its morphologically similar sibling species were also represented, *S. antinorii* and *S. coronatus* Millet, 1828. The inclusion of samples from different races, karyotypic groups and species allows the effect of chromosomal differences to be assessed globally rather than relying on individual case studies.

Material and methods

Forty three samples belonging to *Sorex araneus* (24 karyotypic races), *S. antinorii* (formerly the Valais race) and *S. coronatus* were studied, totalling 495 individuals (Fig. 1, Tab. 1). The karyotype of most of the samples was known directly from previous research by other authors (see Acknowledgements); the karyotypes of the remaining samples were inferred from their geographic position. The karyotype of one of the latter (Neusiedler See, Austria) was ambiguous, belonging either to the Drnholec race or to an Ulm×Drnholec hybrid population (Zima *et al.*, 2003; J. Zima, pers. comm.). Only immature animals were included in the analysis to minimize the effect of age differences on our results. In shrews, an immature individual is one that has reached its adult size, but which has not overwintered and reproduced (Churchfield, 1990). In other words, juvenile and aged individuals were excluded. Age determination was based on tooth wear, thus excluding also individuals with excessively worn teeth. Sexual dimorphism is negligible in shrews so the sexes were pooled.

The skull, mandible and first lower molar of each specimen were photographed in ventral, lateral and functional occlusal views, respectively. The shape of each specimen was represented with two-dimensional Cartesian landmarks (Fig. 2). Lower molars were photographed and landmarked five times each then averaged to minimize orientation error (Polly, 2003).

Centroid size was calculated for each specimen as the square root of the sum of the squared distances between the landmarks and the centroid (Bookstein, 1991). Centroid size was used because it is mathematically independent of shape; linear measurements such as skull length may be biased if they lie along major axes of shape variation. Centroid size was calculated for skulls and mandibles, but not molars because of the difficulty of accurately fitting a scale bar into those photographs.

Each sample was Procrustes superimposed and projected orthogonally into shape tangent space (Rohlf,

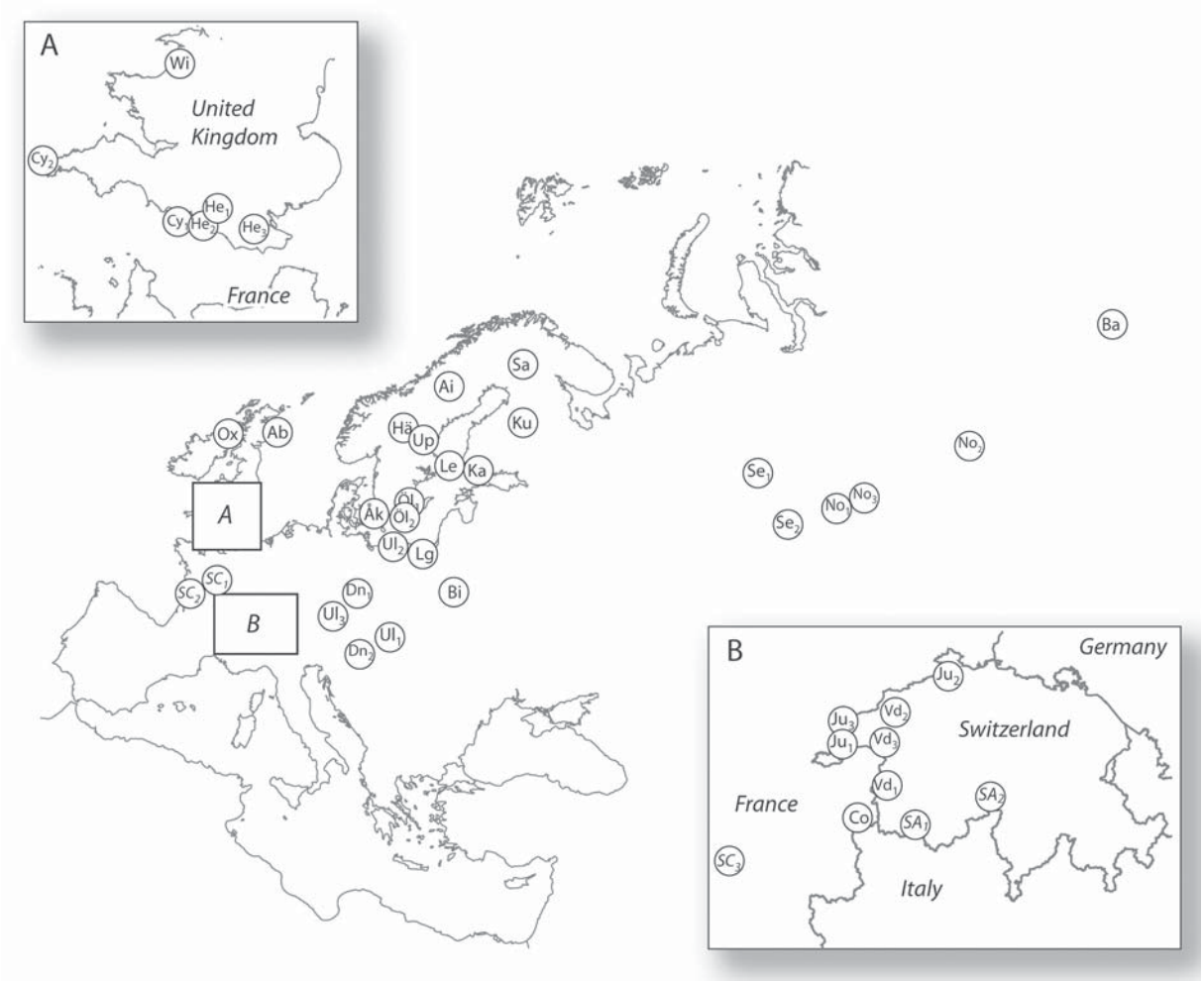

Figure 1. Map showing the location of samples used in this study. The karyotypic race of each sample is indicated by abbreviation (see Tab. 1).

Table 1. Samples used in this study. Latitude and longitude are in decimal degrees.

Abbr	Taxon	Location	Lat	Long	n	Group
SC_1	*S. coronatus*	La Boue, Loir-et-Cher, France[3]	47.55	1.62	7/7/6	CORO
SC_2	*S. coronatus*	Guè de Velluire, Vendée, France[3]	46.37	–0.92	8/7/8	CORO
SC_3	*S. coronatus*	Col de la Croix Perrin, Vercors, France[2]	44.97	5.42	9/9/10	CORO
SA_1	*S. antinorii*	La Monta Arolla, Valais, Switzerland[2]	46.03	7.49	10/9/10	ANTI
SA_2	*S. antinorii*	Haslital, Switzerland[2]	46.58	8.33	21/18/21	ANTI
Ab	Aberdeen race	Dumbreck, Scotland, UK[7]	57.35	-2.18	17/15/18	WEKG
Ai	Abisko race	Vilhelmina, Lappmark, Sweden[5]	64.62	16.66	7/7/7	NEKG
Åk	Åkarp race	Everlöv, Skåne, Sweden[5]	55.66	13.48	15/11/15	WEKG
Ba	Baikal race	Baikal, Siberia, Russia[1]	53.30	109.20	5/5/4	SKG
Bi	Białowieża race	Białowieża Forest, Poland[4]	52.70	23.92	43/31/34	EEKG
Cy_1	Chysauster race	Arreton, Isle of Wight, UK[7]	50.67	–1.25	9/9/9	WEKG
Cy_2	Chysauster race	Chysauster, Cornwall, UK[7]	50.17	–5.52	9/7/10	WEKG
Co	Cordon race	Les Houches, Haute-Savoie, France[2]	45.89	6.80	10/9/10	ACRO

Table 1 (continued).

Abbr	Taxon	Location	Lat	Long	n	Group
Dn_1	Drnholec race	Filipov, Bohemia, Czech Republic[1]	50.83	14.37	14/13/16	WEKG
Dn_2	Drnholec race	Neusidler See, Austria[2]	47.89	16.65	8/9/9	WEKG
Ha	Hällefors race	Bergobacken, Transtrand, Sweden[5]	61.06	13.19	16/9/16	WEKG
He_1	Hermitage race	Alice Holt Forest, Surrey, UK[6]	51.21	–0.79	9/9/9	WEKG
He_2	Hermitage race	Hunston, Surrey, UK[7]	50.80	–0.82	16/9/16	WEKG
He_3	Hermitage race	Twyssenden, Kent, UK[7]	51.10	0.46	12/9/12	WEKG
Ju_1	Jura race	Bassins les Pralets, Vaud, Switzerland[2]	46.47	6.25	7/8/8	WEKG
Ju_2	Jura race	Bellelay, Bern, Switzerland[2]	47.27	7.17	4/4/4	WEKG
Ju_3	Jura race	Chalet à Roch, Vaud, Switzerland[2]	46.56	6.23	4/4/4	WEKG
Ka	Kalvitsa race	Tvärminne Station, Finland[2]	59.87	23.22	14/14/14	NEKG
Ku	Kuhmo Race	Sonkajärvi, Finland[2]	63.79	27.45	3/3/3	NEKG
Lg	Łęgucki Młyn race	Górowo Iłowieckie, Poland[4]	54.30	20.52	15/14/12	EEKG
Le	Lemland race	Åland, Eckero, Finland[2]	60.21	19.62	19/19/19	NEKG
No_1	Novosibirsk × Serov interracial hybrids	Kurgan, West Siberia, Russia[1]	55.95	65.95	3/3/3	NEKG
No_2	Novosibirsk race	Yurga, West Siberia, Russia[1]	55.60	84.95	3/4/4	NEKG
No_3	Novosibirsk race	Abatskii, West Siberia, Russia[1]	56.25	70.67	4/5/5	NEKG
$Öl_1$	Öland race	Karum, Öland, Sweden[5]	56.88	16.80	8/8/6	WEKG
$Öl_2$	Öland race	Southern tip, Öland, Sweden[5]	56.21	16.40	7/5/6	WEKG
Ox	Oxford race	Islay, Scotland, UK[7]	55.63	-6.15	8/4/8	WEKG
Sa	Savukoski race	Luosto, Lappmark, Sweden[5]	67.21	26.74	3/3/3	NEKG
Se_1	Serov race	Serov, Urals, Russia[1]	59.90	60.22	3/4/5	NEKG
Se_2	Serov race	Chelyabinsk, Siberia, Russia[1]	55.95	61.25	8/7/4	NEKG
Ul_1	Ulm race	Belanské Tatry, Slovakia[1]	49.14	19.01	20/17/20	WEKG
Ul_2	Ulm race	Stary Kraków, Poland[4]	54.45	16.58	15/14/12	WEKG
Ul_3	Ulm race	Ruda, Bohemia, Czech Republic[1]	49.68	14.17	6/6/4	WEKG
Up	Uppsala race	Boda, Hälsingland, Sweden[5]	60.99	15.23	13/13/12	WEKG
Vd_1	Vaud race	Bex, Vaud, Switzerland[2]	46.25	7.00	6/6/6	WEKG
Vd_2	Vaud race	Champ-Pittet, Vaud, Switerland[2]	46.78	6.67	14/14/14	WEKG
Vd_3	Vaud race	Le Jourat, Vaud, Switzerland[2]	46.58	6.68	12/11/12	WEKG
Wi	Wirral race	Dolgellau, Wales, UK[7]	52.75	-3.88	13/12/12	WEKG

Sample sizes (n) are for lower molars / skulls / mandibles. Group abbreviations: CORO — *S. coronatus*; ANTI — *S. antinorii*; WEKG — western European karyotypic group; EEKG — eastern European karyotypic group; NEKG — northern European karyotypic group; SKG — Siberian karyotypic group; ACRO — acrocentric race. Samples are from: [1]Institute of Vertebrae Biology, Czech Academy of Sciences, Brno, Czech Republic; [2]Institute of Zoology and Animal Ecology, University of Lausanne, Switzerland; [3]Muséum d'histoire naturelle, Genève, Switzerland, [4]Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland; [5]Naturhistoriska riksmuseet, Stockholm, Sweden; [6]Department of Biological Sciences, Queen Mary, University of London, UK; [7]Department of Biology, University of York, York, UK.

1990; Rohlf & Slice, 1990). Sample means were then calculated and jointly superimposed. Principal components scores were used as shape variables for statistical analysis. Scores were calculated by subtracting the mean from each sample (or group of sample means), calculating the covariance matrix of the residuals, and projecting the residuals onto their principal component axes (Dryden & Mardia, 1998). Shape variables were used for the discriminant function analysis described below. Principal components plots were used to explore shape variation for geographic and taxonomic associations.

Isolation by distance was measured by plotting pairwise morphometric distance (Euclidean distance between centroid sizes or Procrustes distance between sample means) against geographic distance. A linear regression line was used to illustrate the relationship,

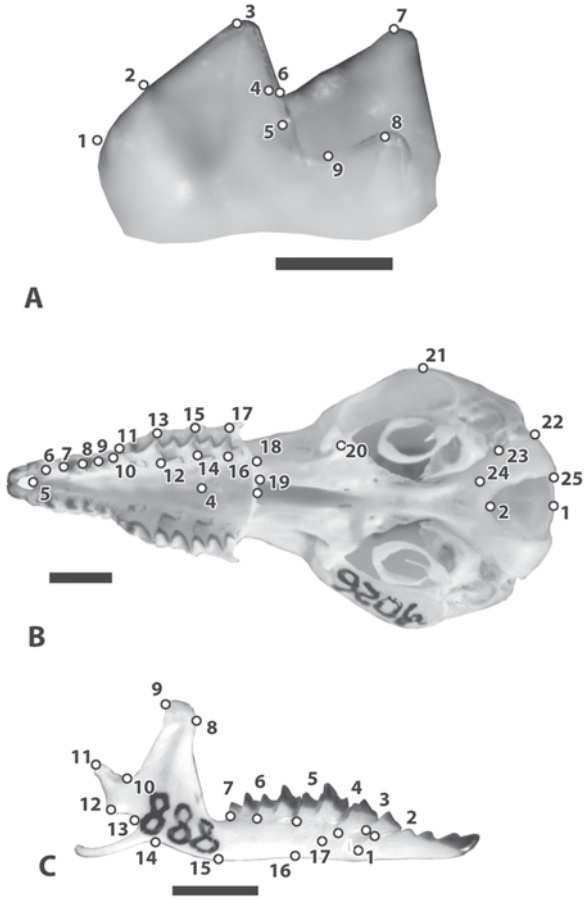

Figure 2. Geometric landmarks used in this study: landmarks of the lower first right molar in occlusal functional view (mesial to the right and buccal to the top) (A); landmarks of the skull in ventral view (B); landmarks of the mandible in lateral view (C). Scale bar in A is 0.5 mm, scale bars in B and D are 2.0 mm.

but significance was assessed using a Mantel test using 100,000 random permutations (Manly, 1991). The correlation (R) between morphological and geographic distance was estimated using the normalized Mantel-Z statistic. *Sorex coronatus* samples were not included in the isolation by distance study, but *S. antinorii* was because it was long considered to be a karyotypic race of *S. araneus* (Brünner *et al.*, 2002).

Structuring at the levels of population, race, group and species was assessed using F-statistics. F_{ST}, the proportion of between-group to within-group variance (Wright, 1951; Lande, 1992; Spitze, 1993), was estimated for skull centroid size, skull shape, mandibular shape and molar shape. To remove sample size bias, a jackknife procedure was used (Weir and Cockerham, 1984). One thousand jackknife iterations were performed in which a random member of each group was dropped and the F_{ST} statistic recalculated (Manly, 1991). By iteratively dropping all samples, the jackknife procedure minimizes the effects of small sample sizes and helps incorporate their effects into the standard error. The jackknife statistic (F_{ST}*) was calculated as

$$F_{ST}* = \frac{(n-1-k)SSB*}{(k-1)(SSW*+SSB*)} \quad , \qquad (1)$$

where SSB* was the sum of squared distances between group means and the grand mean, SSW* was the sum of squared distances between individuals and their group mean, n was the sample size, and k was the number of groups. The asterisk (*) indicates that the statistic was calculated from the jackknifed sample. F_{ST} was calculated as the mean of the 1,000 F_{ST}* estimates, and the standard error of F_{ST} was estimated as their standard deviation. Spitze (1993) proposed the notation Q_{ST} for F_{ST} calculated from quantitative data such as the ones in this study to distinguish it from F_{ST} calculated from molecular data, for which he proposed the notation G_{ST}; some authors have adopted Spitze's notation for Q_{ST}, but not G_{ST}. To avoid misunderstanding and the proliferation of data-specific notations, F_{ST} was used here for quantitative data. Note that the data in this study are phenotypic, not genotypic.

A randomization test was also used to assess whether karyotypic races are morphologically differentiated from one another. If races are differentiated, then morphometric distances within a race should be smaller than those between random populations when geographic distance is held constant. To test this, geographic effects were first removed by regressing pairwise Procrustes distances between sample means onto geographic distance. Then the residual Procrustes distances of pairs belonging to the same race (n=18) were compared to a distribution of 100,000 distances among 18 random pairs. Random pairs in any one sampling could be from different races or the same race depending on chance. Races were considered to be differentiated if the real distances between pairs of the same race were smaller than 95% of the random distribution (α=0.05). *Sorex coronatus* and *S. antinorii* were excluded from this test.

Discriminant function analysis (DFA) was used to determine the accuracy with which specimens can be assigned to karyotypic group based on morphology. DFA finds canonical axes that best separate pre-defined groups, unlike PCA which finds an axis system whose first axis is aligned along the greatest variance in the data. Cross-validation determines the accuracy with which each specimen is classified by the discriminant functions. The *Sorex araneus* samples were classified into the western European karyotypic group (WEKG), the eastern European karyotypic group (EEKG) and the northern European karyotypic group (NEKG) (Searle & Wójcik, 1998; Polyakov *et al.*, 2001). *S. coronatus* (CORO) and *S. antinorii* (ANTI) samples were grouped by species. Note that the groupings of the Siberian races are still problematic (Polyakov *et al.*, 2001). The acrocentric Cordon race and the Baikal race (Siberian karotypic group) were excluded from this part of the analysis because only one sample was available for each. Only those shape variables that collectively accounted for 90% of the variation in each data set were

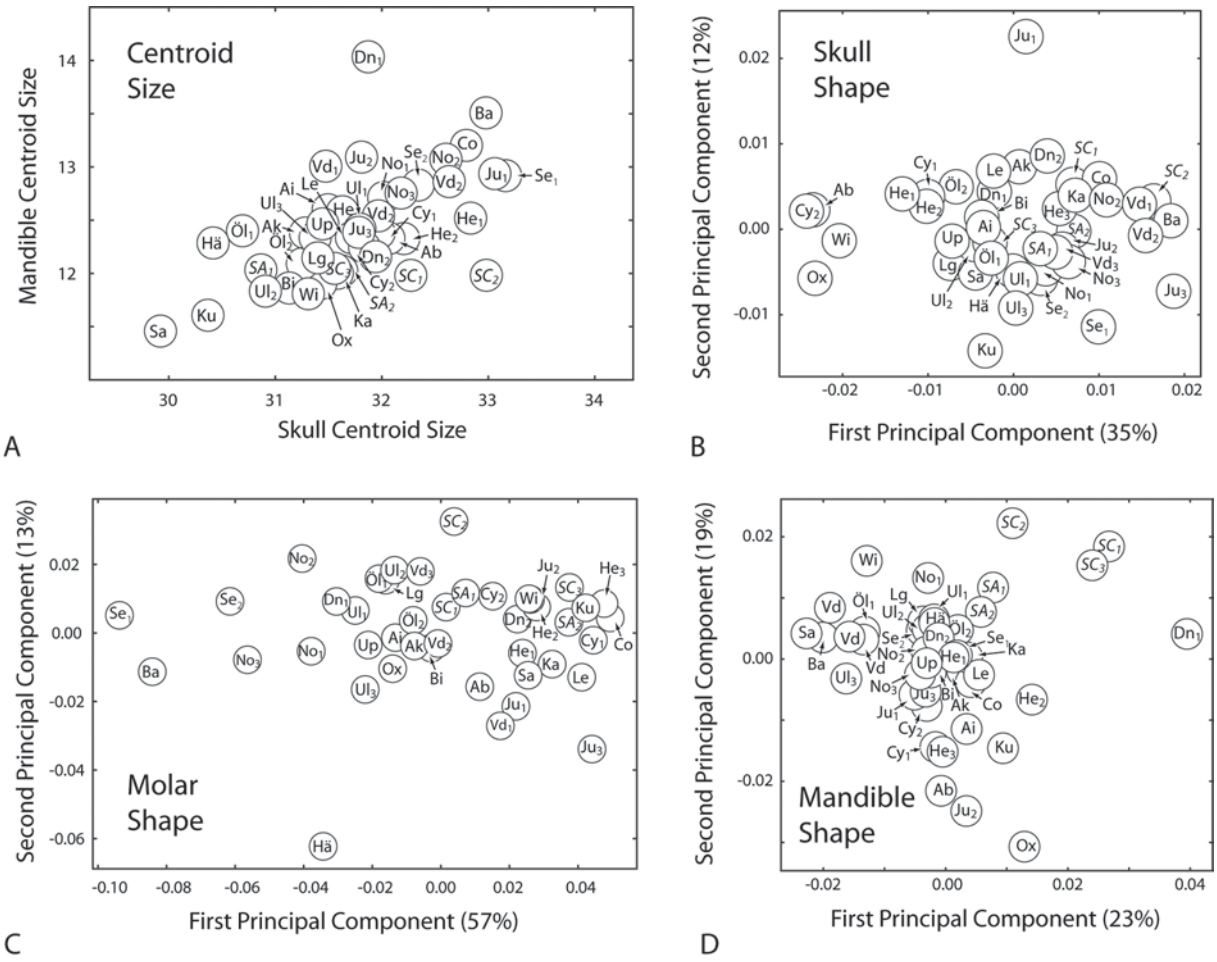

Figure 3. Morphological variation in four traits: variation in mandible and skull centroid size (A); variation on the first two principal components of skull shape (B); variation on the first two principal components of molar shape (C); variation on the first two principal components of mandible shape (D). Abbreviations as in Fig. 1 and Tab. 1.

used for DFA classification: for molars it was the first six PCs, for skulls it was the first 11, and for mandibles it was the first 10. Skull and mandible centroid sizes were combined for analysis of size data. This analysis was performed using sample means, not individual shrews.

All analyses except DFA were performed in Mathematica© 5.2. The DFA was performed in Statistica© 6.0. Landmark coordinates were collected with tpsDig 1.40 by F. James Rohlf.

Results

Geographic patterns of variation

Size. Skull and mandible size varied considerably among species, karyotypic groups and races, with no clear structure at any level (Fig. 3A). The species were not separated by size, with both *Sorex antinorii* and *S. coronatus* falling within the range of *S. araneus. S. coronatus* was a marginal outlier in Fig. 3A because it has a proportionally larger skull than mandible. Samples from the same karyotypic race were usually similar in size, but not exclusively so (*e.g.,* Novosibirsk), but others showed considerable variation within the race (*e.g.*, Jura). The Filipov Drnholec sample (Dn_1) was an outlier for mandible size: this was almost certainly because of an artefact in the original photography; the Filipov mandibles were still joined at the symphysis preventing them from being laid flat, distorting their size and shape through parallax (see also Fig. 3D). Many of the largest shrews were from high-altitude sites: Baikal, Cordon, Jura, Serov, Novosibirsk and Vaud samples. Previous studies have noted increased size at high altitudes (Homolka, 1980). The smallest shrews came from northern sites: Savukoski, Kuhmo, Hällefors and Öland. The correlation between skull centroid size and latitude of R=-0.427 suggests a weak latitudinal trend in which animals are smaller at high latitudes. The same pattern was previously reported in *S. araneus* by Ochocińska & Taylor (2003), who discussed it as a departure from Bergmann's rule.

Molar shape. Molar shape showed a strong east-west cline (Fig. 3C). The eastern races of Serov, Baikal, and Novosibirsk were at the negative end of PC 1 and the western races of Cordon, Hermitage, Chysauster, and Jura races at the positive end. PC 1 had a strong correlation with longitude, R=–0.718. Some structuring was evident at the karyotypic level. Samples from the same race clustered loosely near one another (*e.g.*, Novosibirsk, Ulm, Öland, Hermitage), and samples of *S. coronatus* and *S. antinorii* clustered near their conspecifics on the periphery of *S. araneus*. Despite the clustering, no karyotypic race grouped together exclusive of others. Variation in molar shape was nearly univariate, with 57% concentrated on the first PC (Fig. 4), suggesting that the genetic variation underlying the cline is simple. Axes other than PC 1 explained very little variance and mostly differentiated single samples (such as the Hällefors race on PC 2 in Fig. 3C).

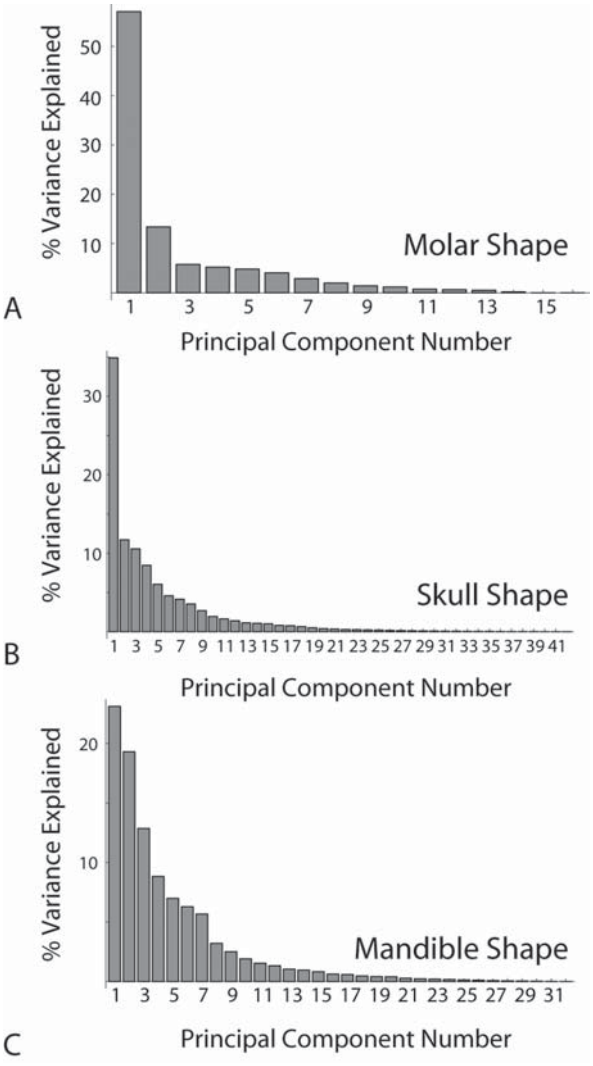

Figure 4. Percentage of total variance explained by individual principal components in molar shape (A), skull shape (B) and mandible shape (C).

Skull shape. Skull shape had a weak east-west cline largely driven by the several western British races clustering at the negative end of PC 1 (Fig. 3B). The Siberian races were not at the opposite end suggesting that the trend is primarily in the western European karyotypic group. The correlation between PC 1 and longitude was substantially lower than in molars (R=0.425). Separation of samples along PC 1 was on the basis of the proportional length of the rostrum and spacing of the antemolars: taxa at the negative end had short rostrums and small, closely spaced antemolars; the ones at the positive end had long rostrums and large antemolars.

A north-south cline in skull shape that has many of the morphological features seen in the east-west cline identified here was described in Scandinavia by Sulkava *et al.* (1985). In Scandinavia, northern shrews had long rostrums and large antemolars, while those to the south had shorter rostrums with closely spaced antemolars. It is likely that the Scandinavian cline is part of the larger east-west gradient identified here, but happens to run north-south through Scandinavia.

The east-west trend in skull shape is probably associated with a cline in which the frequency of missing fifth upper antemolars (U^5) increases westward. (Note that in shrews teeth between the first incisor and fourth premolar are called antemolars because their individual homologies with incisors, canines and premolars are contested). Polymorphic absence of that tooth has been reported before in German, Swedish, Finnish and British populations at the level of about 1–2%, climbing as high as 52% on the island of Islay (Reinwald, 1961; Skarén, 1964; Schmidt, 1967; Corbet & Southern, 1977; Hausser *et al*., 1990). Absence of U^5 was noted in five of the samples studied here: Aberdeen race from Dumbreck, Białowieża race, Chysauster race (both samples), Öland race from the south of the island and Ulm race from Stary Krakow (samples sizes were too small to accurately estimate the frequencies). In animals missing U^5 the rostrum was notably shorter, making it likely that the variation in skull shape identified here is correlated with polymorphism in U^5. Note that the Oxford race from Islay, which has the highest described frequency of missing U^5, falls at the extreme negative end of PC 1.

Mandible shape. No obvious geographic or taxonomic pattern below the species level was found in mandible shape (Fig. 3D). Mandible shape sorted the three species: *Sorex coronatus* was well separated in having a posteriorly projecting condyle and anteriorly projecting coronoid process, features noted by Hausser & Jammot (1974) as being distinctive of the species. *S. antinorii* samples was also separated from *S. araneus*, but less so than *S. coronatus*. The differences in the mandible of *S. antinorii* were similar but less extreme than those in *S. coronatus*. Within *S. araneus*, mandible shape showed little correlation with karyotypic race, with group or with geography. Many of the British races (but not the Wirral) had negative values on PC 2 and so grouped towards the bottom centre of the plot. The Vaud samples clustered together at the negative end of PC 1, but were near the Savukoski, Öland and Baikal races.

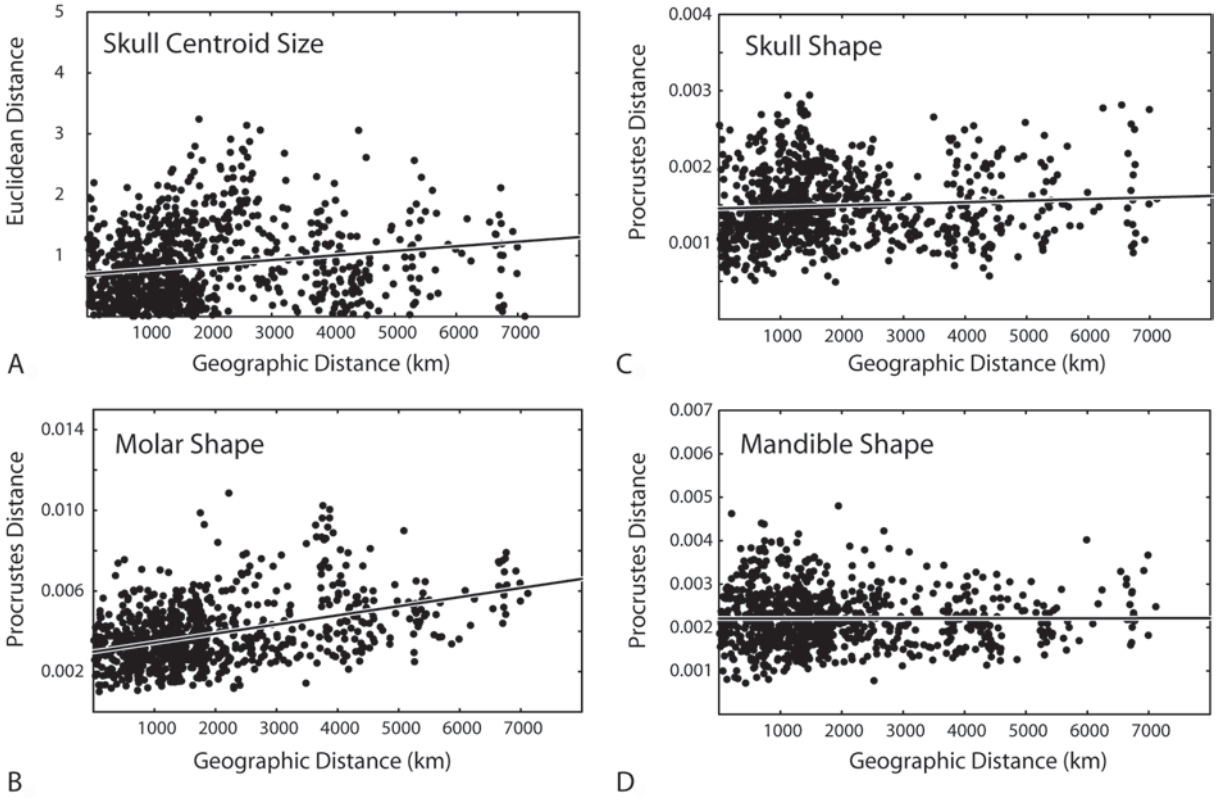

Figure 5. Isolation by distance in skull centroid size (A), skull shape (B), molar shape (C) and mandible shape (D).

The Drnholec sample from Filipov was an outlier at the positive end of PC 1, probably due to biased measurement as discussed above. Mandible shape variation was more complicated than the other structures, with only 23% of the variation explained by the first PC. PCs 2–7 also explained appreciable portions of the variance.

Isolation by distance

Isolation by distance was assessed by comparing the morphological distance between sample pairs to their geographic distance from one another (Fig. 5). Association was tested using Mantel tests on the two distance matrices. Skull centroid size had a weak, but positive association with geographic distance (R=0.18, p=0.00). Lower molar shape had a stronger association with geographic distance (R=0.44, p=0.00), while skull shape had a marginal one (R=0.07, p=0.02). Mandible shape did not have a significant association with geographic distance (R=–0.00, p=0.51). The geographic structuring in skull size is related to latitude and the structuring in molar shape to longitude, as described above.

Morphological structuring and karyotypic races

Structuring in morphology was measured with F-statistics. F_{ST} was measured at four hierarchical levels: between populations of the same race, between races, between karyotypic groups, and between species. F_{ST} is the amount of differentiation between groups compared to variation within them; the greater the value, the greater the structuring. The greatest structuring was found between populations of the same race and between karyotypic groups (Tab. 2). Mandible shape was most structured between populations and between species, but not between races or between karyotypic groups. Structuring between races was weak but significant for all four traits.

Table 2. Divergence in quantitative characters at four hierarchical levels.

	F_{ST}			
	Skull size	Molar shape	Skull shape	Mandible shape
Between populations of the same races	0.11 (±0.019)	0.11 (±0.014)	0.09 (±0.024)	0.08 (±0.012)
Between races	0.04 (±0.004)	0.04 (±0.001)	0.01 (±0.002)	0.01 (±0.001)
Between karyotypic groups	0.13 (±0.019)	0.15 (±0.009)	0.05 (±0.005)	0.04 (±0.003)
Between species	0.06 (±0.011)	0.07 (±0.004)	0.04 (±0.004)	0.08 (±0.004)

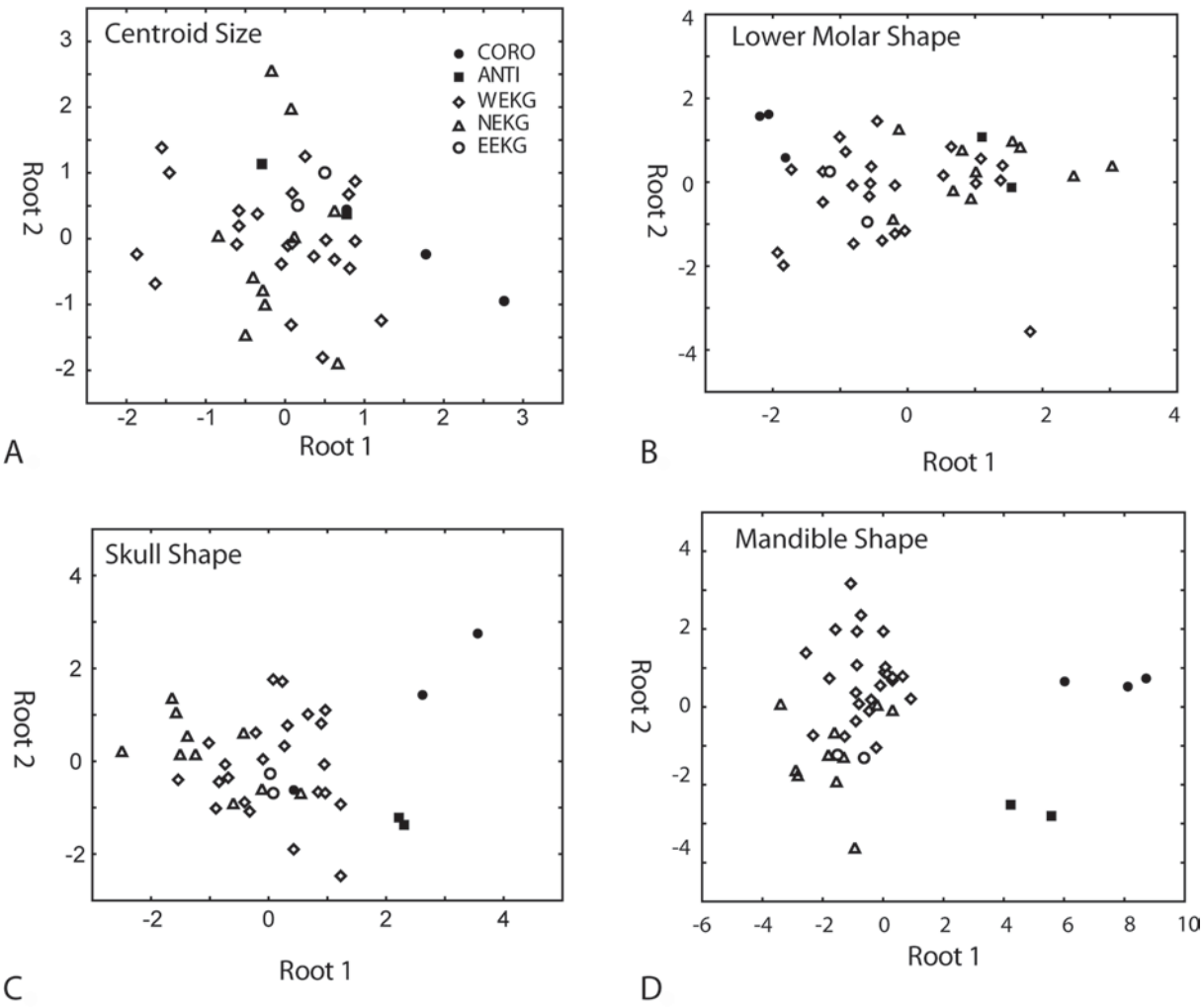

Figure 6. Discriminant function analysis results for karyotypic groups: skull centroid size (A); skull shape (B); molar shape (C); mandible shape (D). Abbreviations as in Tab. 1.

The randomization test showed that the association of morphology and karyotypic race was small but significant for shape but not size. Skull centroid size had a narrowly insignificant association with race (p=0.057), but molar shape, skull shape, and mandible shape all had a strong association (p=0.009, 0.005, and 0.0001 respectively). Thus, differences between races thus appear to be significant, even though the differentiation between them is small as measured by F_{ST}.

Discriminant function analysis and identification of fossils

Large F_{ST} values between karyotypic groups for molar shape suggests that it is at this hierarchical level that fossil taxa can best be identified. The majority of fossil mammalian specimens are isolated teeth. Skulls and mandibles are seldom preserved intact and postcranial bones or non-molar teeth are often difficult to identify taxonomically at the species level. Discriminant Functions Analysis (DFA) with cross-validation was used to test the accuracy with which specimens can be classified at the karyotypic group level using centroid size, molar shape, skull shape and mandible shape (Fig. 6, Tab. 3). Molar and mandible shape were best

Table 3. Percentage of samples referred correctly to karyotypic group by canonical Discriminant Function Analysis. Abbreviations are the same as in Tab. 1.

	Percent correct			
	Centroid size	Lower molars	Skulls	Mandibles
CORO	33.3%	66.7%	66.7%	100.0%
ANTI	0.0%	50.0%	100.0%	100.0%
WEKG	100.0%	87.5%	95.8%	95.8%
NEKG	0.0%	50.0%	60.0%	70.0%
EEKG	0.0%	0.0%	0.0%	0.0%
Total	61.0%	70.7%	80.5%	85.4%

for classifying specimens (80.5% and 85.4% accurate overall respectively) and centroid size was worst (61.0% accurate overall). Mandibles were able to discriminate the three species 100% of the time. In cases where the eastern European group was misclassified, it was classified as the western European group.

Discussion

The remarkable karyotypic diversity of *Sorex araneus* has often been contrasted to its comparative morphological homogeneity. The results presented here demonstrate that the morphological structuring exists in the species, though the actual differences are small and more easily measured than observed.

Morphological structuring is complicated and the pattern differs from trait to trait. Structuring as measured by F_{ST} is greatest at the population and karyotypic group levels for three out of four of the traits (mandible shape being the exception) and weakest at the level of the karyotypic race. This means that differences between populations within a race are often greater than differences between populations from different races or even different species. Large-scale east to west clines exist in skull and molar shape, but not in mandible shape. Mandible shape has no obvious clines within *S. araneus*, but differs more than skulls and molars between species. Overall, the greatest structuring at all levels occurs in molar shape, possibly because tooth phenotypes are not susceptible to structural remodelling after mineralization, unlike skulls and mandibles (Polly, 2001, 2003; Caumul & Polly, 2005). The genetic variance of molars is thus probably higher proportional to the environmental variance than in skull and mandible shape; non-genetic ecophenotypic responses to local environmental factors probably obscure structuring in the latter traits.

High local variation in morphological size and shape is consistent with molecular studies that found high local genetic diversity within races (Lugon-Moulin *et al.*, 2000; Bannikova *et al.*, 2003; Ratkiewicz *et al.*, 2003) and with morphometric studies that found greater morphometric differences within races than between them (Searle & Thorpe, 1987; Wójcik *et al.*, 2000; Banaszek *et al.*, 2003). The between population F_{ST} values measured here (0.08–0.11) are comparable to those measured between populations using microsatellite and allozyme data (0.03–0.14; Fivaz *et al.*, 2003; Ratkiewicz *et al.*, 2003; Andersson *et al.*, 2004). The implications are important for morphometric studies related to karyotype: when only two samples are being compared, the differences are more likely to be local than to be karyotypic. For example, the findings by Polly (2001) of significant differences in molar shape between the Aberdeen and Oxford karyotypic races may really reflect population-level rather than race-level differentiation. To adequately assess difference between races, many samples from each may be required like in the studies by Polyakov *et al.* (2002) and Okulova *et al.* (2004). One supposes that population-level variation is transient, generated by stochastic (or selectional) changes in small local populations and reabsorbed by gene flow in the metapopulation, because the F_{ST} values are lower at the level of karyotypic race, the next rung in the hierarchy. If the population-level variation were permanent, then we would expect differences between races, karyotypic groups and species to be greater. The generation of transient local variation probably ensures rapid phenotypic change in certain conditions, such as founder effects. The population on the island of Islay in western Scotland may be an example, where the frequency of polymorphic absence of the upper fifth antemolar reaches 52% (Corbet & Southern, 1977).

Morphological differentiation between karyotypic races is small, but significant. The morphological F_{ST} values ranged from 0.01 to 0.04, but all were significantly greater than 0. Furthermore, the differences between populations within races were significantly smaller than differences between random populations from different races. Even though racial differences are easily obscured by population-level variation, it appears that karyotype boundaries do influence morphological variation. The effect of karyotype was much greater at the group level than at the level of individual races. F_{ST} between karyotypic groups ranged from 0.04 in mandible shape to a stunning 0.15 in molar shape. This pattern suggests significant genetic divergence among the karyotypic groups, probably corresponding to deeper phylogeographic divergences among the groups than among their constituent races. The northern and western karyotypic groups were as differentiated from one another as they were from the species *Sorex coronatus* and *S. antinorii* in all traits except mandibular shape. Interestingly, the eastern karyotypic group was not well differentiated from the western group, suggesting that the split between the two is more recent than between the western and northern groups. Ratkiewicz *et al.* (2002) found that there was little cytochrome *b* differentiation between these two groups and concluded that their chromosomal differences were recent.

The pattern of morphological structuring identified here has implications for the determination of fossil material to subspecific groupings within *S. araneus*. The low level of differentiation between races of the same karyotypic group compared to the population-level variability probably means that fossil specimens, whose sample size is typically small, cannot be determined to the level of race. They can, however, be reasonably accurately assigned to karyotypic groups. The dating of the Oxford-Hermitage race split by Polly (2001) may need to be re-examined in this light, because that study included only members of the western karyotypic group. Furthermore, that study linked fossil samples from the Late Glacial and Riss-Würm interglacial periods to the Oxford and Hermitage clades respectively but only included one sample from the Hermitage race. If the differentiation in that sample was transient and not representative of the race as a whole, then the association with the interglacial fossil sample may have been spurious. Further studies are required to confirm or refute those results.

Conclusion

Do chromosomal mutations lead to speciation? The results presented here suggest that morphological divergence in the common shrew is, on a broad scale, correlated with karyotypic divergence. Morphological structuring in relation to karyotype is present in *Sorex araneus* at several hierarchical levels. Morphological differentiation is strongest at the level of karyotypic group and there are small but significant differences between individual races. This hierarchical pattern suggests that the karyotypic groups originated before their constituent races, though the timescale of origination cannot be resolved with the data presented here. Mandible shape appears to evolve more quickly than molar or skull shape because the differences between species are larger in the former than the latter. Molar shape, which probably has the highest genetic component of any of the traits examined in this study, has structuring at all levels, more so than size, skull shape or mandible shape. The presence of structuring suggests that genetic differences are significant among karyotypic races, and the hierarchical pattern suggests that the genetic differences have built up in successive stages. The view that current diversity within *S. araneus* is being homogenized because of gene flow across secondary hybrid zones (Bengtsson & Frykman, 1990) appears to be incompatible with the patterns of morphological structuring found here.

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