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Allozyme Differentiation in Four Sympatric Species of European Shrews (Soricidae: Mammalia)

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Key Word Index—*Sorex*; *Neomys*; shrews; allozyme electrophoresis; biochemical systematics; evolution; sympatric species.

Abstract—Four species of shrews from the Białowieża Primeval Forest, eastern Poland, were analyzed electrophoretically: *Sorex araneus* Linnaeus, 1758, *Sorex minutus* Linnaeus, 1766, *Neomys anomalus* Cabrera, 1907, and *Neomys fodiens* (Pennant, 1771). Two of the 25 loci studied were monomorphic for the same allele in all species, nine loci were monomorphic for the same allele in both *Sorex* species and an alternate allele was fixed in the two species of *Neomys*. Only one locus (*Pgm-3*) was diagnostic with a different allele in all species. The mean heterozygosity values in *S. araneus* and *N. fodiens* (0.069 and 0.061) were significantly higher than those in *S. minutus* and *N. anomalus* (0.021 and 0.029). The Nei's genetic distances were small between the two *Neomys* species (0.123) and between the two *Sorex* species (0.330). The highest Nei's distance was found between *S. minutus* and *N. anomalus* (2.487).

Introduction

The common shrew *Sorex araneus* Linnaeus, 1758, the pygmy shrew *Sorex minutus* Linnaeus, 1766, the Mediterranean water shrew *Neomys anomalus* Cabrera, 1907, and the European water shrew *Neomys fodiens* (Pennant, 1771) occur sympatrically in the Białowieża Primeval Forest, eastern Poland. They are competitive species and exhibit clear differences in social behaviour, feeding habits, habitat selection, and space utilization (Churchfield, 1990; Krushinska and Rychlik, 1993; Krushinska *et al.*, 1994; Rychlik, 1995).

To date, few papers describing allozyme variation in shrews have been published. Results of most studies have been reviewed by Heikkilä (1989). Among European species, *S. araneus* is the best studied electrophoretically (e.g. Frykman *et al.*, 1983; Frykman and Bengtsson, 1984; Searle, 1985; Hausser *et al.*, 1991; Ratkiewicz *et al.*, 1994; Wójcik and Wójcik, 1994). Electrophoretic comparisons between different species of shrews have been conducted also (Gębczyński and Jacek, 1980; Catzeflis *et al.*, 1982; Catzeflis, 1984a, b; Frykman and Simonsen, 1984; George, 1986, 1988).

Allozyme variation between the four species of shrews from Białowieża have been previously studied by Gębczyński and Jacek (1980). Their results differ (at some loci) from those obtained in other studies (Catzeflis *et al.*, 1982; Catzeflis, 1984a, b; Frykman and Simonsen, 1984; Wójcik and Wójcik, 1994). Thus, the specific aims of the present paper were: (i) to obtain new information on the allozyme variation in these sympatric species of shrews, and (ii) to assess whether

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there are any substantial genetic differences between the Białowieża population and other European populations of shrews.

Material and Methods

A total of 199 shrews from Białowieża Primeval Forest (52°42'N, 23°52'E), eastern Poland, were analyzed electrophoretically: *S. araneus* (117), *S. minutus* (41), *N. anomalus* (18), and *N. fodiens* (23). The following enzymes were screened: aconitase (ACO, E.C. 4.2.1.3), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), adenylate kinase (AK, E.C. 2.7.4.3), esterase (ES, E.C. 3.1.1.1), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), glucose-phosphate isomerase (GPI, E.C. 5.3.1.9), glutamate-oxaloacetate transaminase (GOT, E.C. 2.6.1.1), α-glycerophosphate dehydrogenase (GPD, E.C. 1.1.1.8), hexokinase (HK, E.C. 2.7.1.1), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), lactate dehydrogenase (LDH, E.C. 1.1.1.27), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40), mannose phosphate isomerase (MPI, E.C. 5.3.1.8), nucleoside phosphorylase (NP, E.C. 2.4.2.1), phosphoglucomutase (PGM, E.C. 2.7.5.1), phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44), sorbitol dehydrogenase (SDH, E.C. 1.1.1.14), xanthine dehydrogenase (XDH, E.C. 1.2.3.2).

Kidney tissue was used for electrophoretic analysis. The samples were run on 94 × 76 mm Titan III cellulose acetate plates (Helena Laboratories Co., Beaumont, Texas, U.S.A.) at 200 V and room temperature. For more details of this method see Searle (1985), Richardson *et al.* (1986), Hebert and Beaton (1989), and Wynne *et al.* (1992).

Genotype and allele frequencies were calculated directly from banding patterns present on the plates. Presumptive alleles were designated by locus symbols with lower case letters of the alphabet. The nomenclature of alleles for *S. araneus* in the present study was made compatible with those used by Searle (1983, 1985, 1986) and Wójcik and Wójcik (1994). The *Ldh-1* locus is equivalent to *Ldh-A* in Harris and Hopkinson (1976).

The mean heterozygosity values (*H*) were calculated from the genotype frequencies according to direct-count method and the method given by Nei (1978). The genetic distance coefficients between pairs of the species were calculated according to the method of Nei (1978). These calculations were performed using the BIOSYS-1 computer program (Release 1.7) of Swofford and Selander (1989). The Fitch–Margoliash method (Fitch and Margoliash, 1967) was used to construct an unrooted tree from Nei's genetic distances by the FITCH program in the software package PHYLIP (Felsenstein, 1994).

Results

Only two loci *Got-2* and *Gpd-1* were monomorphic and fixed for one allele in all four species (Table 1). Nine loci *Ak-2, Got-1, Gpi, G-6-pd, Ldh-1, Mdh-1, Np, Sdh,* and *Xdh* were monomorphic with allele *a* in *Sorex* and allele *b* in *Neomys*. Only the *Pgm-3* locus was fully diagnostic such that the most common allele was different in all species examined. The following loci were found to be the most variable: *Adh, Es-1, Idh-2, Mpi,* and *Pgm-3* in *S. araneus*; *Adh* in *S. minutus*; *Es-1* and *Me* in *N. anomalus*; and *Es-1, Me, Mpi,* and *Pgm-1* in *N. fodiens* (Table 1).

The mean heterozygosity values and percentage of polymorphic loci in *S. araneus* and *N. fodiens* were significantly higher than those in *S. minutus* and *N. anomalus* (Table 2).

The genetic distances of Nei (1978) between the species (Table 3) ranged from 0.123 (between *N. anomalus* and *N. fodiens*) to 2.487 (between *S. minutus* and *N. anomalus*). The two species of *Sorex* are well separated from both *N. anomalus* and *N. fodiens* in the unrooted tree derived by the Fitch–Margoliash method (Fig. 1).

Discussion

All animals examined in this investigation came from the same populations studied by Gębczyński and Jacek (1980) and Gębczyńsk (1985). We found clear differences between our results and those of Gębczyński and Jacek (1980). They reported a high degree of polymorphism at the *Ldh-1* and *Mdh-1* in *Sorex* and *Neomys*. We found no polymorphism at these two loci. Gębczyński and Jacek (1980) observed the same common allele at *Ldh-2* and *Me* in *Sorex* and *Neomys*. We found that the most frequent allele at *Ldh-2* and *Me* was different in *Sorex* and *Neomys*. Similar differences were observed between our data and those of Gębczyńsk (1985) concerning allozyme variation in *S. minutus*. These

TABLE 1. LOCI AND ALLELES DETECTED IN THE FOUR SHREW SPECIES. WHERE MORE THAN ONE ALLELE HAS BEEN FOUND AT THE LOCUS WITHIN A SPECIES, ALLELIC FREQUENCIES ARE GIVEN

Locus	*Sorex araneus* $n=117$*	*Sorex minutus* $n=41$	*Neomys anomalus* $n=18$	*Neomys fodiens* $n=23$
Aco-1	*a*	*a* 0.96 *b* 0.02 *c* 0.01	*d*	*d*
Adh	*a* 0.71 *b* 0.24 *c* 0.04	*b* 0.10 *d* 0.90	*e*	*e*
Ak-1	*a* 0.99 *b* 0.01	*c* 0.96 *d* 0.04	*e*	*e*
Ak-2	*a*	*a*	*b*	*b*
Es-1	*a* 0.76 *b* 0.21 *d* 0.02 *e* 0.01	*b* 0.96 *d* 0.04	*f* 0.83 *g* 0.17	*f* 0.83 *g* 0.17
Got-1	*a*	*a*	*b*	*b*
Got-2	*a*	*a*	*a*	*a*
Gpd-1	*a*	*a* 0.99 *b* 0.01	*a*	*a*
Gpd-2	*a*	*a*	*b*	*a*
Gpi	*a*	*a*	*b*	*b*
G-6-pd	*a*	*a*	*b*	*b*
Hk	*a*	*b*	*a*	*a*
Idh-1	*a*	*a* 0.99 *b* 0.01	*c*	*c* 0.91 *d* 0.09
Idh-2	*a* 0.87 *b* 0.13	*a* 0.98 *c* 0.02	*b*	*b*
Ldh-1	*a*	*a*	*b*	*b*
Ldh-2	*a* 0.99 *c* 0.01	*a* 0.98 *b* 0.01 *c* 0.01	*b* 0.97 *c* 0.03	*b*
Mdh-1	*a*	*a*	*b*	*b*
Me	*a*	*a*	*b* 0.56 *c* 0.44	*b* 0.52 *c* 0.46 *d* 0.02
Mpi	*a* 0.76 *b* 0.18 *c* 0.05 *d* 0.01	*a* 0.01 *g* 0.99	*a*	*h* 0.76 *i* 0.24
Np	*a*	*a*	*b*	*b*

TABLE 1—*CONTINUED*

Locus	*Sorex araneus* $n=117$*	*Sorex minutus* $n=41$	*Neomys anomalus* $n=18$	*Neomys fodiens* $n=23$
Pgd	*a* 0.92 *b* 0.07 *d* 0.01	*e* 0.98 *f* 0.02	*g* 0.94 *h* 0.06	*e* 0.04 *g* 0.96
Pgm-1	*a*	*a* 0.01 *c* 0.99	*d*	*c* 0.02 *d* 0.85 *e* 0.09 *f* 0.04
Pgm-3	*a* 0.73 *b* 0.22 *c* 0.01 *d* 0.04	*f* 0.99 *g* 0.01	*h*	*a* 0.09 *c* 0.91
Sdh	*a*	*a*	*b*	*b*
Xdh	*a*	*a*	*b*	*b*

*$n=116$ for the *Es* and *Idh* loci; $n=25$ for the *G-6-pd* locus.

differences may reflect difficulties in reading zymograms, or different electrophoretic conditions.

Our results are in general agreement with those found by most others studying allozyme variation in shrews. The mean heterozygosities for *S. araneus* and *N. fodiens* are relatively higher than those obtained in other investigations (Fig. 2). However, they are similar to those in two *S. araneus* samples from Hungary and one *N. fodiens* sample from Italy (Catzeflis, 1984a, b). The mean heterozygosity we found in *S. minutus* is lower than the values calculated by others, but is similar to that from Finland (Catzeflis, 1984b). The heterozygosity value we obtained for *N.*

TABLE 2. GENETIC VARIABILITY AT 25 LOCI IN THE FOUR SHREW SPECIES (STANDARD ERRORS IN PARENTHESES)

Species	Sample size *n*	Percentage of polymorphic loci**	Mean heterozygosity	
			Direct-count	H-W expected***
Sorex araneus	117*	24.0	0.069 (0.026)	0.082 (0.031)
Sorex minutus	41	4.0	0.021 (0.005)	0.026 (0.008)
Neomys anomalus	18	12.0	0.029 (0.016)	0.037 (0.022)
Neomys fodiens	23	24.0	0.061 (0.024)	0.074 (0.028)

*$n=116$ for the *Es* and *Idh* loci; $n=25$ for the *G-6-pd* locus.

**A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

***Biased estimate (see Nei, 1978).

TABLE 3. MATRIX OF GENETIC DISTANCE COEFFICIENTS FOR FOUR SHREW SPECIES (NEI, 1978, UNBIASED ESTIMATE). THE ESTIMATES ARE BASED ON DATA FROM 25 ENZYME LOCI

Species		1	2	3	4
1	*Sorex araneus*	—			
2	*Sorex minutus*	0.330	—		
3	*Neomys anomalus*	1.798	2.487	—	
4	*Neomys fodiens*	1.702	2.047	0.123	—

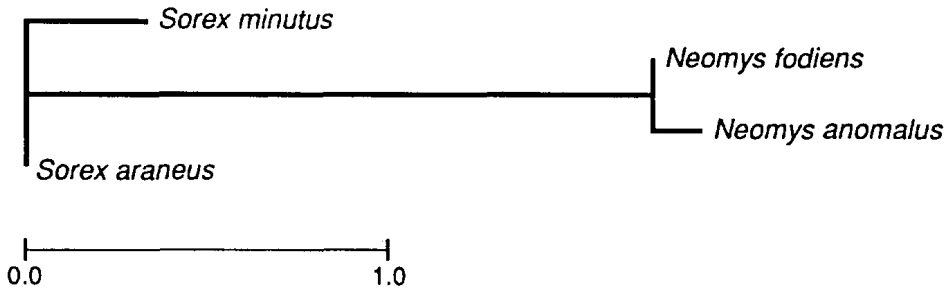

FIG. 1. AN UNROOTED TREE COMPUTED FROM THE NEI'S GENETIC DISTANCES BY THE FITCH–MARGOLIASH METHOD.

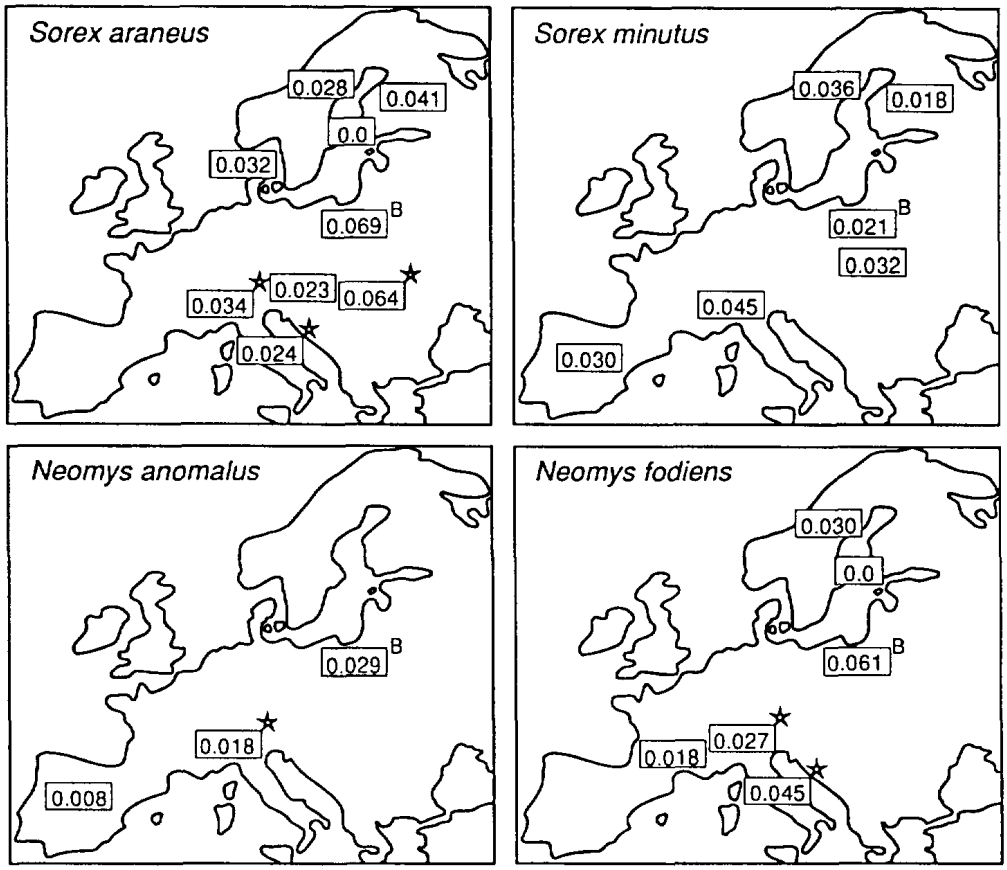

FIG. 2. MEAN HETEROZYGOSITIES IN DIFFERENT POPULATIONS OF *SOREX ARANEUS, SOREX MINUTUS, NEOMYS ANOMALUS,* AND *NEOMYS FODIENS.* ASTERISKS INDICATE AVERAGE VALUES OF DIFFERENT SAMPLES FROM HUNGARY, ITALY AND SWITZERLAND. Data come from Catzeflis (1984b), Frykman and Simonsen (1984), and this study (B).

anomalus is higher than those from Switzerland and Spain found by Catzeflis (1984a, b; see Fig. 2).

It is generally agreed that habitat generalists possess more genetic variation (expressed as mean heterozygosity) than habitat specialists (Nevo, 1978). Our results do not contradict this statement. *S. araneus* is a habitat generalist in the Bial owieża Primaval Forest (Aulak, 1970). It has higher heterozygosity than both *S. minutus* and *N. anomalus* which are habitat specialists. *N. fodiens* also seems to be a habitat specialist, although it occurs in more habitat types than *N. anomalus* (Aulak, 1970). Therefore, the relatively high heterozygosity of *N. fodiens* cannot be easily explained by the above hypothesis.

Mainland populations are known to have higher heterozygosities than island populations of the same species (Berry, 1986). We suspect that this is also true in shrews. As shown on Fig. 2, higher heterozygosities have been found in mainland populations of *S. araneus* and *N. fodiens* from Sweden, Finland and Poland than in populations from the Aland Islands in Finland (Catzeflis, 1984a, b; Frykman and Simonsen, 1984; Heikkilä, 1989; this study).

There is geographical variation in the frequency of the most common allele at some loci. The most common allele at the *Mpi* locus in *S. araneus* is different in northern Sweden than in southern Sweden, Britain and Poland (Frykman *et al.*, 1983; Searle, 1985; Wójcik and Wójcik, 1994). No variation has been observed at the *Adh* locus in *S. araneus* in northern and central Sweden, little variation in southern Sweden, and clear polymorphism in Poland and France (Frykman *et al.*, 1983; Hausser *et al.*, 1991; Ratkiewicz *et al.*, 1994; this study). The *Me* locus in *N. fodiens* is monomorphic in Sweden and Finland, but clearly polymorphic in France, Italy, Switzerland and Poland (Catzeflis, 1984b; Frykman and Simonsen, 1984; George, 1986; this study).

Relatively high genetic differentiation has been observed in *S. araneus* (Catzeflis, 1984b; Searle, 1985; Hausser *et al.*, 1991; Wójcik and Wójcik, 1994; this study). This species is one of the most karyotypically variable mammals known. There are numerous chromosome races over the wide Palearctic range of *S. araneus* (e.g. Searle, 1988; Wójcik, 1993). It has been suggested that the striking differences in karyotype between chromosome races of the species are not associated with any substantial differentiation at electrophoretic loci (Frykman *et al.*, 1983; Searle, 1985; Wójcik and Wójcik, 1994). Only one chromosome race of *S. araneus* from southern Alps and Italy is clearly differentiated genically and morphologically. It is believed that this race evolved during a long isolation in Italy (Hausser *et al.*, 1991).

The genetic distances (Nei, 1978) we estimated between the two *Sorex* species and between the two *Neomys* species are small. The distance between *S. araneus* and *S. minutus* (0.33) is almost the same as the distance (0.32) calculated by Frykman and Simonsen (1984) and only slightly higher than those (0.28, 0.23) estimated by Catzeflis *et al.* (1982) and Catzeflis (1984b). The distance we measured between *N. anomalus* and *N. fodiens* (0.12) is lower than those estimated between the three *N. anomalus* populations from Switzerland and Spain and the six *N. fodiens* populations from Switzerland, Italy, France and Finland (0.15–0.26) reported by Catzeflis (1984a, b).

S. araneus and *S. minutus* clearly have different karyotypes (e.g. Dannelid, 1994; Zima *et al.*, 1996) but display small allozyme differences. *N. anomalus* and *N. fodiens* have very similar karyotypes (e.g. Dannelid, 1994; Zima *et al.*, 1996) and are very close electrophoretically. These results fit a common pattern showing that evolutionary processes run relatively independently at the karyotypic and genotypic levels (e.g. Wilson *et al.*, 1977; Qumsiyeh and Chesser, 1988; Bogdanowicz and Owen, 1992).

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