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Biochemical Variation in Four Species of Insectivora

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Biochemical variation was examined at 11 loci in four species of shrews inhabiting the Białowieża Primeval Forest. *Sorex araneus* and *Sorex minutus* are genetically distinct from sympatric *Neomys fodiens* and *Neomys anomalus*. Genetic similarity is closest between the two *Sorex* species, whereas genetic distance appears to be greater between *N. fodiens* and *N. anomalus*. There is considerable variation in the level of genic heterozygosity among shrew species, although the mean value of five percent is consistent with that estimated for other mammal species.

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1. INTRODUCTION

Knowledge of the genetic differentiation of species and genetic variation in their populations is rapidly increasing, owing, *inter alia*, to the use of protein electrophoresis. The largest number of studies among mammals have been carried out on rodents, which is understandable both on account of the numbers of species, their wide-spread occurrence and their occupation of extremely differing habitats. Species of the order *Insectivora* are less numerous, although also wide-spread. This is a group of mammals phylogenetically older than *Rodentia* and although inhabiting similar habitats to those occupied by rodents, differ basically in their ecology and physiology. Insectivorous mammals have not been studied at all from the aspect of biochemical variation. There are only fragmentary data on hemoglobin and serum proteins in the mole (Dąbrowski & Skoczeń, 1962) and hemoglobin in two American species of shrews (Johnson & Wicks, 1959).

Our aim was to make a preliminary evaluation of biochemical variation in four sympatric species of *Insectivora*, thus obtaining an estimate of genic heterozygosity in populations and also genetic similarity between different species.

2. MATERIAL AND METHODS

Six different proteins were examined in four species of shrews from the populations in the Białowieża National Park. The animals were caught in conelike

pitfalls in the autumn (Table 1). Blood samples and homogenates of kidneys, liver brown adipose tissue and muscles were prepared according to the method described for rodents by Selander *et al.* (1971). Horizontal starch gel electrophoresis (Smithies, 1959; Selander *et al.*, 1971) was used to fraction all samples, employing a 13% concentration of hydrolysed starch.

Buffer systems and staining methods were slightly modified from those described by Selander *et al.* (1971).

Table 1

Material examined.

The animals were caught in a floodplain forest (*Circaeo-Alnetum*). All shrews were young adults, except one *N. anomalus* caught in November which was an overwintered specimen.

Species	Sept.	Oct.	Nov.	Dec.	Total
Sorex araneus Linnaeus, 1758		3	12	8	23
Sorex minutus Linnaeus, 1766	7	4	15	10	36
Neomys fodiens (Pennant, 1771)	2	7	—	1	10
Neomys anomalus Cabrera, 1907	2	3	2	1	8

3. RESULTS

3.1. Pattern of Variation

The following loci were recognized: LDH, LAP, MDH (NAD-dependent), ME (=MDH NADP-dependent), each representing a double locus, while ES were scored as three loci. We thus have a total of 20 alleles at 11 loci in *Sorex araneus* and *S. minutus* and 26 alleles at 11 loci in *Neomys fodiens* and *N. anomalus*. Of the 11 loci analysed, three are monomorphic and do not vary between species. These include *Mdh-2* locus of malate dehydrogenase (NAD-dependent), and both *Me* loci of malic enzyme.

The allelic frequencies at 8 polymorphic loci of different species of shrews are presented in Table 2. No obvious deviation from Hardy-Weinberg equilibrium was detected.

Lactate dehydrogenase (muscles)

The electrophoretic patterns of LDH showed that they are controlled by two loci, *Ldh-1* and *Ldh-2*. It was found that only *Ldh-1* is polymorphic and four alleles have been detected in the locus among the specimes examined. Identical alleles, although different from alleles characteristic of *Neomys fodiens* and *N. anomalus*, have been found in *Sorex araneus* and *S. minutus* (Table 2).

Ldh-2 appears to be monomorphic and the only evidence of variation was found in one individual of *N. fodiens* in which a homozygote occurred for an allele producing a fast-migrating band.

Table 2
Allelic frequencies at 8 polymorphic loci for four species of shrews.

Locus	Allele	*S. araneus*	*S. minutus*	*N. fodiens*	*N. anomalus*
Ldh-1	a	—	—	0.25	0.50
	b	0.73	0.75	—	—
	c	0.27	0.25	—	—
	d	—	—	0.75	0.50
Ldh-2	a	—	—	0.11	—
	b	1.00	1.00	0.89	1.00
Lap-1	a	—	—	—	0.08
	b	0.63	0.73	0.79	0.59
	c	—		—	0.08
	d	0.37	0.27	0.21	0.25
Lap-2	a	0.53	0.71	0.44	0.40
	b	—	—	0.12	—
	c	0.47	0.29	0.44	0.60
Mdh-1	a	0.05	0.02	0.25	0.14
	b	0.24	0.31	—	—
	c	—	—	0.50	0.72
	d	0.71	0.67	—	—
	e	—	—	0.25	0.14
Es-1	a	0.50	0.43	0.50	0.25
	b	0.50	0.57	0.36	0.62
	c	—	—	0.14	0.13
Es-2	a	0.39	0.28	0.61	0.38
	b	0.57	0.70	0.39	0.62
	c	0.04	0.02	—	—
Es-3	a	0.50	0.45	0.61	0.43
	b	0.50	0.55	0.17	0.57
	c	—	—	0.11	—
	d	—	—	0.11	—

Leucine amidopeptidase (liver)

Two zones of activity, *Lap-1* and *Lap-2*, coded by two loci, were found to be present in shrews. Both are polymorphic and their phenotypes were recognized as single- (homozygous) or double-banded (heterozygous). In *Lap-1* four alleles were found to be present and in *Lap-2* three alleles in all the four species of shrew examined. *N. anomalus* had a heterozygote in *Lap-1* with two faster-migrating bands than those

in the other species. A homozygote appeared, however, in *Lap-2* in *N. fodiens*, with intermediate migration rate of the isozyme. No differences were found in respect of *Lap-1* and *Lap-2* migration between *S. araneus* and *S. minutus* (Table 2).

Malate dehydrogenase (NAD-dependent) (kidneys)

Two forms of this enzyme are demonstrable in *Sorex* and *Neomys*, only one being polymorphic, with five alleles (Table 2). Three alleles were found to be present in each of the species examined, being common to the species of the given genus. Only one *Mdh-1* band migrates towards the anode at the same rate in shrews of the two genera.

Mdh-2 was monomorphic in all the individuals examined.

Malic enzyme (liver)

Two forms of this enzyme (=malate dehydrogenase NADP-dependent) were found to occur, both of which stained only faintly. Both would, however, appear to be monomorphic in the four species of shrews examined.

Esterases (liver, brown adipose tissue)

Esterases were the most variable systems, although they would appear to be less complex in shrews than in rodents. A description is given of three most rapidly-migrating esterases. *Es-1*, 3 alleles: only allele *Es-1*^{c} occurred in both *N. fodiens*, and *S. araneus* and *S. minutus*. Allele *Es-1*^{a} occurs only in the two latter species, and is absent in the two species of the genus *Neomys*.

Es-2, 3 alleles. The slowest-migrating allele (*Es-2*^{c}) appeared only in the two *Sorex* species.

Es-3, 4 alleles. Allele *Es-3*^{c} and *Es-3*^{d} were found only in one individual of *N. fodiens*.

3.2. Genetic Relationships and Variability

The coefficient of genetic similarity (*S*) was calculated for all paired combinations of species, using measurements of genetic distance (*D*) introduced by Rogers (1972), where $S=1-D$. The normalized identity of genes (*I*; Nei, 1972) was also calculated. By these means it was possible to establish that *S* and *I* differ for the different pairs of shrew species (Table 3). The greatest similarity is found between *Sorex araneus* and *S. minutus*, whereas the indexes *S* and *I* for the pair *N. fodiens : N. anomalus* is much smaller. It was not until further

comparison had been made in turn with the two species of the genus *Neomys* that considerable genetic distance was revealed.

Average genic heterozygosity ranges from 4.4% to 5.7% in the four species of shrew examined (Table 4). Most of the heterozygosity in

Table 3

Coefficients of genetic similarity (*S* and *I*), for four species of shrews. Upper figure, *S* (Rogers' genic similarity); lower figure, *I* (Nei's genic similarity).

	S. araneus	*S. minutus*	*N. fodiens*	*N. anomalus*
S. araneus	1.000	0.914 0.986	0.667 0.719	0.690 0.740
S. minutus		1.000	0.578 0.700	0.584 0.734
N. fodiens			1.000	0.780 0.912
N. anomalus				1.000

Table 4

Mean heterozygosity per locus per species in four species of shrews.

Locus	*S. araneus*	*S. minutus*	*N. fodiens*	*N. anomalus*	Avg.
Ldh-1	0.044	0.020	0.000	0.000	0.016
Ldh-2	0.000	0.000	0.000	0.000	0.000
Lap-1	0.089	0.045	0.020	0.036	0.047
Lap-2	0.075	0.050	0.061	0.106	0.073
Mdh-1	0.055	0.088	0.071	0.041	0.064
Es-1	0.086	0.062	0.061	0.053	0.065
Es-2	0.050	0.038	0.079	0.035	0.050
Es-3	0.057	0.076	0.076	0.082	0.073
Avg.	0.057	0.047	0.046	0.044	

shrews depends on loci of *Lap-2*, *Es-1* and *Es-3*, values for which are higher than the mean. The least heterozygous are the two *Ldh-1* and *Ldh-2* loci.

4. DISCUSSION

Preliminary data on protein variability in insectivorous mammals show that it is as great as in rodents. There, are however, also differences between representatives of these orders. For instance, leucine aminopeptidese in rodents (Gaines & Krebs, 1971; Anderson *et al.*, 1976; Nadler *et al.*, 1978) has only one polymorphic form, whereas in shrews both forms are polymorphic. Esterases, however, appear to be less complex in the shrews than in rodent species. It was observed that hemoglobin migrates, although only slightly, towards the cathode with

pH=8.5. This shows that its isoelectric point is different from that in rodents, in which Hb at the same pH always migrates to the anode. Similar characteristics of Hb in *Sorex obscurus* and *S. vagrans* have been given by Johnson & Wicks (1959). In the mole Hb moved as a single band (Dąbrowski & Skoczeń, 1962).

Rogers' coefficient of genic similarity in rodents, *e.g.* in the cotton rats *Sigmodon hispidus* and *S. arizonae*, is on an average 0.7634 (Johnson *et al.*, 1972), for 11 species of *Dipodomys* its range is from 0.31 to 0.89, with a mean of 0.61 (Johnson & Selander, 1971). In four species of mice of the *Peromyscus boylii* species group the coefficient comes within limits of 0.501 to 0.945 (Kilpatrick & Zimmerman, 1975) and in 5 species of the genus *Bursarius S* is 0.603, with a range of 0.514 to 0.735 (Penney & Zimmerman, 1976). *I* in rodents is usually slightly larger than *S* (Nevo *et al.*, 1974), and this same is observed in shrews.

When *S. araneus* and *S. minutus* are compared, the degree of *S* and *I* indexes in shrews are shown to be strikingly high in comparison with rodents. They exceeded all indexes of this kind, such as have been calculated for two different species. The differences between *N. fodiens* and *N. anomalus*, are, however, similar to those already described for a large number of different rodent species. It must, however, be emphasised that morphological differences between *Sorex* species are distinct, *e.g.* the body weight of *S. araneus* is more than twice greater than that of *S. minutus*, and the ranges of extreme dimensions do not overlap. In the case of *N. fodiens* and *N. anomalus* differences are smaller, resulting, in the possibility of mistaken identification of these two species by taxonomists. The characteristic given in the key for Differentiating *N. anomalus* from *N. fodiens* is »a more distinct row of hairs on the tail« and »slightly smaller body measurements«. Other morphological features, *e.g.*, skull measurements, are similar. Furthermore, our knowledge of the ecology of these four species is fairly limited, but it is known that they occur sympatrically in many regions. Their food niches appear to be clearly separated, particularly those of the *Sorex* species. The relatively considerable genetic distance between *Sorex* and *Neomys* finds confirmation in their morphological differences.

Genic heterozygosity in shrews ranges around five percent. In rodents, in the same way as in a wide variety of vertebrates, mean heterozygoisty is from 4.0 to 6.5% (Selander & Johnson, 1973; Avise *et al.*, 1974). Contributions of individual loci to overall heterozygosity in shrews are different, as it is in many rodent species (Johnson *et al.*, 1972).

It is to be expected that further studies of biochemical variation in

different populations of shrews will extend the number of loci examined and improve our knowledge about their genic relationships (Sarich, 1977). It also will allow to reveal both geographical and biotopical trends in variation. This assumption may be made not only on the strength of the information so far obtained in relation to other mammals, but also of data on chromosome variation in the common shrew (Fedyk, 1980). The enormous seasonal differences in numbers lead to the assumption that frequency of genes is also subject to cyclic fluctuations in shrews, as has been shown for rodents (Gaines & Krebs, 1971). It is to be expected that in this way it will prove possible to throw light on the case of many species of *Insectivora*, some of which are very similar to each other. Intraspecies systematics, *e.g.*, of chromosome races in *S. araneus* (Fedyk, 1980) will also acquire additional data on protein variation.

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ZRÓŻNICOWANIE GENETYCZNE U CZTERECH GATUNKÓW *INSECTIVORA*

Streszczenie

Zbadano zmienność 6 białek kodowanych przez 11 loci, z których 8 było polimorficznych (Tabela 2). Obiektem badań były cztery gatunki owadożernych (Tabela 1) łowione w Puszczy Białowieskiej. Zebrane wstępne dane o genetycznym zróżnicowaniu ryjówek i rzęsorków pozwalają stwierdzić, że różnice te są zdumiewająco małe pomiędzy *Sorex araneus* i *S. minutus* (Tabela 2), co potwierdzają współczynniki podobieństwa gentycznego (Tabela 3). Natomiast różnice pomiędzy oboma gatunkami z rodzaju *Neomys* są większe (Tabela 3). Stwierdzono także, iż stopień heterozygotyczności w badanych populacjach ryjówek nie przekracza 5% (Tabela 4) i jest podobny jak u wielu innych gatunków ssaków.

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