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Genetic and morphological variation among populations of the bank vole *Clethrionomys glareolus* from north-eastern Poland: the seasonal aspect

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Abstract

The relationship between genetic and morphological variation was studied in five populations of the bank vole (*Clethrionomys glareolus*) from NE Poland. For the assessment of genetic polymorphism 37 enzyme loci were investigated. The proportion of polymorphic loci (P) ranged from 0.162 to 0.270 and the average observed heterozygosity (H_0) from 0.074 to 0.095 in populations studied. F-statistics indicated the existence of seasonal variation in allele frequency in four populations ($F_{ST} = 0.011–0.018$, $p < 0.05$). The differentiation among vole populations was greater in autumn ($F_{ST} = 0.032$, $p < 0.0001$) than in spring ($F_{ST} = 0.023$, $p < 0.0001$). Morphometric variation in 3 groups of parameters (I – body size, II – size of internal organs, III – cranial and mandibular traits) was subjected to principal component analysis (PCA). There was a very clear separation of season in all three groups of parameters in each population. PCA analysis revealed differences among vole populations in body size, organ size and cranium size and shape in spring and only in body size and cranium shape in autumn. No correlation was found between Rogers' genetic distances and Mahalanobis distances, as calculated from 3 groups of morphological characters in both seasons ($r = |0.01–0.43|$, NS; Mantel's test). The data suggest that there is no equivalent degree of divergence on these two levels of integration in *C. glareolus*.

Key words: *Clethrionomys glareolus*, allozymes, morphometry, differentiation

Introduction

There is abundant geographic variation in both morphology and gene frequency in most species. The extent of geographic variation results from balance of forces tending to produce local genetic differentiation and forces tending to produce genetic homogeneity (Slatkin 1987). Protein electrophoresis has been widely used to describe genetic differences among populations of rodent species (Leitner and Hartl 1988; Gallardo et al. 1992; Gębczyński et al. 1993; Fedorov et al. 1995). The degree of genetic differentiation among populations over wide geographical distances is higher than within a narrow geographic area (Gębczyński et al. 1993; Fedorov et al. 1995). It has also become obvious that there was considerable morphological variation among local populations of the species. Morphological differences between animals from various regions have hitherto mainly been related to the environmental conditions or, as in genetic instances, to the geographic distance and isolation (Haitlinger 1965, 1970; Hansson 1985; Baker 1992; Sara and Casamento 1995).

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Genetic and morphological observations are very rarely carried out on the same material. Thus, there were only a few estimations of the relationships between genetic and morphometric divergence among populations in mammals (HARTL et al. 1993; KITCHENER et al. 1994). On the other hand, there are numerous studies dealing with this problem in other groups of animals (LAZARIDOU-DIMITRIADOU et al. 1994; KYRIAKOPOULOU-SKLAVONOU et al. 1991; BAKER 1992; LOBO 1995).

The intrapopulation variability may have an effect on differences among populations. There is considerable evidence that body mass, organ weights, and gut morphology of rodents can change seasonally in response to changes in feeding habits, reproductive state, ambient temperature or photoperiod (HAMMOND 1993; NAGY and NEGUS 1993; HAMMOND and DIAMOND 1994; BORKOWSKA 1995; CAMPBELL and MACARTHUR 1996). Likewise, a spring generation of rodents differs in allele frequency from an autumn generation (FEDYK and GĘBCZYŃSKI 1980). Thus, seasonal variation may also be reflected in morphological and genetic differentiation among bank vole populations.

The bank vole, *Clethrionomys glareolus* (Schreber, 1780) is one of the most common Palearctic rodent species. It has a wide geographical distribution from the British Isles to Lake Baikal, and from Kola Peninsula to Asia Minor (RACZYŃSKI 1983). The genetic differences among bank vole populations have been determined in Austria (LEITNER and HARTL 1988) and Poland (GĘBCZYŃSKI et al. 1993). Morphological differences were found between eastern and western (HAITLINGER 1965) as well as between mountain and lowland vole populations in Poland (HAITLINGER 1970).

The purpose of this study was to determine variability in allozymes and morphology (body size, size of internal organs, and skull dimension) among the bank vole populations over short geographic distances. Next, the seasonal aspects of the interpopulation divergence will be taken into account.

Material and methods

A total of 391 individuals of *C. glareolus* was collected from 5 populations in the vicinity of Bialystok (NE Poland 23°07′E, 53°18′N, Tab. 1). The minimum distance between two sites was 10 km, maximum 50 km. Animals were caught in live-traps during two seasons: spring (May–June) and autumn (October–November) in 1995–97. The voles were brought into the laboratory and dissected. A spring generation of the bank vole consisted of over-wintered individuals, while in autumn the populations were only made up of current-year animals.

Samples of blood plasma, kidney, liver, and salivary gland were taken from each vole and stored at –85 °C until used for electrophoresis. Tissues were homogenized in phosphate buffer (0.01 M, pH 7.5) and then centrifuged at 12 000 rpm for 15 min at 4 °C. Protein electrophoresis was performed on (1) starch gel following the running and staining conditions given by SELANDER et al. (1971), HARRIS and HOPKINSON (1976), QUAVI and KIT (1980), and (2) cellulose acetate plates (SEARLE 1985), and (3) agar gel (NIELSEN 1977). Gene products for the following 37 presumptive enzyme loci were analysed. Locus were the following (E. C. number are given in parentheses): αGpd-1, αGpd-2, βGpd-1, and βGpd-2 (1.1.1.8), Sdh (1.1.1.14), Ldh-1 and Ldh-2 (1.1.1.23), Mdh-1 and Mdh-2 (1.1.1.37), Me-1 and Me-2 (1.1.1.40), Idh-1 and Idh-2 (1.1.1.42), Pgd (1.1.1.44), Dia (1.6.2.2), Cat (1.11.1.6), Sod-1 and Sod-2 (1.15.1.1), Aat-1 and Aat-2 (2.6.1.1), Pgm-1, Pgm-2, and Pgm-3 (2.7.5.1), EstB3 and EstD (3.1.1.1), Amy1-2 (3.2.1.1), Pep-2 and Pep-3 (3.4.11), Acy (3.5.1.14), Ald-1 and Ald-2 (4.1.2.13), Acon-1 and Acon-2 (4.2.1.3), Mpi (5.3.1.8), Pgi (5.3.1.9), Alb, and Prot. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Alleles at polymorphic loci were designated alphabetically with increasing anodal migration of the corresponding allozymes.

BIOSYS-1 (SWOFFORD and SELANDER 1989) was used to calculate observed average heterozygosity (H_0) and χ^2 contingency test for homogenity. If significant deviation from Hardy-Weinberg equilibrium was found the fixation index F_{IS} was calculated. The deviation of F_{IS} from zero was tested by $\chi^2 = NF_{IS}^2$, where N is the total sample size (NEI 1977). Wright's F_{ST} was calculated to quantify the

amount of genetic differentiation between seasons in each population and among populations in spring and autumn, separately. An F-statistic value was considered to be significantly different from zero if statistically significant heterogeneity among samples at the same hierarchical level was found (χ^2 test). Pairwise Rogers' genetic distances (D_R) were estimated from allozyme data and clustered using the UPGMA method (Swofford and Selander 1989).

Morphometric variation in the 5 populations of the bank vole was assessed by taking 39 measurements on 349 individuals older than 3 months (age was determined according to Pucek and Zejda 1968). Morphological characters were divided into three groups:

I – body size (5 external measurements: total body mass TBM to the nearest 0.1 g, and head and body length HBL, tail length TL, hind foot length HFL, ear height EH to the nearest 0.01 mm; Pucek 1981);

II – organ size (13 internal measurements: dry mass of stomach STM, small intestine SIM, large intestine LIM, caecum CM, liver LM, kidneys KM, spleen SM, heart HM, lungs LUM, scraped mucosa SMM to the nearest 0.001 g, and length of small intestine SIL, large intestine LIL, and caecum CL to the nearest 0.01 cm; Myrcha 1964; Diamond and Karasov 1984; Hammond and Diamond 1994);

III – skull dimension (21 cranial measurements: condylobasal length CBL, total cranium length TCL, basal length BL, rostral width RW, interorbital width IW, zygomatic width ZW, mastoid width MW, palatal height PH, skull height per auditory bullae SPB, skull height between auditory bullae SBB, upper diastema length UDL, mandibule length ML, mandibular ramus height MH, recorded by dial caliper to the nearest 0.01 mm, and length of nasal bones NBL, length of frontal bones FBL, length of sagital crest SCL, length of interparietal bone IBL, incisive foramen length IFL, palatal length PL, upper molar series length from the alveoles UMSL, lower molar series length from the alveoles LMSL, recorded by binocular microscope with micrometer ocular to the nearest 0.01 mm; Haitlinger 1965; Viro and Niethammer 1982).

Morphological data were quantitatively compared in three groups separately by the use of principal component analysis (PCA, STATISTICA, StatSoft. Inc. 1995). The scores of the first principal component were used to calculate Mahalanobis distance (D^2) in a pairwise fashion between all populations in both seasons. To test differences among populations studied and seasons, ANOVA test and Fisher's least significant difference tests (L.S.D.) were performed on the first three factors of PCA (PC1, PC2, PC3). Mantel's (1967) test was used to test relationships between Rogers' genetic distances and Mahalanobis morphological distances among vole populations in both seasons. The analysis was performed using the TFPGA computer programme (Miller 1997).

Results

Genetic analysis

Fourteen of the 37 loci examined were found to be polymorphic in the bank vole from NE Poland, as defined using the 0.95 common allele frequency: Ldh-2, Me-2, Dia, Cat, Aat-2, Pgm-1, Pgm-2, Pgm-3, EstB3, EstD, Amyl-2, Pep-2, Acy, Mpi. But only five loci: Me-2, Dia, Pgm-3, EstB3, and Amyl-2 were polymorphic in every population studied and in both seasons. The percentage of polymorphic loci (P) ranged from 0.162 to 0.270 and the mean observed heterozygosity (H_0) from 0.070 to 0.095 in the bank vole populations (Tab. 1). There were no significant differences in observed heterozygosity H_0 between seasons in each population and among populations either in spring or in autumn ($p > 0.05$; Kruskal-Wallis test). Two populations in spring (BIA, ZED) and two others in autumn (PRZ, SZE) showed significant departures from Hardy-Weinberg equilibrium in a few loci (Tab. 2). There was a significant deficit of heterozygotes in all these populations.

To measure genetic differences between spring and autumn generations F_{ST} values were calculated (Tab. 3). In four populations mean F_{ST} values were statistically significant indicating that above 1 per cent of genetic variation in a *C. glareolus* population was attributable to differences between seasons. There was a low but significant genetic differen-

Table 1. Sample size (N), percentage of polymorphic loci (P) and observed heterozygosity (H_0) in spring and autumn generations of *C. glareolus*. Abbreviations of the populations are in parentheses.

Population		Spring generation			Autumn generation		
		N	P	H_0	N	P	H_0
Bialystok	(BIA)	44	0.216	0.074	71	0.243	0.075
Suprasl	(SUP)	31	0.216	0.081	38	0.270	0.079
Przewalanka	(PRZ)	31	0.243	0.094	41	0.270	0.079
Zednia	(ZED)	26	0.162	0.079	39	0.162	0.070
Szelagowka	(SZE)	29	0.216	0.081	41	0.243	0.086

Table 2. Allele frequencies at 14 polymorphic loci in spring (SG) and autumn (AG) generations of *Clethrionomys glareolus* from NE Poland. Asterisks indicated deviation from Hardy-Weinberg equilibrium.

Locus Allele	Bialystok		Suprasl		Przewalanka		Zednia		Szelagowka	
	SG	AG	SG	AG	SG	AG	SG	AG	SG	AG
Ldh-2										
a	0.977	0.965	1.000	1.000	0.968	1.000	0.981	1.000	0.983	0.902
b	0.023	0.035	0.000	0.000	0.032	0.000	0.019	0.000	0.017	0.098
Me-2										
a	0.798*	0.887	0.900	0.776	0.758	0.902	0.788	0.859	0.862	0.854
b	0.202*	0.113	0.100	0.224	0.226	0.037	0.212	0.141	0.138	0.146
c	0.000	0.000	0.000	0.000	0.016	0.061	0.000	0.000	0.000	0.000
Dia										
a	0.000	0.007	0.016	0.000	0.000	0.000	0.000	0.000	0.017	0.000
b	0.580	0.579	0.339	0.289	0.549	0.512	0.365*	0.423	0.414	0.512
c	0.420	0.414	0.645	0.711	0.419	0.450	0.635*	0.577	0.569	0.488
d	0.000	0.000	0.000	0.000	0.032	0.038	0.000	0.000	0.000	0.000
Cat										
a	0.000	0.007	0.017	0.053	0.000	0.000	0.000	0.038	0.000	0.000
b	1.000	0.993	0.983	0.947	1.000	1.000	1.000	0.962	1.000	1.000
Aat-2										
a	0.045*	0.021	0.017	0.053	0.000	0.000	0.000	0.000	0.000	0.000
b	0.955*	0.979	0.983	0.947	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1										
a	1.000	1.000	1.000	0.987	0.855	0.902	1.000	1.000	0.948	0.976
b	0.000	0.000	0.000	0.013	0.145	0.098	0.000	0.000	0.052	0.024
Pgm-2										
a	0.000	0.014	0.033	0.053	0.081	0.061	0.000	0.000	0.017	0.012
b	0.886	0.928	0.919	0.868	0.919	0.939	0.962	0.987	0.862	0.854
c	0.014	0.058	0.048	0.079	0.000	0.000	0.038	0.013	0.121	0.134
Pgm-3										
a	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.083	0.164	0.167	0.171	0.081	0.134*	0.135	0.244	0.225	0.390
c	0.119	0.236	0.233	0.092	0.339	0.232*	0.327	0.192	0.155	0.146
d	0.298	0.257	0.150	0.184	0.177	0.146*	0.173	0.128	0.241	0.184
e	0.500	0.336	0.433	0.553	0.403	0.488*	0.365	0.436	0.379	0.280
f	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. (Continued)

Locus Allele	Bialystok		Suprasl		Przewalanka		Zednia		Szelagowka	
	SG	AG	SG	AG	SG	AG	SG	AG	SG	AG
EstB3										
a	0.761	0.791	0.600	0.750	0.694	0.720	0.673	0.718	0.690	0.598
b	0.239	0.209	0.317	0.237	0.306	0.280	0.308	0.282	0.293	0.378
c	0.000	0.000	0.083	0.013	0.000	0.000	0.019	0.000	0.017	0.024
EstD										
a	0.875	0.887	0.862	0.921	0.806	0.793	0.808*	0.962	0.948	0.878
b	0.125	0.113	0.138	0.079	0.194	0.171	0.192*	0.038	0.052	0.122
c	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.000
Amy1-2										
a	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.667*	0.843	0.750	0.908	0.733	0.859	0.812	0.784	0.727	0.868*
c	0.333*	0.157	0.229	0.092	0.267	0.141	0.188	0.216	0.273	0.132*
Pep-2										
a	0.102	0.086	0.065	0.013	0.016	0.063	0.000	0.000	0.071	0.159*
b	0.898	0.900	0.935	0.974	0.984	0.913	0.981	1.000	0.875	0.817*
c	0.000	0.014	0.000	0.013	0.000	0.024	0.019	0.000	0.054	0.024*
Acy										
a	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.045	0.028	0.000	0.158	0.000	0.037	0.000	0.077	0.000	0.012
c	0.955	0.972	0.967	0.842	1.000	0.963	1.000	0.923	1.000	0.988
Mpi										
a	0.000	0.000	0.000	0.000	0.016	0.013	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.065	0.074	0.019	0.000	0.034	0.000
c	0.989	0.936	0.968	0.971	0.903	0.913	0.981	1.000	0.949	1.000
d	0.011	0.064	0.032	0.029	0.016	0.000	0.000	0.000	0.017	0.000

Table 3. Mean F-statistic values at all loci for seasonal generations in five populations of *Clethrionomys glareolus* from NE Poland. Asterisks denote statistical significance for F_{ST} as determined by the χ^2 test (see text); *$p < 0.05$; **$p < 0.01$; NS = nonsignificant. Site abbreviations as in Tab. 1.

Population	F_{IS}	F_{IT}	F_{ST}
BIA	0.089	0.100	0.012**
SUP	0.004	0.022	0.018**
PRZ	0.033	0.043	0.011*
ZED	0.007	0.018	0.011*
SZE	–0.048	–0.036	0.012 NS

tiation among the bank vole populations in spring (mean $F_{ST} = 0.023$; $p < 0.0001$) in eight loci (Dia, Aat-1, Pgm-1, Pgm-2, Pgm-3, EstB3, Pep-2, Acy). However, in autumn the mean F_{ST} value was higher ($F_{ST} = 0.032$; $p < 0.0001$) and 16 single locus F_{ST} values were statistically significant (Ldh-1, Ldh-2, Me-2, Idh-1, Pgd, Dia, Cat, Aat-1, Aat-2, Pgm-1, Pgm-2, Pgm-3, EstD, Pep-2, Acy, Mpi). Rogers' genetic distance estimates based on pairwise comparisons of the five sites ranged from 0.018 to 0.029 in spring and from 0.023 to 0.039 in autumn, indicating that populations were very similar to one another. The linkage distances were so low that any groupings in the clusters could be considered to be random associations. However, two geographical nearest populations (SUP and ZED)

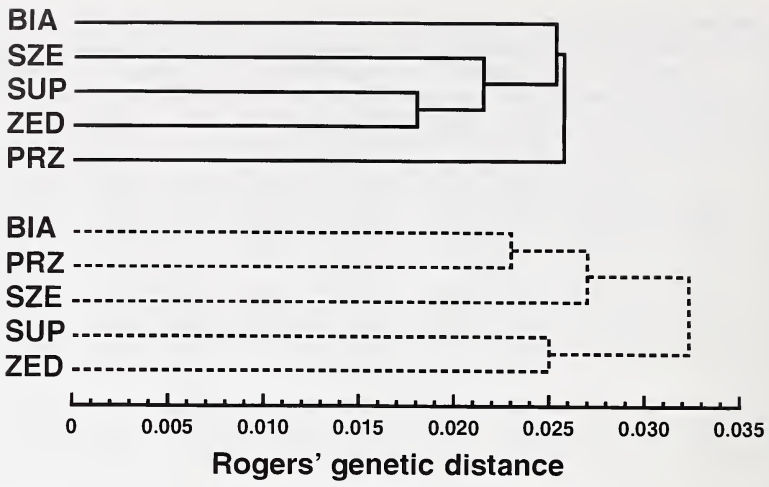

Fig. 1. Phenogram generated by UPGMA cluster analysis based on Rogers' genetic distances among five populations of *C. glareolus* in spring (solid lines) and autumn (broken lines). Site abbreviations as in Tab. 1.

clustered together in both seasons (Fig. 1). Correlation of two genetic distance matrices from various seasons revealed that genetic differentiation among the bank vole populations in spring was not covered by interpopulation differentiation in autumn ($r = 0.06$, $Z = 0.01$; NS; Mantel's test).

Morphological variation

Seasonal variation was conspicuous in all the populations studied. The first principal component (PC1) explained over 50% of the total variation in body size (I) and about 40% in organ size (II) and cranium dimension (III) in each population. Nevertheless, the between-season variation in three groups of morphological parameters described by PC1 was significant in all populations of the bank vole (Tab. 4).

Principal component analyses of *C. glareolus* revealed significant differences in body size (group I) among populations both in spring (ANOVA on PC1: $F = 7.24$; $p < 0.0001$) and autumn (ANOVA on PC1: $F = 10.12$; $p < 0.0001$). However, the first PC based on group II of morphological parameters divided the bank vole populations only in spring ($F = 2.78$; $p < 0.05$) but not in autumn ($F = 1.01$; NS). The first component (PC1) based on craniometric data (group III) explained 36% of total amount of phenotypic variability in spring and about 32% in autumn (Tab. 5). The differences observed among the bank vole populations also referred to skull dimension. The PC1s were of the size-type with all coefficients positive in both seasons, which suggested an influence of overall skull size on group separation. The second factor (PC2) based on craniometric parameters accounted for 8.8% of the variation in spring. In this season it loaded positively on measurements of skull and mandibule length (CBL, TCL, BL, UDL, ML) and negatively on parameters of skull width (RW, IW, ZW) and height (PH, SPB, SBB; Tab. 5). Thus, PC2 could be interpreted as a shape-type component in spring. In autumn, however, the second (PC2) and the third (PC3) factors which explained 24.3% of the total variance together represented 'shape' variation (Tab. 5). Analyses of variance confirmed that in spring the bank vole populations differed significantly in cranium size (PC1: $F = 6.45$; $p < 0.0001$) and cranium shape also (PC2: $F = 4.18$; $p < 0.01$, PC3: $F = 13.59$; $p < 0.0001$).

Table 4. Seasonal variation in body size (I), organ size (II), and skull dimension (III) in five populations of Clethrionomys glareolus from NE Poland revealed by one-way ANOVA on PC1. EV = eigenvalue, V = per cent of total variance, p = significance level for between-season comparison in ANOVA. Only factor loading values greater than absolute 0.60 are presented. Site abbreviations as in Tab. 1. Acronyms are described in the text.

Population		EV	V	Factor loadings	p
BIA	I	2.67	53.44	HBL (0.91), TBM (0.85), TL (0.74)	0.0000
	II	4.61	35.43	LM (0.84), KM (0.78), SIM (0.77), SMM (0.70)	0.0000
	III	6.56	31.23	BL (–0.96), CBL (–0.95), TCL (–0.92), UDL (–0.81), ZW (–0.78), MW (–0.76)	0.0003
SUP	I	3.18	63.68	HBL (0.92), TBM (0.88), TL (0.82)	0.0000
	II	6.68	51.42	STM (0.91), KM (0.87), LIM (0.83), SIM (0.82), HM (0.80), CM (0.78), LUM (0.74)	0.0000
	III	8.45	40.26	TCL (0.97), CBL (0.95), BL (0.94), NBL (0.85), ZW (0.83), ML (0.83), UDL (0.82), IFL (0.72)	0.0009
PRZ	I	3.13	62.66	TBM (–0.94), HFL (–0.92), EH (–0.82), TL(–0.77)	0.0000
	II	5.89	45.29	SIM (0.89), STM (0.83), SMM (0.82), LUM (0.82), CM (0.80), LIM (0.75)	0.0000
	III	6.99	33.30	CBL (0.88), BL (0.86), MW (0.77), ZW (0.73), FBL (0.72), UDL (0.71)	0.0000
ZED	I	2.76	55.22	TBM (–0.93), HBL (–0.93), TL (–0.84)	0.0000
	II	6.11	47.01	STM (0.91), CM (0.84), KM (0.83), SIM (0.82), LIL (0.70)	0.0000
	III	9.04	43.07	TCL (–0.94), CBL (–0.93), BL (–0.93), ZW (–0.84), NBL (–0.84), MW (–0.80), UDL (–0.77), FBL (–0.76), IFL (–0.74)	0.0000
SZE	I	3.24	64.84	HBL (0.93), TBM (0.85), EH (0.84), TL (0.78)	0.0000
	II	4.59	35.30	STM (0.90), CM (0.85), KM (0.82), HM (0.75)	0.0000
	III	7.93	37.78	BL (0.93), CBL (0.92), TCL (0.92), UDL (0.88), ML (0.88), ZW (0.80), NBL (0.80), MW (0.72), IFL (0.71)	0.0106

However, two 'shape' components (PC2 and PC3) only separated vole populations in autumn (ANOVA on PC1: $F = 1.46$; NS, PC2: $F = 19.71$; $p < 0.001$, PC3: $F = 11.76$; $p < 0.0001$).

Mahalanobis distances (D^2) calculated from the first principal component based on group I of morphological parameters ranged from 0.046 to 1.456 among populations in spring and from 0.001 to 1.618 in autumn. The differences in organ and cranium size (PC1 based on group II and III) were not found among populations in autumn. Thus, in this season the Mahalanobis distances among populations based on these two groups of variables ranged only from 0.0–0.587. Likewise, D^2 index also reached the low values when we analysed differences among populations in size of voles internal organs (group II; D^2 range 0.023–0.426) and cranium size (group III, D^2 range 0.022–1.317) in spring. Cluster diagrams generated on the basis of Mahalanobis distances from group I of morphological parameters were similar in spring and autumn ($r = 0.71$; Mantel's test) but the correlation coefficient was not significant ($Z = 2.16$; $P = 0.0560$, Fig. 2 A). There was no significant correlation in Mantel's test between matrices of Mahalanobis distances in spring and autumn based on group II ($r = -0.34$, $Z = 0.10$; NS, Fig. 2 B) and group III of morphological parameters ($r = -0.28$, $Z = 0.75$; NS, Fig. 2 C).

Table 5. Component loadings for the first three principal components (PC1, PC2, PC3) analysed in spring and autumn generations of *Clethrionomys glareolus*. Five populations are included in the analysis and PCA is based on 21 craniometric variables. EV = eigenvalue, V = per cent of total variance. Acronyms are described in the text.

Variables	Spring generations			Autumn generation		
	PC1	PC2	PC3	PC1	PC2	PC3
CBL	0.900	0.245	–0.001	0.914	0.164	–0.086
TCL	0.890	0.137	–0.026	0.893	0.200	–0.028
BL	0.916	0.217	–0.058	0.885	0.151	–0.129
RW	0.486	–0.018	0.156	0.570	0.101	0.080
IW	0.501	–0.250	0.206	0.189	0.273	–0.185
ZW	0.752	–0.117	0.057	0.647	0.419	–0.248
MW	0.739	–0.279	0.115	0.711	0.316	–0.216
PH	0.461	–0.178	–0.076	0.437	–0.516	0.351
SPB	0.554	–0.595	0.259	0.421	0.090	0.414
SBB	0.451	–0.688	0.211	0.297	0.014	0.556
UDL	0.722	0.346	0.313	0.777	0.090	0.026
ML	0.804	0.092	–0.049	0.709	0.108	–0.048
MH	0.450	–0.099	–0.254	0.424	0.406	–0.362
NBL	0.647	0.264	–0.106	0.708	–0.275	–0.187
FBL	0.658	–0.041	0.184	0.404	–0.705	–0.136
SCL	0.149	–0.023	–0.060	0.043	0.605	0.358
IBL	0.004	–0.060	0.030	0.269	–0.073	0.524
IFL	0.570	0.479	–0.094	0.502	–0.587	–0.060
PL	0.225	0.348	–0.191	0.205	–0.071	0.546
UMSL	0.230	–0.294	–0.799	0.313	–0.671	–0.167
LMSL	0.375	–0.249	–0.718	0.261	–0.808	–0.256
EV	7.58	1.85	1.62	6.64	3.31	1.74
V	36.09	8.82	7.71	31.63	15.75	8.40

Table 6. Correlation between Rogers' genetic distances D_R and Mahalanobis distances D^2 calculated from PC1 of groups I, II, and III of morphological parameters in spring (SG) and autumn (AG) generations of *Clethrionomys glareolus*. r = correlation coefficient, Z = statistic value, p = probability level after randomly permuting the values in one of the distance matrices 1 000 times, Mantel test.

Comparison	Generation	r	Z	p
$D_R \times D^2$ (I)	SG	0.07	0.10	NS
	AG	–0.38	0.16	NS
$D_R \times D^2$ (II)	SG	0.43	0.04	NS
	AG	0.01	0.02	NS
$D_R \times D^2$ (III)	SG	0.17	0.10	NS
	AG	0.23	0.05	NS

Correlation between genetic and morphological divergence

Cluster diagrams generated on the basis of Rogers' distances and Mahalanobis distances (in three groups of parameters separately) were not similar either in spring or in autumn, suggesting no equivalent degree of divergence on the two levels of integration. To quantify the lack of this relationship correlation coeffcients between D_R matrices and D^2 matrices were calculated, with regard to season and group of morphological parameters (Tab. 6). The correlation coefficients (r) were low and not significant in all cases of Mantel's test.

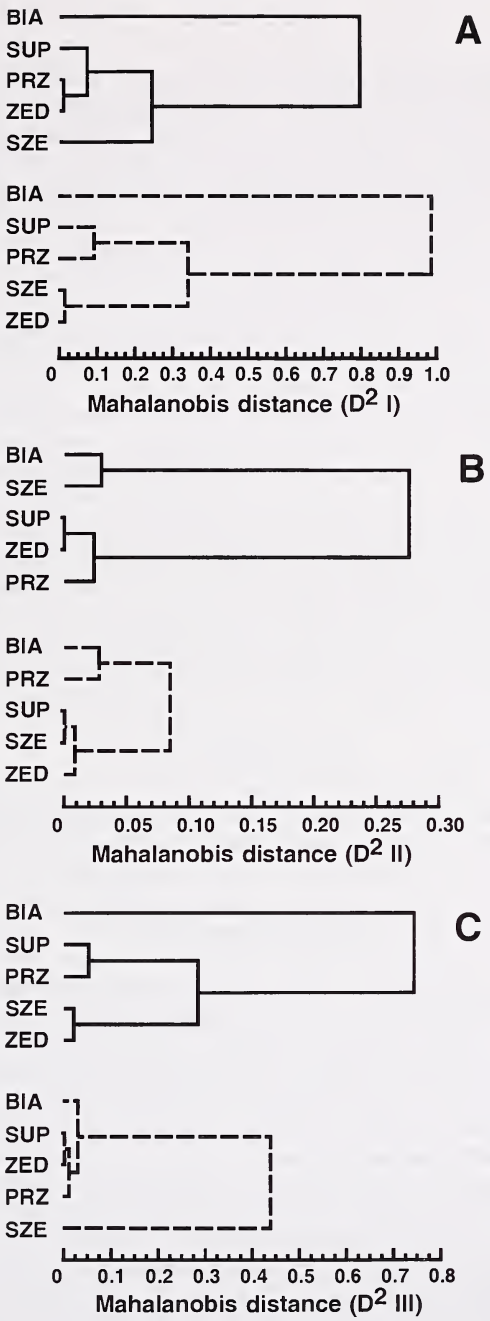

Fig. 2. UPGMA cluster analysis using Mahalanobis distances based on PC 1 from morphological data of (a) group I (D^2I), (b) group II (D^2II) and (c) group III (D^2III) among *C. glareolus* populations in spring (solid lines) and autumn (broken lines). Site abbreviations as in Tab. 1.

Discussion

There are three general areas where the present data are noteworthy. Firstly, the bank vole populations differed from each other over short geographical distances both genetically and morphologically. Secondly, the genetic and morphological divergence among populations varied between seasons. Finally, there was no correlation between genetic and morphological differentiation of the populations in both seasons.

The results of the analyses on allozyme variation in *C. glareolus* indicated that genetic polymorphism is not relatively high in this species. Percent of polymorphic loci in the populations studied approximated the values obtained for other populations of the bank vole in Poland (FEDYK and GĘBCZYŃSKI 1980; GĘBCZYŃSKI et al. 1993; GĘBCZYŃSKI and RATKIEWICZ 1998). However, the observed heterozygosity (H_0) slightly exceeded H_0 values obtained both in Poland ($H_0 = 0.073$; GĘBCZYŃSKI and RATKIEWICZ 1998) and in Austria ($H_0 = 0.075$; LEITNER and HARTL 1988).

Morphological analysis supported that the bank vole populations exhibited body size, organ, and cranium size variation within its distribution, even over short geographical distances. It is known that *C. glareolus* from various geographical regions differs considerably in intestinal morphology and body size (HANSSON 1985). Comparison of Western and Eastern Polish bank voles revealed the presence of several skull characters differing among the populations (HAITLINGER 1965). Likewise, mountain populations of *C. glareolus* differed from lowland populations in respect to dimensions and proportions of the body and skull (HAITLINGER 1970). According to HANSSON (1985), all geographical differences in *C. glareolus* are related to ecogeographical rules, to possible demographic patterns, and to various adaptations following different modes of feeding (i. e., more granivores or more foliovores animals). However, morphological differences observed among the bank vole populations over short geographical distances seem to be due to adaptations to various local habitats.

Genetic structure of the population changes between seasons in four out of five bank vole populations. Differences in allele frequency between spring and autumn generations of *C. glareolus* have been already noted (FEDYK and GĘBCZYŃSKI 1980; GĘBCZYŃSKI and RATKIEWICZ 1998). It is interesting that seasonal intrapopulation variability strongly affected divergence among vole populations. Thus, the differentiation among autumn populations was greater than among those in spring. Furthermore, correlation of two genetic distance matrices from spring and autumn revealed that genetic differentiation among the bank vole populations was not equivalent in various seasons. Smaller differences among vole populations in spring than in autumn suggested elimination of rare heterozygotes from the populations. Winter mortality can reach 77% of autumn numbers in *C. glareolus* (PUCEK et al. 1993). However, it did not cause between-season changes in H_0 value, as GĘBCZYŃSKI and RATKIEWICZ (1998) noted, but decreased the F_{ST} value, the measure of genetic differences among populations. Next, throughout breeding season the intrapopulation genetic variability increased. It seems that two processes, dispersion and mating system, can be responsible for increasing the intrapopulation variability, and consequently increasing F_{ST} value among populations in autumn. The dispersal rates of *C. glareolus* individuals vary significantly with seasons, being the highest in early summer and in autumn (GLIWICZ 1988). Additionally, *C. glareolus* was characterized by a promiscuous mating system, and multiple paternity was common in natural populations (RATKIEWICZ and BORKOWSKA 1999). Hence, there was a deficit of homozygotes at all loci, showing significant departures from Hardy-Weinberg equilibrium.

Seasonal variation in morphological parameters of the bank vole occurred in each population studied. The dynamic aspect of body mass and gut size of small herbivores was widely noted and explained as a physiological response to fluctuating environmental conditions (HAMMOND 1993; BORKOWSKA 1995; CAMPBELL and MACARTHUR 1996). Con-

trary to the genetic data, the differentiation in body size among bank vole populations was similar in spring and autumn. Therefore, it seems that body size could be an indicator of morphological divergence among populations of the bank vole. However, variation in size of internal organs strongly depended on a state of sexual activity (Hammond and Diamond 1994) or food availability (Campbell and MacArthur 1996). This is why the differences in group II in morphological parameters appeared among populations only in spring. The divergence in cranium dimension demonstrated seasonal variation also. Thus, cranium size and shape divided the populations in spring. However, individual variation related to age in craniometric parameters (Haitlinger 1965) is high in autumn populations, which consisted of relatively young animals. Thus, the 'shape' components (PC2 and PC3) only separated vole populations in this season.

Genetic and morphological differentiation patterns were discordant in *C. glareolus*. The analyses revealed that in both seasons allozymic variation did not correspond to morphological variation specified either by body size, or by size of internal organs, or by cranium dimension. Among the studies that have combined electrophoretic and adequate morphometric data to examine intraspecific population differentiation patterns, discordant genetic and morphological differentiation was found in amphibians (Kyriakopoulou-Sklavounou et al. 1991) and birds (Baker 1992). The authors suggested that genetic patterns were haphazard, while the morphological differences were due to either climatic adaptation or random divergence through founder effects. On the other hand, changes in environmental conditions, either temporarily or permanently, strongly influence both genetic and morphological variance among populations of small mammals. Thus, it seems that a temporal variation in the genetic and morphological constitution of a population has the potential for revealing the agents responsible for microevolutionary change.

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Zusammenfassung

Genetische und morphologische Differenzierung zwischen Rötelmauspopulationen (Clethrionomys glareolus) aus dem Nordosten Polens: Saisonale Aspekte

Bei 5 Rötelmauspopulationen (*Clethrionomys glareolus*) aus dem Nordosten Polens wurde die genetische und morphologische Variabilität untersucht. Zur Abschätzung der genetischen Variabilität wurden 37 Enzymloci mittels Proteinelektrophorese untersucht. Der Polymorphiegrad (P) reichte von 0,162 bis 0,270 und der durchschnittliche beobachtete Heterozygotiegrad (H_0) von 0,047 bis 0,095. Berechnungen zur F-Statistik ergaben das Auftreten von saisonalen Unterschieden in den Allelfrequenzen von vier Populationen ($F_{ST} = 0{,}011–0{,}018$, $p < 0{,}05$). Die Differenzierung zwischen den Rötelmauspopulationen war im Herbst ($F_{ST} = 0{,}032$, $p < 0{,}0001$) größer als im Frühling ($F_{ST} = 0{,}023$, $p < 0{,}0001$). Die morphometrische Variation in drei Gruppen von Parametern (I – Körpergröße, II – Größe innerer Organe, III – Schädelmaße) wurde mittels Hauptkomponentenanalyse (PCA) untersucht. In allen drei Parametergruppen zeigten sich klare saisonale Unterschiede. Die PCA ergab Unterschiede zwischen den Rötelmauspopulationen hinsichtlich der Körpergröße, der Größe innerer Organe sowie der Schädelgröße und -form für den Frühling, während sie sich im Herbst nur in bezug auf die Körpergröße und die Schädelform unterschieden. Genetische Distanzen nach Rogers und Mahalanobis-Distanzen, berechnet für 3 Gruppen morphologischer Merkmale in beiden Jahreszeiten, waren nicht miteinander korreliert ($r = |0.01–0.043|$, NS; Mantel-Test). Nach unseren Daten zeigen bei der Rötelmaus die beiden untersuchten Merkmalssysteme keinen vergleichbaren Grad an Differenzierung zwischen Populationen.

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