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Epigenetic and dental variation of the common vole, *Microtus arvalis* (Mammalia: Rodentia) in the Czech Republic

Jitka UHLÍKOVÁ

Department of Zoology, Faculty of Science, Charles University, Viničná 7, CZ-128 44 Praha 2, Czech Republic; e-mail: uhlik.jan@atlas.cz

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A b s t r a c t. Epigenetic and dental variation was studied in ten populations of the common vole (*Microtus arvalis*) from the Czech and Slovak Republic. A total of 1,536 specimens were scored for 34 cranial non-metric traits. The dependence on sex, size and correlation between traits was evaluated. The mean measure of divergence was used to express the interpopulation differences. The clustering of population generally agreed with their supposed subspecific status. The revealed epigenetic differentiation was not in concordance with the pattern of occlusal variation of M_1 and M^3. These results are discussed in respect of estimates of genetic and phenetic differentiation within and among populations.

Key words: subspecies, non-metric skull traits, molar occlusal variation

Introduction

The distribution range of the common vole, *Microtus arvalis* (Pallas, 1779), extends from the Atlantic coast of France to central Russia. The range is almost continuous with the exception of isolated populations in Iberia (Z i m a 1999, C r u z et al. 2002). Twenty six subspecies are assumed to occur in Europe (N i e t h a m m e r & K r a p p 1982). The status of most of them is questionable.

On the territory of the former Czechoslovakia, K r a t o c h v í l (1959) recognized three subspecies, namely *M. a. arvalis*, *M. a. duplicatus* and *M. a. levis* (Fig. 1). Discrimination of the subspecies was based on phenotypic traits, such as body size, fur colour, skull measurements and morphology of the upper third molar.

Epigenetic traits have been used to asses genetic relatedness between animal populations (e.g. B e r r y & R o s e 1975, H a r t l et al. 1993, K r y š t u f e k 1990, M a r k o w s k i & M a r k o w s k a 1988, P a t t o n et al. 1975, S i k o r s k i 1982, S m i t h 1981, Z i m a 1989). Although these traits are controlled by both genetic and nongenetic factors (S j ö v o l d 1977), it is claimed that they are highly heritable in nature, and may be employed in phylogenetic studies (B e r r y 1975).

This study aims to reveal the pattern of epigenetic skeletal and dental variation in Czech vole populations and to compare epigenetic divergence among them with their supposed subspecific affiliation.

Material and Methods

The study is based on examination of 1,536 skulls of *Microtus arvalis*. The individuals were collected in nine localities of the Czech Republic: Bližná (B), Český Dub (CD), Fládnice (F), Kopřivnice (K), the Krkonoše Mts (Kr), Olomouc (O), Praha-Řeporyje (R), Nový

Drahov (S), Velemín (V) and one locality of the Slovak Republic: Buzica (Z) (Fig. 1, Table 1). Besides being weighted and measured, all individuals were autopsied and their sexual condition determined. Specimens captured in the mountain range of the Krkonoše Mts (Kr) originated from two localities. The first site was the eastern grass slope below the peak of Sněžka mountain, the highest mountain of the Czech Republic (eight individuals collected), and the second site, Bílá louka, was in the northern grass slopes of Mt Luční hora and Mt Studniční hora (19 individuals collected). The distance between these two sites was about 4 km. Material from these two localities was pooled into one sample.

The studied material is deposited in collections of the Department of Zoology, Charles University, Prague.

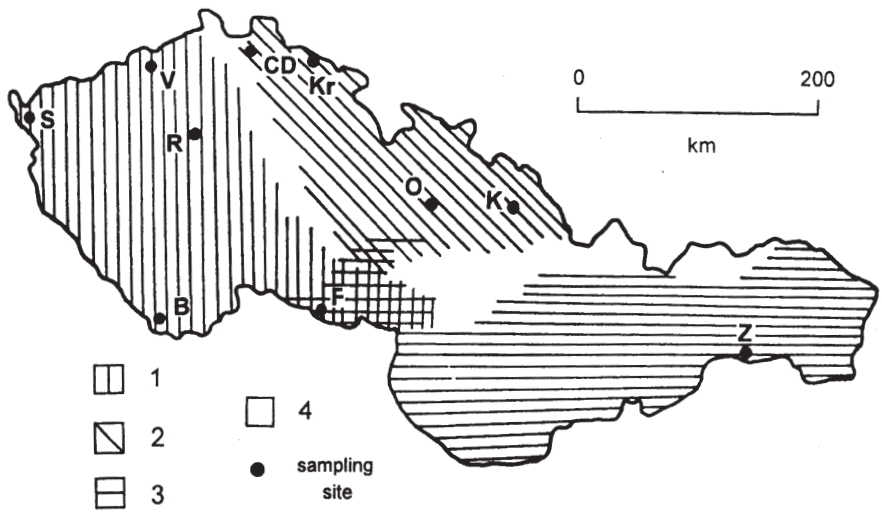

Fig. 1. Map of former Czechoslovakia showing the sampling sites of *Microtus arvalis*. See Material and Methods for the abbreviations of localities. 1 – *Microtus arvalis arvalis*, 2 – *M. a. duplicatus,* 3 – *M. a. levis,* 4 – populations of uncertain subspecific status (adapted after K r a t o c h v í l (1959)).

Table 1. Collecting localities of the specimens studied. N – number of individuals examined.

Locality	District town	Geographic coordinates		Altitude (m)	N	Time of capture
Bližná (B)	Český Krumlov	48°43´N	14°05´E	765	250	Oct. 1998
Český Dub (CD)	Liberec	50°39´N	14°59´E	325	130	Oct. 1997
Fládnice (F)	Znojmo	48°48´N	15°59´E	268	178	Nov. 1997
Kopřivnice (K)	Nový Jičín	49°35´N	18°07´E	320	158	Dec. 1997
Krkonoše (Kr)*	Trutnov	*N:*50°44´N *Bl:*50°43´N	*N:*15°44´E *Bl:*15°41´E	N: 1595 Bl: 1460	N: 8 Bl: 19	Aug. 1999–2001
Olomouc (O)	Olomouc	49°36´N	17°15´E	218	206	Oct. 1998
Řeporyje (R)	Praha	50°01´N	14°17´E	315	156	Oct.-Nov. 1997
Nový Drahov (S)	Cheb	50°08´N	12°24´E	445	146	Oct. 1997
Velemín (V)	Litvínov	50°32´N	13°58´E	287	155	Nov. 1997
Buzica (Z)	Košice	48°32´N	21°04´E	211	130	Sept. 1999

* Individuals captured in Krkonoše Mts originated from two localities: Sněžka (N) and Bílá louka (Bl).

Epigenetic variation

The specimens were scored for 34 non-metric traits. All the traits were scored as discrete variables. Most of them were similar to those desribed in other rodents by Andersen & Wiig (1982), Berry & Searle (1963), Kratochvíl (1959) and Ventura & Sans-Fuentes (1997). Because of damage of skulls, it was necessary to asess an expression of a bilateral trait on each side (L-left, R-right) separately as an individual character, and a total set of scored traits comprised 60 units. There is a disagreement in the literature as to the appropriateness of this artificial doubling of sample size. The theoretical considerations involved are discussed in Green et al. (1979) and Sjövold (1973, 1979).

In the available literature the descriptions of morphological characteristics of epigenetic traits, in particular of foramen, is lacking (e.g. Bauchau 1988). Therefore a study was made to examine the kind of structure (nerve or blood vessel) that passes through evaluated openings. Four adult specimens, two males and two females, were deeply anesthetized with an overdose of diethyl ether and then transcardially perfused with warm heparinized saline followed by 4% paraformaldehyde in 0,1 M phosphate buffer (PB; pH 7,4). This was followed by post-fixation rinse of transfer ink after twenty minutes Detailed dissection of a head was subsequently carried out. Application of transfer ink enabled black blood vessels to be distinguished from white nerves. In the following survey of traits examined (Fig. 2), the structure passing through evaluated foramens is given as *B* (blood vessel) or *N* (nerve).

Trait no.

1: shape of the posterior end of nasal bones – pointed, bow or straight (bilateral)
2: position of the posterior end of nasal bones in regard to sutura incisivofrontalis – in front of or behind
3: shape of sutura coronalis – bow or rectangular
4: shape of sutura lambdoidea – shape bow, obtuse angle or brace
5: foramen – absent or present (bilateral), B
6: foramen – absent or present, B
7: foramen – absent or present (bilateral), B
8: foramen – absent or present (bilateral), B
9: foramen – single or double (bilateral), B
10: foramen – absent or present (bilateral),B
11: foramen – two or four foramens (bilateral), N, B
12: foramen – absent or present (bilateral), B
13: foramen – absent or present,
14: sutura between os basisphenoidale and os basioccipitale – absent or present
15: foramen – single or double (bilateral), N, B
16: position of foramen no. 15 in regard to lower margin of foramen ovale – above or below
17: foramen – absent or present (bilateral), B
18: shape of processus zygomaticus maxillae – bow or straight (bilateral)
19: foramen – single or double (bilateral), N, B
20: foramen – absent or present (bilateral), B
21: foramen – absent or present (bilateral), B
22: foramen – absent or present (bilateral), ?N – In this region nervus opticus compresses the skull bone; it is possible that the nerv causes perforation.
23: foramen – absent or present (bilateral), B

24: foramen – single or double (bilateral), B 25: foramen – absent or present (bilateral), B
26: shape of foramen – isosceles ( ) or rectangular ( ) triangle (bilateral)
27: foramen – single or double (bilateral), B
28: shape of processus paracondylaris – bow or straight (bilateral)
29: shape of foramen ovale – bow or pointed
30: foramen mentale – absent or present (bilateral), N
31: foramen – absent or present (bilateral), B
32: foramen – single or double (bilateral), N, B
33: foramen – absent or present (bilateral), B
34: foramen – absent or present (bilateral), B

Traits Nos. 2, 3, 4, 16, 18, 26, 28, 29 are not true threshold chracters, because their variants may intergrade. However, such traits have also been used in studies of epigenetic variation (e. g. Berry & Searle 1963, Petras 1967, Rees 1969, Sikorski & Bernshtein 1984, Markowski & Markowska 1988).

All the traits were examined for dependence that might influence the overall pattern of intersample epigenetic differences. In specimens from Olomouc traits were checked for intertrait correlations (the chi-square test with the Bonferroni adjustment). After this procedure, 16 characters (1R, 7R, 9R, 10R, 11R, 12R, 17R, 18R, 21L, 22R, 23R, 24R, 26R, 28R, 32R, 33R) with pairwise associations of character states (α<0.0001) were excluded from further analysis.

In the Olomouc sample, logistic regression was used to assess the dependence of frequencies of traits on sex, size and molar morphotype. The length of diastema was used as a measure of size. Three traits (8L, P<0.05; 11L, P<0.05; 21L, P<0.01) revealed dependence on sex but no significant differences were found between samples in number of males and females (the chi-square test, P=0.205). Three traits (17P, P<0.05; 21L, P<0.05; 29, P<0.01) revealed significant dependence on size. Significant differences were found among samples in their size composition (ANOVA, F=12.64, P<10^{-6}). After excluding skulls (altogether 13 skulls) with diastema length smaller than 5.6 mm, the size composition of samples appeared homogenous (ANOVA, F=2.50, P=0.114). Thus no traits were omitted from further analyses because of size- and sex-dependence of their character states.

The index of epigenetic variability (I_v) was calculated after Smith (1981):

$$I_v = \sum_{i=1}^{h} [(p_i \cdot q_i) / h]$$

p_i – the frequency of the i^{th} character
$q_i = 1 - p_i$
h – number of traits examined (h = 36, see Results)
The mean measure of divergence (MMD) proposed by C. A. B. Smith and adapted by Constandse – Westermann after Finnegan & Cooprider (1978) was calculated between the samples:

$$MMD = 1/h \cdot \sum_{i=1}^{h} [(\theta_{1i} - \theta_{2i})^2 - (1/n_{1i} + 1/n_{2i})]$$

h – the number of traits examined (h = 36)
$\theta_{1i} = \arcsin (1 - 2p_{1i})$
$\theta_{2i} = \arcsin (1 - 2p_{2i})$
$p_{1(2)i}$ – observed frequency of a trait *i* in the sample *1 (2)*

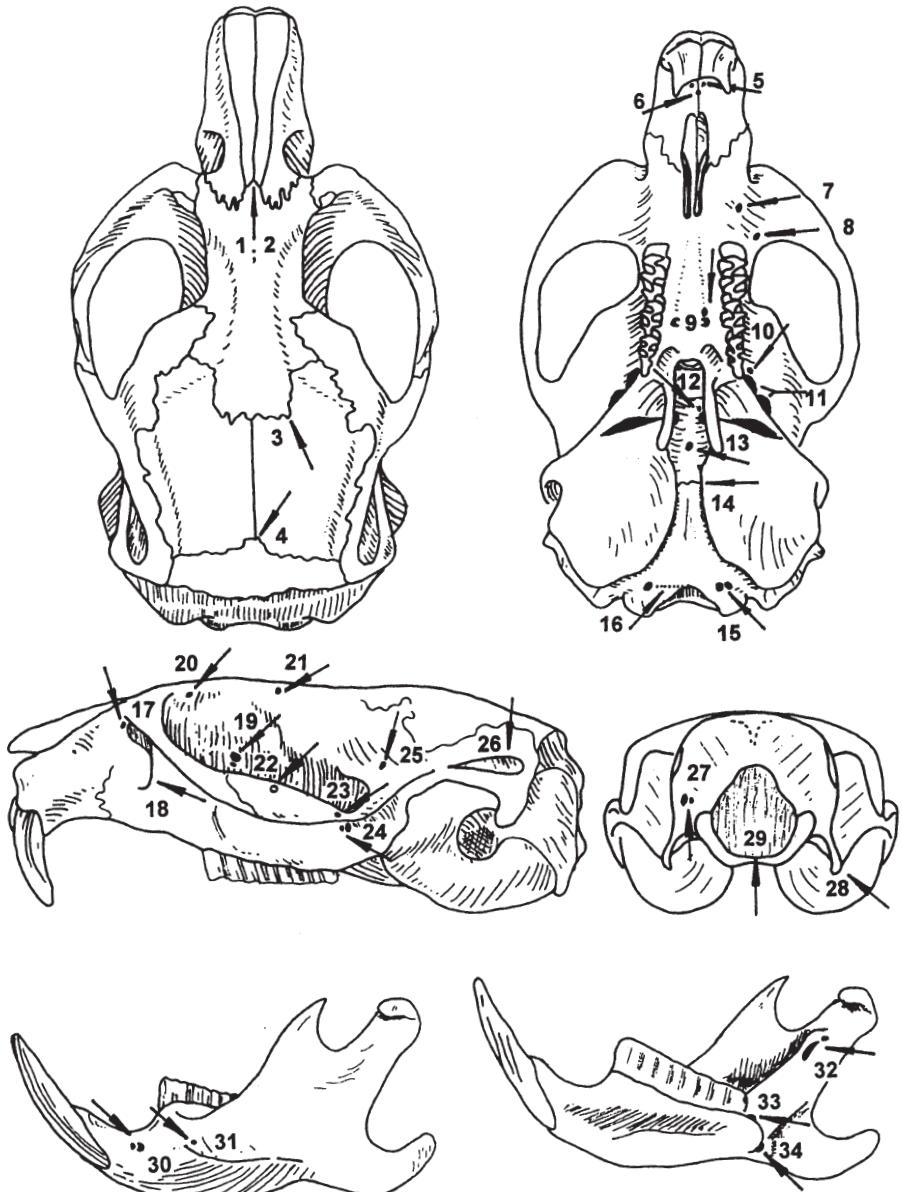

Fig. 2. Position of non-metric traits scored in the common vole skull (for the trait description see Material and Methods). Variants present and double are illustrated. Foramen no. 22 is hidden below the zygomatic arch. Dorsal, ventral, lateral and caudal aspects of cranium; lateral and medial aspects of the lower jaw.

If p reached extreme proportions (0 or 1), Bartlett´s Adjustment was performed (S j ö v o l d 1973):

p = 0 is replaced by p = 1/4n

p = 1 is replaced by p = 1 - 1/4n

n – the number of observations for a given trait in a sample

For calculating the MMD, the phenotypic expression was dichotomized in the traits 1, 4 (the first two listed variants were pooled).
The values of MMD were statistically significant at 0.05 level if the value was two times higher than the standard deviation of the MMD:

$$SD_{MMD} = (2/h \cdot \sum_{j=1}^{h} [1/(n_{1i} + 1/2) + 1/(n_{2i} + 1/2]^2)^{1/2}$$

The measure of uniqueness (MU) was calculated for each sample as the sum of its epigenetical distances from the other samples:

$$MU_k = \sum_{j=1}^{u} MMD_{kj}$$

j – the number of samples, which where compared with k^{th} sample
Based on the mean measure of divergence a dendrogram of epigenetic similarity was constructed. The cluster analysis was done by use of the neighbour-joining method. The correspondence between geographic proximity and epigenetic similarity was estimated by the Mantel test.

Dental variation

Morphological variability of the dental pattern of the first lower molars (M_1) and the third upper molars (M^3) was evaluated according to Kratochvíl (1959) and Angermann (1971) on both sides of skull. The variation of the grinding surface of M_1 was based on morphology of the anteroconid complex. Seven morphological variants were recorded (Fig. 3). The enamel tooth pattern of vole M^3 was evaluated and six morphological variants were distinguished (Fig. 4). Variants 1, 2 – 4, 5, 6 differed in the number of lingual and buccal triangles. Variants 2–4 had a different shape of posterior cup: v. 2-no aboral field, v. 3-aboral field present, v. 4-aboral field is longer than the last lingual triangle.

Differences among populations in molar shape variation were tested using the chi-square test. The cluster analysis was conducted with the neighbour-joining method. Matrices on which trees of M_1, M^3 and MMD were based were compared by the Mantel test.

Results

Epigenetic variation

The frequencies of 36 non-metric traits are given in Table 2. Traits that revealed no variation (2, 5LR, 6, 13, 14, 30LR) and sixteen characters with a high degree of correlation (see above) were excluded from a total set of 60 traits. The results of the chi-square test did not show significant differences between samples in seven traits. The correlation was estimated in all the 1,326 pairwise comparisons, and it was significant in 17 pairs (1.28%) ($P<10^{-6}$). These 17 pairs involved 14 correlations between left and right variant of a trait.

Values of the index of epigenetic variability varied from 0.138 to 0.173 with the maximum in the sample from Český Dub (Table 3). The differences between individual samples were not distinct, the lowest I_v values revealed two samples from mountain ranges (B, Kr).

Table 4 shows the MMD-matrix used in the NJoin cluster analysis (Fig. 5) as well as the values of standard deviation (SD) and measure of uniqueness (MU). From a total amount of 45 pairwise comparisons of the epigenetic distance MMD, 35 of them (77.8%) showed

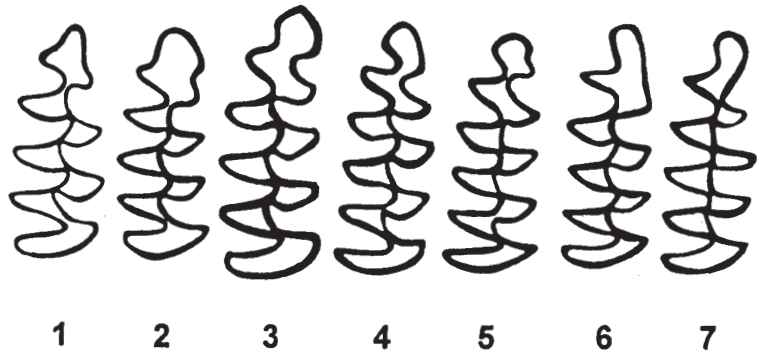

Fig. 3. Morphological variability of M_1. The right M_1 is illustrated.

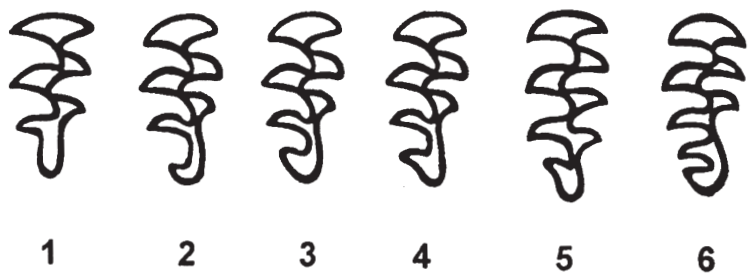

Fig. 4. Morphological variability of M^3. The left M^3 is illustrated.

significant differences. The maximum value of MU revealed the sample from Český Dub. No evidence of a relationship between epigenetic and geographical distances was found ($r = 0.08$, $P = 0.32$). Two clusters of samples are obvious (Fig. 5). The first one consists of seven samples (B, CD, O, Kr, R, S, V), with a distinctly separated position of the sample B. These samples originated from regions populated supposedly by the subspecies *M. a. arvalis* and *M. a. duplicatus* (K r a t o c h v í l 1959). The other group of samples F, K, Z – with a distinct position of the sample F – originated from the eastern sites of the area studied where K r a t o c h v í l (1959) supposed the occurrence of the subspecies *M. a. duplicatus*, *M. a. arvalis* and especially *M. a. levis*

D e n t a l v a r i a t i o n

The distribution of the molar shape variants of the skull left side in the populations studied is shown in Table 5. Significant heterogeneity of molar variation among samples was found (M_1L: $\chi^2 = 144.8$, $P<10^{-6}$; M_1R: $\chi^2 = 141.5$, $P<10^{-6}$; M^3L: $\chi^2 = 214.6$, $P<10^{-6}$; M^3R: $\chi^2 = 210.8$, $P<10^{-6}$).

Results of cluster analysis were identical for both sides of a given tooth, thus only two dendrograms for M^3 and M_1, respectively, are shown (Fig. 6). No evidence of a relationship between dental and epigenetic variation was found (M_1L: $r = -0.007$, $P = 0.52$, M^3L: $r = -0.12$, $P = 0.34$).

Table 2. Frequencies (%) of epigenetic traits in the samples evaluated. L, R - left and right variant of a trait; G – straight; E – rectangular; C – brace; P – present; D – double; B – below; M – impedance triangel; N – pointed. * Differences in the trait frequencies were not significant ($\alpha = 0.05$).

Locality	B		CD		F		K		Kr		O		R		S		V		Z	
Trait: variant	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n
1L: G	0.101	237	0.083	120	0.102	176	0.114	149	0.074	27	0.060	199	0.195	149	0.153	144	0.153	150	0.165	115
3: E	0.498	201	0.616	99	0.455	121	0.309	97	0.556	27	0.512	166	0.585	106	0.598	112	0.436	117	0.381	105
4: C	0.921	215	0.945	109	0.959	123	0.925	120	1.000	27	0.966	174	0.851	114	0.907	118	0.957	138	0.905	116
7L: P	0.731	234	0.675	120	0.663	175	0.450	140	0.407	27	0.705	193	0.589	141	0.643	140	0.521	140	0.651	109
8L: P	0.746	228	0.376	117	0.741	174	0.619	139	0.778	27	0.738	191	0.551	138	0.493	134	0.561	139	0.899	109
8P: P	0.741	228	0.311	119	0.700	170	0.613	137	0.815	27	0.704	186	0.551	138	0.477	132	0.551	138	0.766	107
9L: D	0.556	243	0.418	122	0.606	175	0.346	153	0.615	26	0.528	197	0.408	147	0.479	142	0.333	144	0.559	118
10L: P	0.289	180	0.359	92	0.241	112	0.265	83	0.222	27	0.406	165	0.537	95	0.288	104	0.385	104	0.224	98
11L: D*	1.000	173	1.000	92	1.000	109	0.988	82	1.000	27	0.975	162	0.989	94	1.000	102	0.990	102	1.000	98
12L: P	0.556	198	0.431	102	0.600	120	0.188	117	0.600	25	0.560	166	0.632	106	0.574	108	0.347	118	0.400	105
15L: D	0.513	199	0.486	109	0.504	121	0.411	124	0.440	25	0.576	170	0.393	117	0.405	111	0.397	121	0.396	106
15P: D	0.461	206	0.536	110	0.469	128	0.476	124	0.360	25	0.556	169	0.448	116	0.495	111	0.361	122	0.453	106
16: B	0.896	192	0.533	105	0.879	116	0.487	117	0.960	25	0.804	163	1.000	98	0.822	107	0.782	119	0.924	105
17L: P	0.965	227	0.892	120	0.942	172	0.922	141	1.000	27	0.973	184	0.929	140	0.993	141	0.908	141	0.981	107
18L: G*	0.040	225	0.076	118	0.018	170	0.029	137	0.074	27	0.026	191	0.044	136	0.081	136	0.029	140	0.028	109
19L: D*	0.023	219	0.017	117	0.024	166	0.015	133	0.000	27	0.039	181	0.015	131	0.015	135	0.008	132	0.038	106
19P: D*	0.009	213	0.026	117	0.024	164	0.048	126	0.000	27	0.028	179	0.015	131	0.022	134	0.008	132	0.038	105
20L: P	0.168	220	0.167	114	0.133	166	0.098	132	0.259	27	0.139	187	0.162	130	0.170	135	0.154	130	0.299	107

Table 2. (continued)

Locality	B		CD		F		K		Kr		O		R		S		V		Z	
Trait: variant	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n
20P: P*	0.156	212	0.173	110	0.180	161	0.089	123	0.185	27	0.181	182	0.197	127	0.195	133	0.163	129	0.265	102
21P: P	0.688	205	0.553	103	0.365	148	0.127	110	0.519	27	0.594	170	0.500	122	0.476	126	0.336	125	0.642	95
22L: P*	0.077	209	0.127	110	0.058	154	0.049	122	0.111	27	0.084	167	0.080	125	0.053	132	0.111	126	0.140	100
23L: P	0.972	177	0.830	88	0.949	118	0.908	87	0.926	27	0.962	156	0.937	95	0.919	111	0.870	108	0.854	89
24L: D	0.480	223	0.607	112	0.526	156	0.692	120	0.444	27	0.538	186	0.540	124	0.466	133	0.266	128	0.578	116
25L: P	0.941	185	0.634	93	0.944	108	0.785	93	0.889	27	0.919	161	0.915	106	0.934	106	0.857	119	0.921	101
25P: P	0.944	180	0.570	93	0.883	103	0.793	92	0.889	27	0.866	157	0.911	101	0.919	99	0.841	107	0.917	96
26L: M	0.780	164	0.605	76	0.729	85	0.866	82	0.440	25	0.750	144	0.843	89	0.907	86	0.879	107	0.622	90
27L: D	0.558	208	0.533	105	0.650	123	0.516	128	0.440	25	0.618	173	0.583	120	0.593	113	0.554	112	0.745	110
27P: D	0.574	204	0.430	107	0.598	132	0.504	123	0.440	25	0.604	169	0.564	117	0.549	113	0.520	123	0.761	109
28L: G	0.192	203	0.686	105	0.355	121	0.496	127	0.100	20	0.405	173	0.546	119	0.661	112	0.331	124	0.545	112
29: N	0.040	201	0.067	104	0.114	114	0.034	117	0.000	25	0.062	161	0.086	116	0.063	112	0.118	119	0.037	108
31L: P	0.126	246	0.323	130	0.091	175	0.120	158	0.259	27	0.102	205	0.090	156	0.241	145	0.129	155	0.119	126
31P: P	0.232	246	0.377	130	0.166	175	0.146	158	0.370	27	0.224	205	0.103	156	0.290	145	0.187	155	0.269	130
32L: D	0.539	245	0.488	125	0.581	172	0.404	151	0.556	27	0.591	203	0.503	153	0.686	140	0.437	151	0.565	124
33L: P	0.260	246	0.480	127	0.554	175	0.703	158	0.296	27	0.385	205	0.321	156	0.729	144	0.677	155	0.134	127
34L: P	0.967	245	0.969	127	0.966	174	0.949	157	1.000	27	0.990	205	0.987	156	0.973	146	0.935	155	0.992	127
34P: P	0.984	245	0.984	126	0.954	175	0.936	157	1.000	27	0.995	204	0.994	155	0.986	145	0.907	150	0.992	127

Table 3. Epigenetic variability (I_V) in the populations studied.

Locality	B	CD	F	K	Kr	O	R	S	V	Z
I_v	0.138	0.173	0.144	0.145	0.140	0.146	0.148	0.150	0.155	0.143

Table 4. Mean measure of divergence (MMD) and standard deviation of MMD (in italics) between samples examined. MU - Measure of uniqueness; significant values of MMD in bold.

Locality	CD	F	K	Kr	O	R	S	V	Z	MU
B	**0.164**	0.027	**0.163**	0.020	0.014	**0.054**	**0.083**	**0.087**	**0.047**	0.659
	0.020	*0.017*	*0.019*	*0.060*	*0.015*	*0.018*	*0.018*	*0.018*	*0.020*	
CD		**0.135**	**0.112**	**0.164**	**0.107**	**0.143**	**0.087**	**0.104**	**0.180**	1.196
		0.023	*0.025*	*0.066*	*0.021*	*0.025*	*0.025*	*0.024*	*0.026*	
F			**0.091**	0.045	0.013	**0.050**	**0.047**	**0.046**	**0.063**	0.516
			0.022	*0.063*	*0.018*	*0.022*	*0.022*	*0.021*	*0.023*	
K				**0.182**	**0.117**	**0.156**	**0.104**	**0.050**	**0.189**	1.165
				0.065	*0.020*	*0.024*	*0.023*	*0.023*	*0.025*	
Kr					0.044	0.093	0.116	0.103	0.059	0.827
					0.061	*0.065*	*0.065*	*0.064*	*0.066*	
O						**0.047**	**0.053**	**0.072**	**0.047**	0.515
						0.020	*0.020*	*0.019*	*0.021*	
R							**0.056**	**0.075**	**0.081**	0.754
							0.023	*0.023*	*0.025*	
S								**0.054**	**0.121**	0.722
								0.023	*0.025*	
V									**0.143**	0.735
									0.024	
Z										0.931

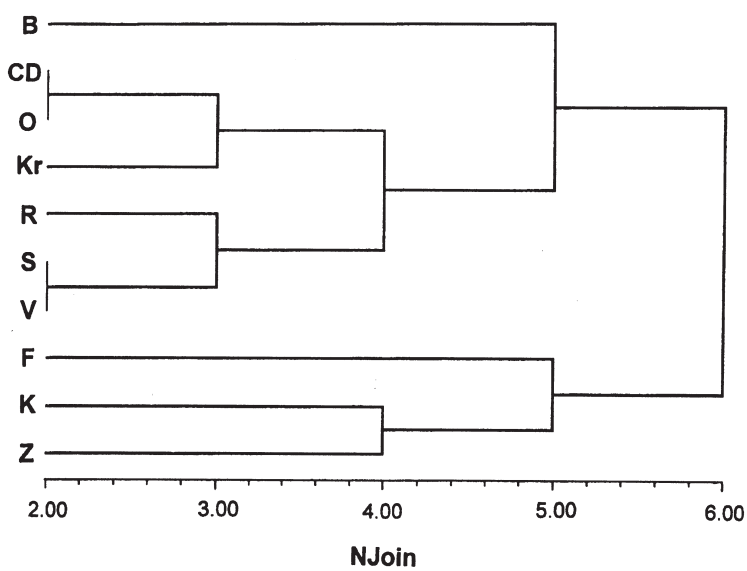

Fig. 5. Dendrogram of epigenetic relationships among *Microtus arvalis* populations (the NJoin method).

Table 5. Frequencies (%) of the M_1 and M^3 morphotypes on the left side of skull (1-7, see Figs 3, 4).

Locality	B		CD		F		K		Kr	
Molar	M_1	M^3	M_1	M^3	M_1	M^3	M_1	M^3	M_1	M^3
Morphotype										
1	3.7	2.4	0.0	0.0	0.0	5.7	0.0	0.0	0.0	0.0
2	0.8	7.8	0.0	14.5	0.0	12.6	0.0	7.1	0.0	0.0
3	14.7	59.6	16.1	55.6	15.4	65.1	6.4	47.7	12.0	62.5
4	68.2	30.2	59.7	29.1	76.6	16.6	75.0	45.2	56.0	37.5
5	10.2	0.0	22.6	0.9	6.3	0.0	18.6	0.0	28.0	0.0
6	2.4	0.0	0.8	0.0	0.6	0.0	0.0	0.0	0.0	0.0
7	0.0	-	0.8	-	1.1	-	0.0	-	4.0	-
n	245	245	124	117	175	175	156	155	25	24

Locality	O		R		S		V		Z	
Molar	M_1	M^3	M_1	M^3	M_1	M^3	M_1	M^3	M_1	M^3
Morphotype										
1	0.5	0.0	2.6	0.7	0.0	0.0	0.0	0.7	0.0	0.9
2	0.0	6.9	0.0	19.5	0.0	29.6	0.0	12.5	0.0	5.1
3	6.9	53.7	20.9	62.4	18.1	57.0	10.8	53.5	19.7	58.1
4	76.0	38.4	62.1	17.4	74.3	12.7	77.0	29.2	66.1	35.0
5	15.7	1.0	13.7	0.0	7.6	0.0	12.2	0.0	14.2	0.0
6	0.0	0.0	0.0	0.0	0.0	0.7	0.0	4.2	0.0	0.9
7	1.0	-	0.7	-	0.0	-	0.0	-	0.0	-
n	204	203	153	149	144	142	148	144	127	116

Discussion

The divergence estimate indicated that the major epigenetic split of Czech vole populations is between two groups representing the eastern and the western part of the area studied. Comparing this pattern with the subspecific status of populations proposed by K r a t o c h v í l (1959), the revealed epigenetic similarities are in vague agreement with the supposed distribution pattern of subspecies. The western cluster represents the assumed range of *arvalis-duplicatus* subspecies with a separate position of the population B from the Šumava Mts. The eastern cluster includes populations that should have affinity especially to the *levis* subspecies but, at the same time, this group includes also the "*duplicatus*" population K.

The highest epigenetic uniqueness and, at the same time, the highest value of epigenetic variability was exhibited by the population CD. However, it is difficult to find an unambiguous explanation for this result.

The obtained pattern of M^3 variation, that is used as discriminant criterion for particular subspecies (K r a t o c h v í l 1959), did not correspond either with the hypothetical subspecific allocation of populations or with the epigenetic pattern ascertained. For example, the frequency of the *simplex* form (the morphotype no. 1 on Fig. 3), that had been reported as typical for the north-eastern subspecies *M. a. duplicatus*, was higher in the southern part of Czech Republic, in the epigenetically distant populations B and F.

It should be also mentioned that there is a nomenclatoral problem in the *Microtus arvalis* group. Based on skull characters of the type specimen of *Microtus levis* Miller,

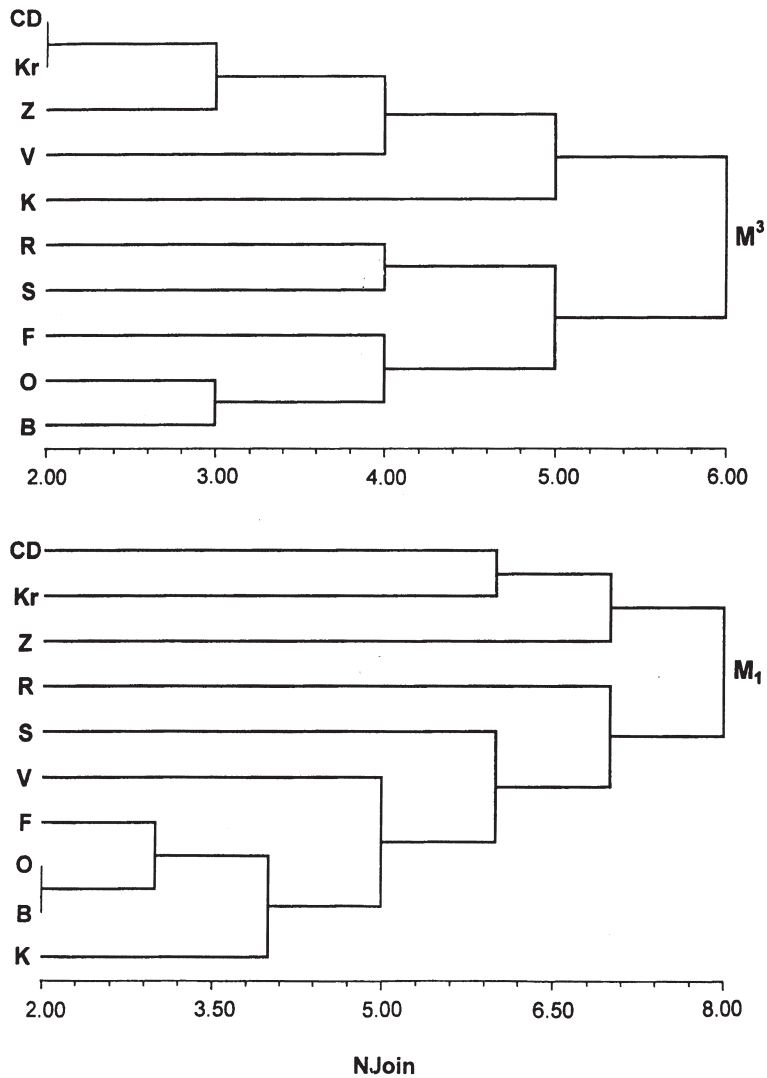

Fig. 6. Phenetic dendrograms (the NJoin method) of M^3 and M_1 for populations of *Microtus arvalis*.

1908, M a s i n g (1999) assumed that the name *Microtus levis* is the valid name of the 54-chromosome species of common vole inhabiting Europe. Therefore, the status of the *levis* subspecies appears questionable, and if M a s i n g (1999) is right, it is not clear what is the subspecific status of the populations from eastern parts of the area under study.

The pattern of intraspecific variation of the common vole in central Europe could be influenced especially by two processes. The first one is called „isolation by distance" (W r i g h t 1934) and the second one is the pattern of development of vole communities in the Pleistocene-Holocene period. The influence of the former factor has not be confirmed in the results obtained, as no pattern of increased epigenetic differentiation with geographical distance was observed. Thus, it seems probable that the latter factor has played a major role in shaping the structure within the species. The glaciations had two main effects on the

present pattern of variation: first, geographical isolation in separate glacial refugia generated divergence between populations. Second, species responded to the glacial-interglacial periods by distributional changes that affected the geographical design and amount of diversity (E h r i c h et al. 2000). Taking into account the period from the Würm-Riss interglacial, *Microtus arvalis* was widely distributed in moderate climatic zones of Europe until the Upper Pleistocene (K o r d o s 1990). It was the predominant rodent species in the Early Würm but later, particularly in the Middle and Late Würm, the frequency of its occurrence decreased. With Holocene warming and the spreading of woodland, the frequency of its incidence became still lower, with new immigrating taxa bound with forest habitats (K o r d o s 1990).The postglacial era of its expansion began with neolithic deforestation that opened a period of strong impact of man´s activity on nature.

K r a t o c h v í l (1959) supposed that founders of individual subspecies populations colonized the territory of the former Czechoslovakia from different directions and in different time horizons in the postglacial period. At that time there was a general perception that central and northern Europe were colonized by range expansion from Mediterranean refugia at the end of the last glaciation. The latest findings show (B i l t o n et al. 1998, H o r á č e k 2000), however, that certain small mammal species started the postglacial recolonization of central and northern Europe from one or more refugia located within their recent ranges. It can be assumed that the genetic backround of today´s populations in the Czech Republic is a mixture of glacial residents and postglacial settlers that derived from unknown but non-Mediterranean refugia. Hence, intraspecific pattern of variation will be obviously different from that proposed by K r a t o c h v í l (1959).

We can conclude that the results obtained do not fit well with the model of subspecific differentiation of Czech common vole and with the traditional perception of the postglacial recolonization of central Europe. It is clear that further analysis would be desirable to confirm the pattern revealed. In particular, molecular markers would be valuable to elucidate genetic variation and history of *Microtus arvalis* in central Europe.

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