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Craniometrical Differentiation of Population of The Flat-headed Vole in North Kazakhstan and South-east Altai

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In this paper we analyzed the morphological differences between populations of the flat-headed vole inhabiting Kazakh Upland and South-east Altai inclusive of the age variation. As used in this paper a comprehensive phenotypic analysis using the methods of multivariate statistics allows detecting steady set of quantitative traits that characterize the conjugate development at different stages of ontogeny. Voles of these 2 populations, despite such contrasting habitat conditions are similar in overall size of the skull. It was found that the voles from Kazakhstan were significantly different from all the others, and the differences between the known Altai populations do not reach the level of subspecies. Our results about the differences of adults are generally consistent and complement the results of previous studies. The main differences between the voles from Kazakhstan and Altai relate to the width of the interorbital space, height of the skull and length of the diastema. No significant differences in the zygomatic width between samples from Kazakhstan and South-east Altai found.

Key words: Flat-headed vole, morphological variability, craniometrical material, craniometrical variability, discriminant analysis, Bayanaul and Altai populations of flat-headed voles.

In recent years, due to the intensive use of molecular biological methods, the number of papers aimed at solving different problems of taxonomy and philogeography has increased. However, clarification or revision of the status of one or another taxon (species, subspecies), nonetheless does not eliminate the problems of morphological differentiation of groups of animals of different taxonomic rank.

Flat-headed vole is common in the mountains of Central and Eastern Kazakhstan, Altai, Mongolian Altai, and in the southwestern Tuva and northwestern Mongolia, comes in North-west China[1]. Rodent bent to dry rocky mountains everywhere and inhabits them, starting from a height of 300 m in the west of the Kazakh highlands up to 2900 m in the South-east Altai and Mongolia. Almost everywhere within the habitat the climate is sharply continental, dry, winter with little snow. Mountain-steppe, or, less commonly, semi-desert vegetation prevails.

Intraspecific taxonomy and geographical variability of the flat-headed vole are poorly studied, which is partly because of the limited area

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and inaccessibility of animals habitats. Research of morphological variability of the flat-headed vole is usually aimed at studying ambiguities questions of the voles' taxonomy[1]. They analyzed only adults. At the same time the issues related to the age variability remain unexplored. Understanding that the phenotypic responses are based on developmental processes[2-7] shifts the emphasis in the study of morphological variability in the direction of the study of population's morphogenetic specifics.

Materials and techniques

Craniometrical material for this study was collected near the village Bayanaul of Pavlodar region of the Republic of Kazakhstan in May - September 2008–2009, July – September 2014 and in the Kosh-Agach district of the Altai Republic of the Russian Federation (sample Saylyugem) in July-August 1990, July 2010 and August 2013 (Fig. 1). For comparison, in addition was used craniometrical material from the Central Altai from collection of ISEA SB RAS.

For the statistical analysis we used 19 dimensional features of the skull: basal length (length from occipital condyle to the most exserting part of the skull over incisors) (BLng), the front length of the skull (SFLng), the length of the upper diastema (UDLng), the length of the upper row of teeth (URTLng), the width of the rostrum (RostrWd), zygomatic width (ZygomWd), the width of the interorbital space (IntorbspacWd), the width of the skull in area of the auditory bullas (SAABWd), the height of the rostrum (RostrHt), the height of the hard palate before the first upper molars (HPBFUMHt), the height of the skull from the third top molars (SH M3), the height of the skull in the area of the auditory bullas (SAABHt), the length of the auditory bullas (ABHt), width of the auditory bullas (ABWd), the length of the lower diastema (LDLng), the length of the lower row of teeth (LRTLng), the length of the mandibula from the alveoli of the incisors to the zygapophysis (MFAIZLng) the length of the mandibula from the alveoli of the incisors to the angular appendices (MFAIAALng), the width of the ramus of the mandibular bone (RMDWd) [1].

Preliminary analysis of the importance of variability factors is made by multivariate analysis of variance (MANOVA). The bootsrap method was used for a statistical significance test of differences between samples.

To assess the differences in the overall sizes of the skull between the samples, the geometric mean of all the measurements of the skull of the specimen[8] was used. We tested values of group geometric means for normality using the Anderson-Darling test. Geometric means of all geographic samples follow a normal distribution ($p > 0.05$). Since the variances of group geometric means were equal according to Levene's F-test ($p > 0,05$), the one-way ANOVA together with the Tukey HSD post-hoc test for unequal sample sizes were used to compare geometric means.

The variability of the morphometric measurements was examined using principal component analysis[9] and canonical discriminant function analysis[10]. The principal components PC and discriminant functions DF were interpreted using the correlation between the morphometric variables and principal components or discriminant functions. Scatterplot of scores for all individuals for the first two principal components and canonical discriminant functions were produced to visualise the extent of differentiation between groups.

All calculations were performed using the statistical package Statistica for Windows, version 6.0 [11] and the program PAST (Paleontological Statistics) ver. 3.01 [12].

RESULTS

Preliminary assessment of the significance of the main factors of variation performed using analysis of variance (Table 1). Analysis of variance of three factors (sex, age, and region) revealed significant differences between age groups and geographical samples, but found no significant sex differences. Previous studies related to the variability of the species *Alticola olchonensis* on a complex 17 craniometrical features also revealed further of sexual differences[13]. Therefore, the significant analysis used samples combined by the sex.

The first principal component in the analysis of samples of different ages can be interpreted as the size and age[14-16]. All the species are located along the first component according to the size of the skull from the youngest to the adult animals. In this case, loadings of various morphometric characters on the first component (correlation coefficients) reflect their variability in the growth process. Features with more pronounced linear growth, such as basal length, the length of the front of the skull, the length of the mandible and zygomatic width have largest connection with the component.

Comparison of loadings (coefficients of the features correlation with the component) on component 1 for the different age samples of voles from Kazakhstan and South-east Altai has shown (Fig. 2), as expected, concordance of variation of most features of the skull with age. Differences between voles from Kazakhstan and South-east Altai relate to the variation of such features as the interorbital width of the skull, the width of the skull in the auditory bullas, as well as the height of the skull in the auditory bullas and the third upper molar (M^3).

For the first two (out of 19 received) principal components of craniometrical variability at the joint analysis of mixed-age samples of flat-headed voles from Kazakhstan and South-east Altai accounted 68.9% (56.3% and 12.6%, respectively) of the total variance. Most features have large positive coefficients of correlation with component 1 (see Table). The largest connection with the component belong to the features with more pronounced linear growth during ontogenesis, such as basal length, the length of the front of the skull, the length of the mandible and zygomatic width. This allows us to interpret the first component as the size and age, reflecting the linear growth of the skull with age[14,15].

Some individuals within the geographic samples are arranged according to size and age characteristics: adults correspond to the highest values of the first component. Herewith, samples of voles from Bayanaul and South-east Altai on component 1 completely overlap (Fig. 3), indicating the similar nature of growth of different parts of

Table 1. Source of variation for a tree-way MANOVA (sex*age*region) of morphometric skull measurements of flat-headed vole

.	$\Lambda_{19,\ 161}$	$F_{19,\ 161}$	Significance *(P)*
sex	0.85	1.4	0.129
age	0.18	36.8	< 0.000001
region	0.35	15.1	< 0.000001
sex*age	0.91	0.7	0.768
sex*region	0.86	1.3	0.210
age*region	0.71	3.5	< 0.00001
sex*age*region	0.94	0.5	0.966

Table 2. The correlation coefficients of morphometric characters with the principal components

	PC 1	PC 2
BLng	0.96	-0.059
SFLng	0.96	0.043
UDLng	0.90	0.213
URTLng	0.77	-0.22
RostrWd	0.78	-0.13
ZygomWd	0.91	-0.18
IntorbspacWd	0.19	-0.74
SAABWd	0.19	-0.35
RostrHt	0.87	-0.06
HPBFUMHt	0.79	-0.05
SH M^3	0.41	0.65
SAABHt	0.21	0.66
ABHt	0.78	-0.33
ABWd	0.77	-0.36
LDLng	0.49	0.62
LRTLng	0.75	0.10
MFAIZLng	0.94	0.10
MFAIAALng	0.92	0.09
RMDWd	0.71	0.18

Table 3. The correlation coefficients of morphometric characters with the discriminant functions

Features	DF 1	DF 2
BLng	-0.02	0.77
SFLng	-0.28	0.57
UDLng	-0.61	0.41
URTLng	0.03	0.65
RostrWd	-0.34	0.37
ZygomWd	0.31	0.68
IntorbspacWd	0.68	0.33
SAABWd	0.08	0.43
RostrHt	-0.18	0.46
HPBFUMHt	-0.22	0.44
SH M^3	-0.73	0.30
SAABHt	-0.65	0.51
ABHt	0.26	0.36
ABWd	0.26	0.44
LDLng	-0.29	-0.14
LRTLng	-0.45	0.36
MFAIZLng	-0.32	0.61
MFAIAALng	-0.46	0.50
RMDWd	-0.25	0.09

the skull with age in two vole populations.

The characters with essentially nonlinear growth, such as interorbital width, height and width of the skull in the auditory bullas are not connected with the 1 principal component (low values of the correlation coefficients) (Table 2). The variability of these characters, as well as the height of the skull in the M^3 area and the length of the lower diastema form the second principal component. The loading of interorbital width and other characters have a different sign, which indicates oppositely-directional variability of these features.

Compliance of direction of component 1 of craniometrical variability of age-mixed sample with component 1 obtained separately for samples from Kazakhstan (r = 0.93, P <0.001) and South-east Altai (r = 0.93, P <0.001) confirm the high correlation coefficients between them.

Two geographic samples significantly differ from each other (bootstrap t = 16.3, p <0.001) in the direction of second principal component. At that, according to the loadings of morphometric variables voles living in the Kazakh upland have a relatively large interorbital width and relatively smaller height of the skull and shorter diastema.

When considering the mixed age samples, component 1 can be regarded as an integral size characteristic. Study of temporal variation of the first size-related component may be useful to assess the differences in the growth rate of individuals across different geographic samples[14,15].

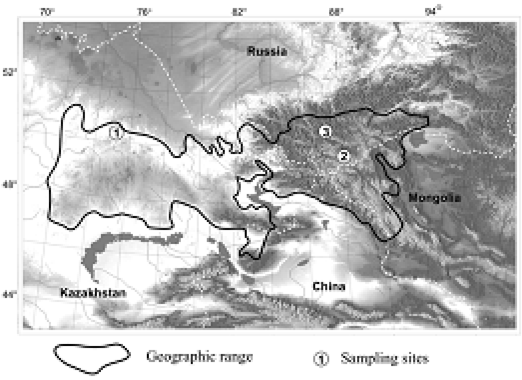

Fig. 1. Range and location of the studied samples of the flat-headed vole. 1 - Bayanaul, 2 - South-east Altai, 3 - Central Altai

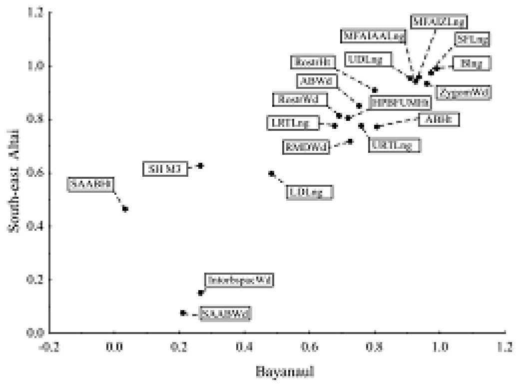

Fig. 2. Correlation coefficients of the morphometric characters with the first principal component for the mixed age samples of voles from Bayanaul and South-east Altai

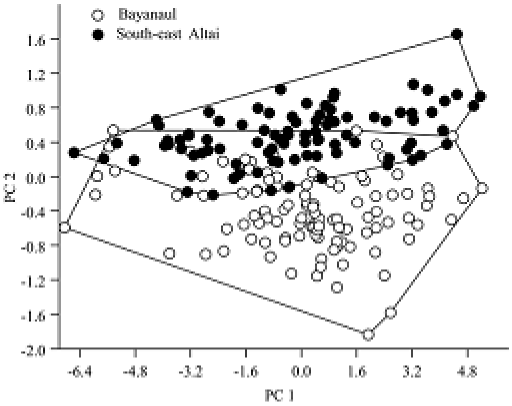

Fig. 3. Distribution of mixed age samples of the flat-headed vole in the space of the principal components

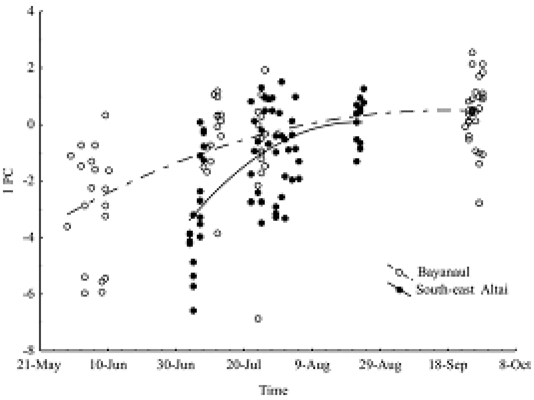

Fig. 4. Distribution of values of the first principal component of underyearlings of the flat-headed voles relative to the time of catching

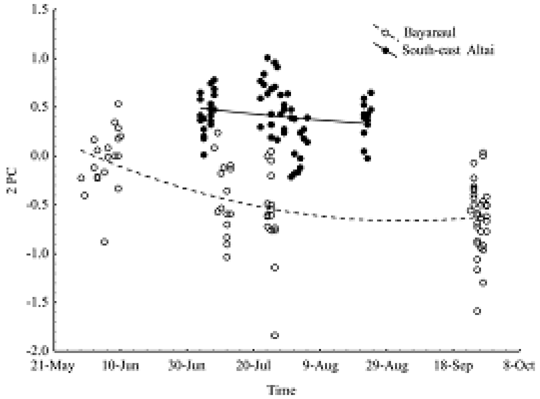

Fig 5. Distribution of values of the 2 principal component of underyearlings of the flat-headed voles relative to the time of catching

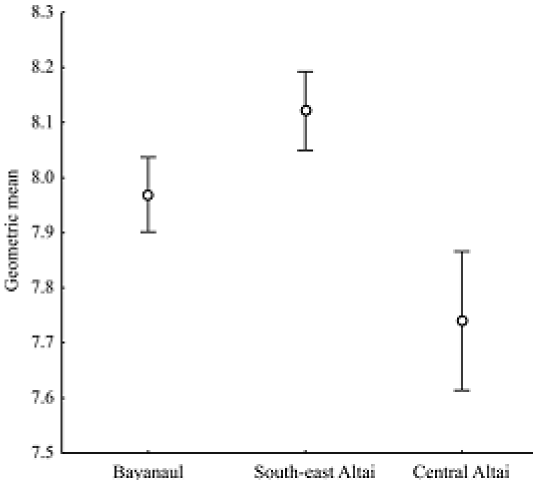

Fig. 6. Differences in the overall size of the skull between the populations of the flat-headed vole. Vertical bars denote 0.95 confidence intervals

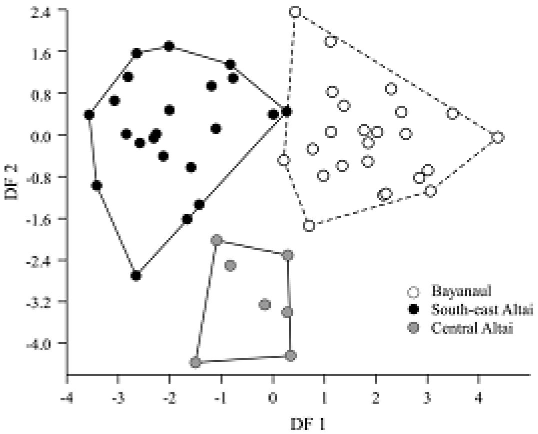

Fig. 7. Distribution of geographic samples of the flat-headed voles in the space of discriminant functions

Figure 4 shows the distribution of the value of component 1 from the previous analysis for underyearlings of both geographic samples relative to the date of catching. It is clearly seen that in June underyearlings of Saylyugem populations are much smaller than the size of the skull than grown up underyearlings of Bayanaul population ($P < 0.001$). However, in the second half of July, these differences are significantly reduced, and in the fall disappear at all. No significant differences on component 1 between Altai underyearlings caught in late August, and underyearlings of Bayanaul population caught in the second half of September were found ($P > 0.05$).

On component 2 that characterize the shape of the skull, differences between underyearlings are expressed during all months (Figure 5). Moreover, these differences are smaller between voles in the first month of life.

According to one-way ANOVA, adult voles of the three geographical populations significantly differ in overall size of the skull (ANOVA: $F_{2,50}=14.9, P < 0.001$) (Fig. 6). Voles of the Central Altai ($P < 0.001$) differ by the smallest overall size of the skull among the analyzed populations. Voles from Kazakhstan and South-east Altai are close in size of the skull, but differences in the geometric mean of all measurements between them highly significant ($P < 0.001$). Voles of the South-east Altai are characterized by the largest skull.

According to the result of canonical discriminant function analysis of three geographic samples of mature animals, 1 discriminant function ($\chi^2 = 89.8$, df = 38, $P < 0.001$, 73.3% of general between-group variance) mainly describes the differences between the samples from Kazakhstan and South-east Altai, which are only slightly overlap in the space of discriminant functions (Figure 7). The Central Altai sample takes the intermediate position between them in the direction of the 1 principal component. According to the correlation coefficients of the morphometric characters with discriminant functions (Table 3), the main differences on component 1 relate to upper diastema length, the height of the skull in the auditory bullas and the third molar (M^3), and interorbital width of the correlation coefficient, which has the opposite sign.

The second discriminant function ($\chi^2 = 32.37$, df = 18, $P < 0.05$, 26.7% of the total intergroup variance) describes the differences of voles from the Central Altai from voles of the South-east Altai and Kazakhstan. Almost all the features have positive correlation coefficients with the second discriminant axis. The largest of them: basal length, the length of the front part, zygomatic width and lower jaw length.

Thus, according to the location of samples in the space of discriminant functions, voles from Kazakhstan differ from voles in South-east and Central Altai by greater width of the interorbital space, less height of the upper diastema, less height of the skull in the auditory bullas and M^3. In addition, voles from Kazakhstan and South-east Altai differ from voles from Central Altai by a larger skull, especially larger basal length and larger length of the front part of the skull and a larger width in the area of the zygomatic archs.

CONCLUSION

Flat-headed vole was first described in 1899 by Kashchenko N.F., professor at Tomsk University from the vicinity of the lake Tenga, Central Altai. Also in 1901 he described the subspecies *Alticola s. desertorum* from Kazakh uplands. The features distinguishing it from the nominative subspecies include more light-colored body and wide-set sygomatic archs. The third subspecies, *Alticola s. depressus*, was described from Chu steppe of South-east Altai by Ognev S.I. (1950) and was diagnosed by narrower interorbital space and bend of the upper line of the skull in the area of eye-pits. Some researchers[1] did not recognize the subspecies *Alticola s. desertorum*, considering it as synonym of the nominative.

The study of the variability of the fragment of the mitochondrial gene cytochrome *b* of the flat-headed vole *Alticola strelzowi* from different parts of the range showed that the species as a whole is characterized by low degree of genetic variability. Was confirmed the separation of the flat-headed vole species into two subspecies - *A. s. strelzowi* and *A. s. desertorum*. Herewith, the level of genetic polymorphism was higher in the latter subspecies. Voles, living on the territory of Kazakh uplands belong to the subspecies *Alticola s. desertorum*, while voles from South-east and Central Altai to the nominative subspecies *Alticola s.strelzowi*[17].

Habitat conditions of Bayanaul and Altai populations of voles differ significantly. Thus, voles' habitats in the territory of Kazakh uplands located at an altitude of about 460 meters above sea level. The annual average temperature here is 3.5°C. The average temperature in January is -13.7°C, minimum -48°C. The average temperature in July is 14.6°C, maximum up to 32.6°C. The average duration of the frost-free period is 98 days. The annual rainfall is 340 mm with variations in some years from 190 to 494 mm. Snow cover melts at the end of March - beginning of April[18,19].

A much more severe climatic conditions observed in the habitats of the flat-headed vole in the South-east Altai[20,21], who were caught in the valleys of rivers Chagan-Burgazy, Bayan Chagan and Ulandryk at altitudes of 2200–2500 meters above sea level. The annual average temperature here is -6.6°C. The average temperature in January is -22.6°C, minimum -62°C. Negative temperatures hold up during the whole spring and autumn, severe frosts occur in summer as well. The average temperature in July is 11.1°C. The frost-free period is only 35-60 days. The amount of precipitation does not exceed 250-300 mm per year. Prolonged freezing period (until May) in the absence of snow cover on the ridge Saylyugem defines significantly later beginning of the flat-headed vole's breeding season in the highlands.

Flat-headed vole's pregnancy period lasts 20 days. Young animals start to run out of the nest and feed themselves at the age of 16-20 days, while achieving weight of 13-18 grams. The interval between the end of the first and beginning of the second pregnancy can be minimum (1 day), or up to 10 days or more. In the period from 29 May to 9 June, in Bayanaul population all caught females had placental spots from two litters in the uterus. Second litter cubs had a weight of 13-18 grams, which corresponds to the age of 16-20 days. Simple calculations show that the second litter born in the period from 10 to 20 May, and the first - in the second half of April (subject to a minimum interval between pregnancies). Thus, the beginning of the breeding season in Bayanaul falls at the end of March - beginning of April, which coincides with the snow cover melting and the start of the growing season.

In the high altitude part of South-east Altai on the Ridge Saylyugem, on 4-5 July all caught females, except one, *were at various stages of pregnancy of the second litter*. We caught young animals (underyearlings) of the second litter since 22 July. It is easy to calculate that the beginning of the breeding in the highlands of South-east Altai begins almost two months later, that is, in late May - early June.

As shown by the analysis, voles of these 2 populations despite such contrasting habitat conditions are similar in overall size of the skull. Earlier in his paper Pozdnyakov et al[22] analyzed ecological and morphological features of the Altai populations compared with voles from Kazakhstan and Tuva[22]. It was found that samples from Kazakhstan significantly differ from all the others, and the differences between the known Altai populations do not reach the level of subspecies. Our results on the differences of adult voles are generally consistent and complement the results of previous studies. The main differences between the voles from Kazakhstan and Altai relate to the width of the interorbital space, height of the skull and length of diastema. We have not found any significant differences in the zygomatic width between samples from Kazakhstan and South-east Altai, as previously reported[22,23].

Detectable size differences between the underyearlings of two populations are mainly due to different time of the beginning of breeding period. However, these differences cancel out by the fall, when their growth is slowing in both populations. Inter-population differences in the skull shape are revealed between individuals of any age. The greatest differences relate to the features having taxonomic significance. These differences keep during the entire ontogeny and are not dependent on the age of individuals.

Thus, voles of Bayanaul and Saylyugem populations having similar skull size differe significantly in shape of the skull. Thus, the greatest differences between the populations relate to the characteristics distinguishing the different Alticola subspecies. More severe natural conditions of Altai high mountains resulted in a significant delay in the breeding period of Altai population.

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