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Original Investigation

Ontogenetic variation in occlusal shape of evergrowing molars in voles: An intravital study in *Microtus gregalis* (Arvicolinae, Rodentia)

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ABSTRACT

In order to reveal patterns of ontogenetic change in occlusal shape of evergrowing molars in arvicolines, an intravital tooth printing method was applied to 20 individuals of *Microtus gregalis* born in captivity. Complexity patterns of the first lower molar were assessed by morphotype analysis of the anteroconid complex. Morphotype dental patterns were monitored using tooth prints at 0.5, 1, 2, 3 months of age, and postmortem. Ontogenetic changes in molar complexity and bilateral symmetry among right and left molars of the same individual during the process of tooth wear were assessed. Our results suggested that morphotype dental patterns could not be clearly established in half-month old animals due to presence of juvenile characters. For animals of 1 month and older, age differences in morphotype dental patterns were non-significant and negligible compared to among-individual variation. Within-individual differences among right and left molars, when present, were not related to age of an animal suggesting that bilateral asymmetry of morphotype dental pattern could be regarded as inherent characteristic of an individual persisting during post-juvenile tooth wear.

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Introduction

Molar teeth of arvicoline rodents represent a traditional model to address taxonomic, phylogenetic, evolutionary, and ecological issues (e.g., Hinton, 1926; Zimmermann, 1958; Guthrie, 1965, 1971; Gromov and Polyakov, 1977; Bolshakov et al., 1980; Koenigswald, 1982; Chaline and Graf, 1988; Barnosky, 1990; Agadjanian, 1996; Maul et al., 1998; Chaline et al., 1999; Borodin, 2009; Vasilyev and Vasilyeva, 2009; Guérécheau et al., 2010; Ledevin et al., 2010; McGuire, 2010; Piras et al., 2010). Despite of wide use of dental characters in evolutionary and ecological studies in arvicolines, relatively little attention is paid to the problem of ontogenetic variation in dental characters and its effect in studies that rely upon tooth morphology. Although the age variation in dental characters is well-known in arvicoline rodents, there are only a few studies examining the effect of age when undertaking correlation analyses between tooth morphology and spatial, temporal and environmental variables (Fominykh et al., 2010; Guérécheau et al., 2010). Those studies have been done on the species with rooted molars for which the age of an individual could be determined based on the stages of root development (Koshkina, 1955; Tupikova et al., 1970; Pankakoski, 1980; Perrin, 1988;

Evdokimov, 1997; Olenev, 2009). In rodents with evergrowing (hypselodont) teeth the crown grows permanently that makes it impossible to establish age classes based on root-crown ratios. Most of the studies that address ontogenetic variation in evergrowing molars in voles (Oppenheimer, 1965; Kaneko, 1996; Sözen et al., 1999; Markova, 2006), or provide short descriptions of juvenile and post-juvenile occlusal enamel patterns of hypselodont molars (Ognev, 1950; Nadachowski, 1982; Borodin, 2009) are based on samples of individuals classified by age so that between-individual variability is not taken into account. Only a few studies addressing ontogenetic variation in occlusal outlines within the same individual are known for hypselodont rodents. In particular, considerable changes in size and depth of re-entrant angles of the occlusal surface were described in *Lemmus trimucronatus* (Cheprakov, 1983, 2010), *Microtus middendorffi* (Kourova, 1985), and *M. agrestis* (Kourova, 1986).

There are numerous evidences of age variation in occlusal outlines in voles with evergrowing molars. First, the salient angles and re-entrant folds have a very different general appearance in young teeth from that which characterizes them in the teeth of adult and senile animals (Hinton, 1926; Nadachowski, 1982; Kourova, 1985, 1986; Sözen et al., 1999): in young teeth the salient angles are relatively narrow (acute), the re-entrant folds are wide and open, but in later stages of wear the salient angles gradually become less acute and the re-entrants separating the prisms become relatively narrow. Second, there are

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Table 1
Individuals of *Microtus gregalis* used for analysis of ontogenetic variation in occlusal shape of the first lower molar, occlusal complexity ratings at 1 month of age and morphotypes of the left (L) and right (R) molars. Id – number of the specimen in the zoological collection of IPAE UB RAS.

Maternal line (female Id)	Litter	Id	Intravital toothprinting			Postmortem observations		
			Number of sessions	Complexity rating at 1 month ^a		Age, months	m1 morphotype	
				L	R		L	R
I (2)	1	7	3	3.0±0.00	1.7±0.33	4	3	3
	1	8	3	2.3±0.36	3.7±0.33	6	3	4
	2	18	3	2.0	2.0	10	1	2
	2	21	3	2.0	1.0	3	2	1
	2	23	3	2.3±0.33	1.0	10	2	1
	2	24	4	2.0	2.0	4	2	2
	2	25	3	2.0	2.0	13	2	2
II (4)	1	11	3	2.0	2.0	6	2	1
	1	12	3	3.0	3.0	4	3	3
	2	36	3	2.0	2.0	12	2	2
	2	37	3	2.3±0.33	2.3±0.36	12	2	2
	2	39	3	3.0	3.0	12	2	2
	2	40	3	1.3±0.33	1.0	8	2	2
	2	46	3	2.3±0.33	3.0	12	3	3
III (6)	1	26	4	2.3±0.33	3.0	12	3	3
	1	29	1	–	–	0.5	juv	juv
	1	30	3	3.0	3.0	5	3	3
	1	32	4	3.0	3.0	6	3	3
	1	34	1	–	–	0.5	juv	juv
	2	42	4	2.0	2.0	12	3	3
	2	45	4	3.0	3.0	7	3	3

^a The average of 3 repeated prints (mean ± standard error); standard error is not calculated when there is no variation among repeated prints.

evidences for the association between age and certain size and shape characters of the anterior cap of m1 and/or posterior lobe of M3 in *Microtus arvalis*, *M. rossiaemeridionalis* (Markova, 2006), *M. guentheri* (Sözen et al., 1999), *Eothenomys smithii*, *E. andersoni*, and *E. shanseius* (Kaneko, 1996). In general, the area of the anterior cap of m1 and posterior lobe of M3 increases with advancing age.

However, the question about the amount of within-individual age-dependent variations compared to variation between individuals still remains unaddressed. Another source of within-individual variation in dental pattern is bilateral asymmetry. It is well-known for arvicoline rodents that occlusal shape patterns are not always identical in right and left molars of the same individual (Yeremina, 1974; Maleeva, 1982; Zejda et al., 1997; Kovaleva et al., 2002; Pozdnyakov, 2007; Polly et al., 2011). Although the age-related variability and bilateral asymmetry are well-known, there are no studies that estimate the amounts of age variation and bilateral variation in molar shape as compared to the amount of between-individual variation. Does the variation due to age or asymmetry bias the results of morphological studies based on molar shape in hypselodont arvicolines? To answer this question, we undertake a case study in *Microtus gregalis*, a vole species with hypselodont molars. As in all arvicolines, occlusal outlines of the molars in *Microtus gregalis* differ in complexity due to development of additional salient and re-entrant angles between the prisms of the crown (e.g., Borodin, 2009).

Within the variety of techniques for comparative morphological analyses of occlusal outlines in arvicolines (Van der Meulen, 1973; Bolshakov et al., 1980; Nadachowski, 1982; Kochev, 1983; Schmidt-Kittler, 1986; Ruiz Bustos, 1996; Hurth et al., 2004; Navarro et al., 2004; Polly et al., 2011, etc.), the simplest is the morphotype-based analysis relied on visual inspection of molar outline pattern. Traditional morphotype approach to analysis of vole dentition takes into account the complexity of the occlusal surface, in particular, the development of additional salient and/or re-entrant angles (e.g., Rörig and Börner, 1905; Angermann, 1973; Bolshakov et al., 1980; Contoli et al., 1992; Markova et al., 2010; Pozdnyakov, 2011). Although this method provides only a rough

estimate of morphological variability, it is a useful tool when one needs to compare data between studies or to combine large data sets in order to reveal general patterns of spatial or temporal variation in arvicolines (Nadachowski, 1982; Smirnov et al., 1986; Smirnov and Fedorov, 2003; Markova et al., 2010). At the same time, it has been shown that morphotype dental patterns in arvicoline voles are heritable and could be regarded as Mendelian traits exhibiting incomplete dominance (Zimmermann, 1935; Angermann, 1973; Stohl, 1984). Although there is still some debate over the applicability of the morphotype approach to analysis of vole dentition because of the continuous pattern of variability between the morphotypes (Ledevin et al., 2010), the numerous studies have shown that this approach takes proper account of the general trend toward increasing crown complexity in the evolution of Arvicolinae (Guthrie 1971; Bolshakov et al., 1980; Smirnov et al., 1986; Chaline and Graf, 1988; Chaline et al., 1999).

In this study, we investigate patterns of ontogenetic variability in occlusal shape of the first lower molar in *Microtus gregalis* using intravital technique of toothprinting. We aim to answer the two questions: 1. Does the occlusal complexity change with wear in evergrowing molars (as assessed by determining morphotype dental patterns)? 2. How are the two sources of within-individual variation – ontogenetic change and bilateral asymmetry – of morphotype dental patterns interrelated when studied in postnatal ontogeny of the same individual?

Material and methods

Material

A total of 20 individuals of narrow-skulled vole *Microtus gregalis* Pall., 1779 from 3 maternal lines were studied (Table 1). Maternal females belonged to the subspecies *M. g. gregalis*, and were trapped in the wild in vicinities of Talitsa settlement (Middle Urals, Russia). For each maternal female, the two F1 litters were available for analysis. All the materials studied were registered as part of the zoological collection housed in the Institute of Plant and Animal

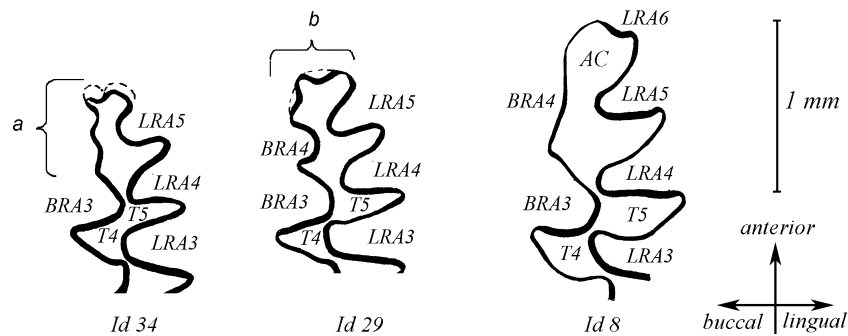


Fig. 1. Anteroconid complexes of the first lower molars of *Microtus gregalis* showing the patterns of occlusal surface in juvenile (Id 29, 34) and adult (Id 8) animals. Id 29, 34 – left molars showing bucco-anterior (a) and anterior (b) patterns of juvenile folding, Id 8 – right molar showing a completely formed outline of the anteroconid, mirrored image; dotted outlines indicate ephemeral peculiarities in the form of the anterior cap of juvenile individuals. Identification numbers and age of individuals are given in Table 1. LRA – lingual re-entrant angles, BRA – buccal re-entrant angles, AC – anterior cap, T – triangular loops of the occlusal surface (designated and numbered according to the scheme of Van der Meulen (1973)).

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Tooth-printing

A method of intravital study of dental morphology in voles (Olenev, 1980) was used to obtain occlusal surface prints of the first lower molars (m1). Tooth prints were taken under conditions of hexenal-induced sleep (4 ml per kg bodyweight). The slices of mastic were used to obtain the prints from right and left tooth rows separately.

Tooth-printing sessions were undertaken at fixed age for each individual (1, 2, and 3 months). For several individuals, tooth printing was also done at 14–16 days, so that the total of 4 printing sessions was taken (Table 1). At least 3 repeated prints were obtained from the same tooth row during one printing session. The tooth prints were checked for imperfections such as blur, contour displacement or bias. In case of imperfect outline contour, additional toothprints were obtained in order to have 3 prints per tooth during one printing session.

Occlusal outlines in slices of mastic were inspected under magnification 2.5×. For each individual, after-death molar shape inspection was also undertaken. The experiment lasted from the birth until the death of an individual. The age at death varied among individuals (Table 1).

Dental variability

In this study, we focused on qualitative traits corresponding to development of additional salient and re-entrant angles on lingual or/buccal side of the anteroconid portion of the first lower

molar (Figs. 1 and 2). Morphotype dental patterns were established based on anteroconid complexity taking into account the presence or the absence of the three elements – BRA4, LRA6 and T6 (Fig. 2). Although the development of these structures could be described both qualitatively and quantitatively, we chose to focus on morphotypes based on additional elements because they reflect both the phylogenetic trend toward increasing crown complexity in arvicolines (Guthrie 1971; Chaline and Graf, 1988) and the iterative addition of cusps after the establishment of lateral cusp topography during the development of arvicoline molars (Jernvall, 2000; Jernvall et al., 2000).

Morphotypes 1–4 (Fig. 2) are ranged from the most simple to the most complex as follows: 1 – BRA4 is absent, T6 is absent, LRA6 is absent; 2 – shallow BRA4 could be distinguished being no deeper than or equal to the width of enamel band, T6 is not yet distinguishable, LRA6 is absent; 3 – BRA4 is well-developed, its depth exceeds the width of enamel band, T6 is clearly distinguishable, LRA6 is absent; 4 – BRA4 is well-developed, T6 is well-developed, LRA6 is present. The order of morphotypes is natural because it reflects the order of appearance of the qualitative traits in evolution of *Microtus gregalis*: morphotypes 1–3 represent the transition from “gregaloid” to “microtid” stage of m1 evolution in the species (Bolshakov et al., 1980; Nadachowski, 1982; Smirnov et al., 1986), whereas the morphotype 4 is more advanced (the additional re-entrant angle LRA6 is only found in m1s with clearly distinguishable BRA4 and T6).

The morphotype numbers represent complexity ratings from 1 to 4. For further analysis, we consider the complexity ratings as equally spaced variables because the morphotypes differ from one another by presence/absence of one additional element of occlusal surface (a salient or re-entrant angle).

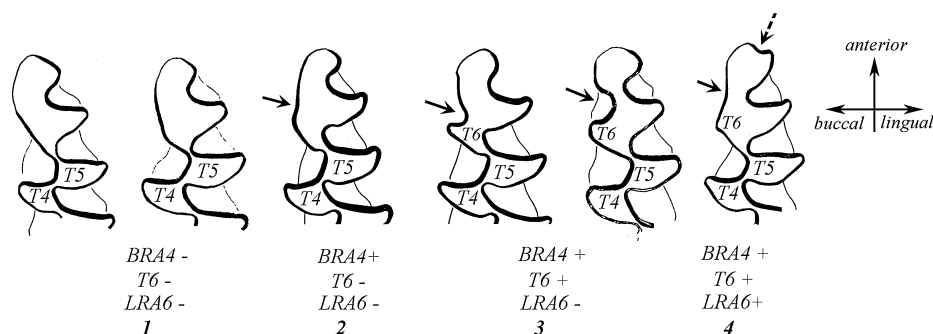


Fig. 2. Scheme of the morphotype dental patterns established in the study based on anterior cap complexity of the first lower molar. Morphotypes are shown regardless of left or right position (right molars are mirrored). 1–4 – complexity ratings of the morphotypes. Solid arrow shows position of buccal re-entrant angle BRA4, dashed arrow shows position of lingual re-entrant angle LRA6.

Table 2
Results of Model I ANOVA comparing the variation in dental complexity due to among-individual differences (*individual*), between-side differences (*side*), among post-juvenile ontogenetic stages (*age*), and due to interaction between explanatory variables. See text for abbreviations.

	Source of variation	SS	DF	MS	F	p
Main effects	<i>Individual</i>	84.44	17	4.97	40.24	0.000
	<i>Age</i>	0.31	3	0.10	0.83	0.479
	<i>Side</i>	0.30	1	0.30	2.45	0.119
Interactions	<i>Individual</i> × <i>age</i>	11.43	51	0.22	1.81	0.002
	<i>Individual</i> × <i>side</i>	18.92	17	1.11	9.01	0.000
	<i>Age</i> × <i>side</i>	0.08	3	0.03	0.22	0.882
	<i>Individual</i> × <i>side</i> × <i>age</i>	6.12	51	0.12	0.97	0.533
Error	<i>Replicates</i>	26.67	216	0.12		

Two sources of within-individual variability were considered – age variability due to growth and wear, and bilateral asymmetry of morphotype dental patterns widely known in *Microtus* species (Yeremina, 1974; Pozdnyakov, 2007).

Dental terminology for this study was adapted from Van der Meulen (1973).

Statistical analysis

Analysis was aimed at testing for the presence of systematic within-individual ontogenetic and bilateral variation in occlusal complexity of the first lower molar, and comparing this variation to the magnitude of among-individual variation.

Sources and patterns of variation in morphotype dental patterns were assessed in the post-juvenile dataset including tooth prints obtained at 1, 2, 3 months of age, and postmortem observations. The dataset for statistical analysis included 18 individuals, for which the three post-juvenile tooth-printing sessions were available for analysis (Table 1).

We used model I ANOVA (Sokal and Rohlf, 1994) to examine the variation in dental complexity that was due to among-individual differences (*individual*, *I*), between-side differences (*side*, *S*), and due to differences among ontogenetic stages related to the age when the printing sessions were undertaken (*age*, *A*) by treating *individual*, *side* and *age* as main effects in a factorial design. The first-order ($I \times S$, $I \times A$, $S \times A$) and second-order ($I \times S \times A$) interaction terms were tested for any individual- or side-related differences in the patterns of ontogenetic variation. Tooth prints obtained from the same tooth row during one printing session were the replicates in this study.

For statistical analyses, a criterion of $\alpha = 0.05$ was used to determine significance. Statistical analysis relied on programs included in STATISTICA 5.5 package (StatSoft Inc, 2001).

The following abbreviations were used: R^2 – coefficient of determination, SS – sum of squares, MS – mean square, *F* – Fisher's *F* statistic, *df* – degrees of freedom, *p* – probability (Sokal and Rohlf, 1994); Id – identification number of the specimen in the zoological collection IPAE UB RAS.

Results

Investigation of occlusal outlines in slices of mastic taken during the first printing session (0.5 month of age) showed that the anteroconid complex of m1 was not yet fully developed exhibiting so-called juvenile folding in the enamel of the anterior cap (Fig. 1). The two different juvenile patterns could be determined: bucco-anterior (Fig. 1, Id 34), and anterior folding (Fig. 1, Id 29). Analysis of the prints taken at the first printing session (0.5 months) showed that one individual (Id 24) exhibited the bucco-anterior pattern of juvenile folding, and four individuals (Id 26, 32, 42, 45) exhibited the anterior folding. Comparison of the prints taken at the juvenile stage with the postmortem observations showed that

the bucco-anterior pattern of juvenile folding of the individual 24 had resulted in the morphotype 2, and the anterior folding pattern had resulted in the morphotype 3 in individuals 26, 32, 42, 45 (Table 1). This result indicates that both the anterior and bucco-anterior patterns of juvenile folding represent the ephemeral peculiarities of the occlusal outline.

The next stage of molar development (1 month of age) is characterized by complete development of BRA4 and LRA6 (when present), and by well-defined morphotype dental pattern. However, the two individuals at 1 month of age (Id 24, Id 32) had retained immature characteristics of their anterior caps, which look slightly waved in shape. That immature trait disappears with age (it is not found in tooth prints of the same molars at 2 months of age and older).

ANOVA results for the post-juvenile dataset are shown in Table 2. The effects included in the model I ANOVA explained substantial amount of variation in dental complexity, as indicated by adjusted $R^2 = 0.75$. The effects of *age* and *side* are not significant, whereas the variation among individuals is highly significant. Statistically significant interactions were found for *individual* and *age*, and also for *individual* and *side*.

Significant interaction *individual* × *side* could be regarded as evidence of non-directional asymmetry (Palmer, 1994). To test for genotype differences over the amount of non-directional asymmetry, *F* ratio could be calculated as $MS_{\text{individual}}/MS_{\text{side} \times \text{individual}}$ (Palmer, 1994). Here, variation among individuals (genotypes) is significant given the amount of non-directional asymmetry ($F_{17;17} = 4.46$, $p = 0.002$). Significant interaction *individual* × *side* suggests that the bilateral pattern of dental variability varies among individuals studied.

Significant interaction *individual* × *age* (along with no significant effect of *age*) suggests that the ontogenetic pattern of dental variability varies among individuals studied. Second-order interaction diagrams (Fig. 3) show that three individuals (Id 24, 39, 45; Table 1) exhibited no morphotype changes in both left and right molars at post-juvenile stages. Nine individuals showed no age-related variability either on the left or right molar with certain changes in the morphotype dental pattern on the opposite side (Id 7, 11, 12, 18, 21, 23, 25, 26, 32). Six individuals showed the similar patterns of age-related change in both left and right molars. Those patterns could be regarded as non-directional (random) variability among observations made at different age (Id 8, 30, 36; Table 1) and as the directional change toward complication (Id 40, 42) or simplification (Id 37) with progressing age. Taken all together, regardless of side or individual, 15 molars out of 36 exhibit no morphotype changes at post-juvenile stages, 11 molars show non-directional differences among ontogenetic stages, 6 molars demonstrate directional change toward complication and 4 molars show simplification with progressing age. Magnitude of variation due to age is not high – differences between ontogenetic stages for the same tooth are usually not higher than ± 1 (Fig. 3). However, the signs of differences among vital and postmortem observations

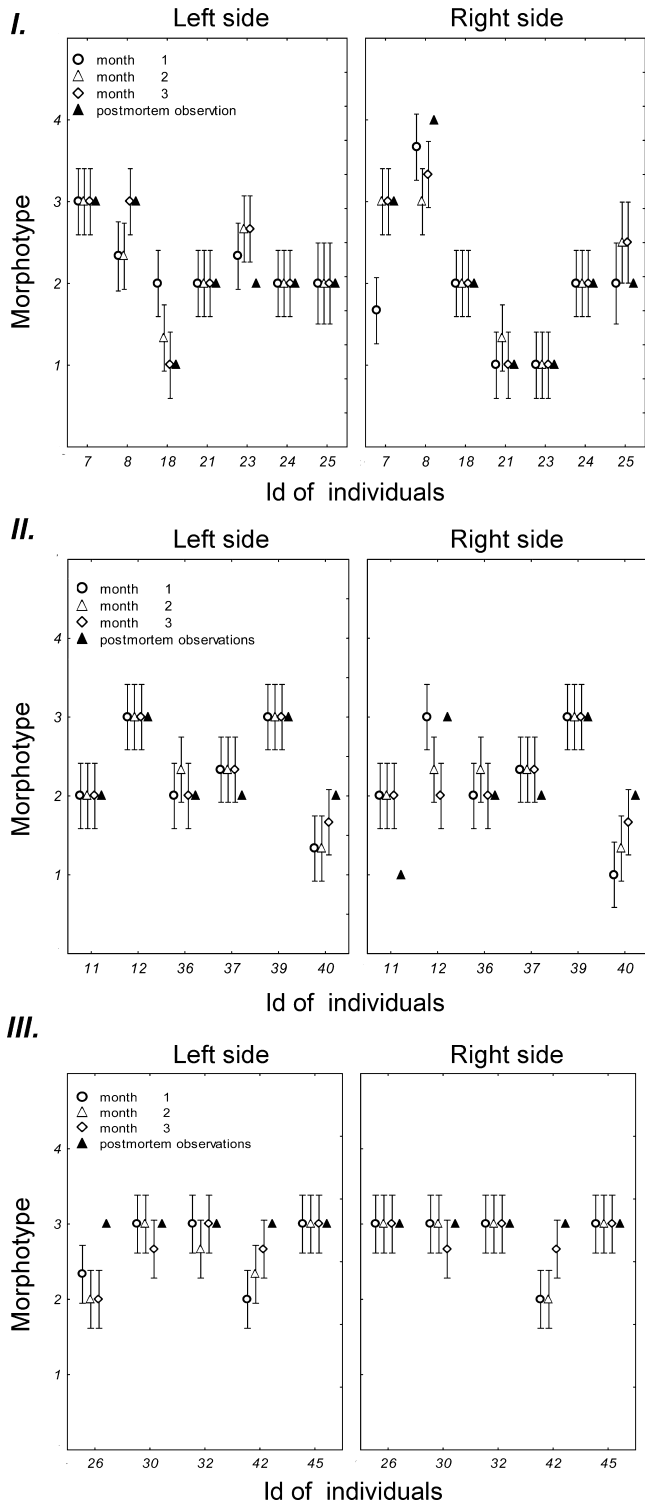


Fig. 3. Dental complexity of the left and right molars of *Microtus gregalis* as it was estimated by tooth prints (months 1, 2, 3), and postmortem. The x-axes represent the identification numbers of individuals; I–III – different maternal lines (see Table 1 for individual identification numbers and maternal lines). Vertical bars denote 0.95 confidence intervals.

were stable for a particular molar. Postmortem observations are always recorded as the most complex or the simplest for a given molar, and the complexity rate observed postmortem never falls between the vital observations for a given molar.

To test for genotype differences over the amount of between-individual differences in ontogenetic patterns of dental variability,

F ratio was calculated as $MS_{\text{individual}}/MS_{\text{individual} \times \text{age}}$. The genotype differences in morphotype dental patterns were found to be significant ($F_{17;51} = 22.17; p < 0.001$).

Since the main effect of *individual* was significant, we examined the differences among individuals who descended from different maternal lines using the one-way ANOVA. The effect of maternal line was found to be significant ($F_{2;351} = 28.16; p < 0.001$). The progenies of female 2 and 4 did not differ in dental complexity on average ($F_{1;252} = 1.98; p = 0.161$), although the progeny of female 6 had significantly more complex molars on average as compared to those of female 2 ($F_{1;232} = 50.50; p < 0.001$) or female 4 ($F_{1;218} = 44.04, p < 0.001$). Fig. 3 shows that individuals descending from female 6 are less variable in dental complexity (only morphotypes 2 and 3 are present), whereas the maternal line I exhibits the highest dental variability (morphotypes from 1 to 4 are present).

Discussion

When analyzing spatial or temporal patterns of variation in hypselodont arvicolines, the common practice is to exclude juvenile individuals from quantitative analyses in order to avoid biased results (Nadachowski, 1982; Borodin, 2009; Markova et al., 2010). Here, we confirm the necessity for caution when dealing with juvenile individuals in samples taken for morphological assessments. The juvenile folding represents the ephemeral peculiarity of the arvicoline molars and disappears at early stages of wear. Accordingly, we conclude that juvenile folding makes it impossible to determine a morphotype based on occlusal outline. Comparison of the tooth prints taken at juvenile stages with the postmortem observations allows us to hypothesize that the two patterns of the juvenile folding found in our dataset might be indicative of the future anteroconid complexity (anterior pattern of juvenile folding might result in relatively more complex pattern whereas the bucco-anterior pattern of the juvenile folding might develop into the simpler morphotypes with the less developed BRA4). However, this hypothesis needs to be tested and the transition from the juvenile to the definitive adult occlusal patterns should be studied in animals from 0.5 to 1 months of age.

Our results show that in narrow-skulled voles of 1 month and older, age-related differences in morphotype dental patterns were non-significant and negligible compared to among-individual variation. Considering the previous results obtained by the use of tooth-printing in *Microtus middendorffi* (Kourova, 1985, 1986), we can conclude that despite substantial change in size and depth of the re-entrant angles and increase in occlusal surface area, the morphotype features of the molars (namely, the presence or absence of additional salient angles) do not change with age.

It is known that the rooted molars of arvicoline species such as *Ondatra zibethica* (Viriot et al., 1993) or *Clethrionomys* species (Emelyanova, 2005; Borodin, 2009) exhibit a well pronounced trend of occlusal simplification during the crown wear. Despite the differences in the life-span, the similar stages of crown and root development could be clearly established for rooted molars of different arvicoline genera based on the extent to which the occlusal surface is formed, the crown is worn, and the roots are differentiated. Substantial variability in the occlusal outline of rooted molars in arvicolines is related not only to the earliest stages when the occlusal surface gradually acquires its definitive appearance but also to adult stages when additional prisms of the crown might get worn out and to the senile stages when the crown is almost completely worn out and the occlusal surface gradually loses its typical outline. The stages of crown wear and root development were recommended to account for occlusal simplification when studying

dental variability in natural populations of arviculines with rooted molars (Borodin et al., 2006; Fominykh et al., 2010).

Here, we find no evidence of age-related occlusal simplification in hypselodont molars at post-juvenile stages. We conclude that when the specimens showing the juvenile dental traits are excluded, the morphotype-based approach to analysis of dental patterns in hypselodont arviculines is expected to be less vulnerable to the age structure of a population (or a sample) than it is known for rooted arviculines (Borodin et al., 2006; Fominykh et al., 2010; Guérécheau et al., 2010).

Analysis of the age-related variability within the same molar and comparison between intravital and postmortem observations suggest that some molars exhibit no morphotype changes at different ontogenetic stages, whereas others appear to vary. Magnitude of morphotypic variability within the same molar (when present) is not high. However, the postmortem observations of dental complexity are always recorded as the most complex or the simplest for a given molar, and never fall between the complexity ratings established by tooth prints at 1–3 months of age. Based on that, we suggest that variability among ontogenetic stages in some individuals reflects the process of dental pattern clarification of a given molar with its growth and wear. In other words, the morphotype dental pattern of a hypselodont molar appears to be more clearly manifested in aged animals.

Within-individual differences among right and left molars, when present, are not related to age of an animal suggesting that bilateral asymmetry of morphotype dental pattern could be regarded as inherent characteristic of an individual persisting during post-juvenile tooth wear (e.g., individuals 8, 18, 21, 23). It should be noted, that in our study almost all individuals with asymmetric dental pattern originate from the same maternal line (I, Table 1, Fig. 3).

Highly significant differences among individuals found in our study as well as the differences in dental complexity among the maternal lines confirm the previous results showing the genetic background of the morphotype dental patterns in arviculines, namely in *Microtus arvalis* (Zimmermann, 1935; Stohl, 1984). Although the question of genetic vs. epigenetic mechanisms regulating tooth development in arviculines is still open (Jernvall, 2000; Salazar-Ciudad and Jernvall, 2002; Ledevin et al., 2010), there are no reasons for doubts in heritability of the characters used to identify the morphotypes (namely, the additional re-entrant or salient angles). Although it is clear that the features of the occlusal pattern of arvicoline molars show rather continuous than discrete patterns of variation (Borodin, 2009; Ledevin et al., 2010), we suppose that the role of genetic factors in dental variability should not be minimized in favor of epigenetic regulation processes.

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References

- Agadjanian, A.K., 1996. Modes of phytophagy in mammals. *Paleontol. J.* 30 (6), 723–729.
- Angermann, R., 1973. Homologous variability of molars in voles (Microtinae). In: Vorontsov, N.N. (Ed.), *Problems of Evolution*. Nauka Publishers, Novosibirsk, pp. 104–118 (in Russian).
- Barnosky, A.D., 1990. Evolution of dental traits since latest Pleistocene in meadow voles (*Microtus pennsylvanicus*). *Paleobiology* 16 (3), 370–385.
- Bolshakov, V.N., Vasilyeva, I.A., Maleeva, A.G., 1980. *Morphotypicheskaya Izmenchivost' Zubov Polevok* (Morphotypical Variability of Vole Dentition). Nauka Publishers, Moscow (in Russian).
- Borodin, A.V., 2009. *Opredelitel' Zubov Polevok Urala i Zapadnoy Sibiri* (Pozdnyy Pleystotsen-Sovremennost' (A Diagnostic Guide to Teeth of Arviculines of the Urals and Western Siberia (Late Pleistocene – Modern Time)). Ural Branch of the Russian Academy of Sciences Publishing, Ekaterinburg (in Russian).
- Borodin, A.V., Davydova, Yu.A., Elkina, M.A., 2006. Odontologicheskie kharakteristiki polevok roda *Clethrionomys* Tilesius, 1850 Visimskogo zapovednika. In: Marin, Yu.F. (Ed.), *Ekologicheskie Issledovaniya v Visimskom Zapovednike*. Middle Urals Publ. House “Novoe Vremya”, Ekaterinburg, pp. 77–85 (in Russian).
- Chaline, J., Brunet-Lecomte, P., Montuire, S., Viriot, L., Courant, F., 1999. Anatomy of the arvicoline radiation (Rodentia): palaeogeographical, palaeoecological history and evolutionary data. *Ann. Zool. Fenn.* 36, 239–267.
- Chaline, J., Graf, J.-D., 1988. Phylogeny of the Arvicolidae (Rodentia): biochemical and paleontological evidence. *J. Mammal.* 69 (1), 22–33.
- Cheprakov, M.I., 1983. Vozrastnaya izmenchivost' formy zhevatelnoy poverkhnosti korennykh zubov zheltobryukhogo lemminga. In: *Ecology, Human and Nature Conservation Problems: Proc. Regional Scientific-and-Practical Conference of the Young Scientists and Professionals* (1–3 of March, 1983), Sverdlovsk, pp. 107–108 (in Russian).
- Cheprakov, M.I., 2010. Izmenchivost' formy zhevatelnoy poverkhnosti korennykh zubov obyknovennogo lemminga (*Lemmus*) (Variation in occlusal shape of molars in common lemmings (*Lemmus*)). *Scientific bulletin of Yamal-Nenets Autonomous Area* 1 (64), 75–82 (in Russian).
- Contoli, L., Amori, G., Nazzaro, C., 1992. Tooth diversity in Arvicolidae (*Mammalia rodentia*): ecochorological factors and speciation time. *Hystrix*, NS 4 (2), 1–15.
- Emelyanova, A.A., 2005. Age variation of the teeth characters of the Bank Vole (*Clethrionomys glareolus* Schreber). *Vestnik Tver State University. Ser. Biol. Ecol.* 1, 88–96 (in Russian).
- Evdokimov, N.G., 1997. Metodika opredeleniya vozrasta obyknovennoy slepushonki *Ellobius talpinus* (Rodentia, Cricetidae) (A method for age determination in the northern mole vole *Ellobius talpinus* (Rodentia Cricetidae)). *Rus. J. Zool.* 76 (9), 1094–1101.
- Fominykh, M.A., Markova, E.A., Borodin, A.V., Davydova, Yu.A., 2010. Intrapopulation variation in odontometric characters of the bank vole *Myodes glareolus* Schreber, 1780 in the Middle Urals. *Rus. J. Ecol.* 41 (6), 535–538.
- Gromov, I.M., Polyakov, I.Y., 1977. The Voles (Microtinae) Fauna of the USSR. *Mammals*, vol. 3. Nauka Publishers, Leningrad, pt. 10 (in Russian).
- Guérécheau, A., Ledevin, R., Henttonen, H., Deffontaine, V., Michaux, J.R., et al., 2010. Seasonal variation in molar outline of bank voles: an effect of wear? *Mamm. Biol.* 75, 311–319.
- Guthrie, R.D., 1965. Variability in characters undergoing rapid evolution, an analysis of *Microtus* molars. *Evolution* 19 (2), 214–233.
- Guthrie, R.D., 1971. Factors regulating the evolution of microtine tooth complexity. *Z. Säugetierkd.* 36, 37–54.
- Hinton, M., 1926. *Monograph of the Voles and Lemmings (Microtinae) Living and Extinct*, vol. 1. Brit. Mus. (Nat. Hist.), London.
- Hurth, E., Montuire, S., Schmittbuhl, M., Schaaf, A., Viriot, L., Chaline, J., 2004. Examination of the morphological fields of three *Mimomys* lineages (Arvicolidae Rodentia) by elliptical Fourier methods. In: López-Martínez, N., Peláez-Campomanes, P., Hernández Fernández, M. (Eds.), *Surrounding Fossil Mammals: Dating, Evolution and Paleoenvironment: Coloquios de Paleontología*, v. ext. 3 (2003), pp. 325–334.
- Jernvall, J., 2000. Linking development with generation of novelty in mammalian teeth. *P. Natl. Acad. Sci. U.S.A.* 97, 2641–2645.
- Jernvall, J., Keranen, S.V.E., Thesleff, I., 2000. Evolutionary modification of development in mammalian teeth: Quantifying gene expression patterns and topography. *P. Natl. Acad. Sci. U.S.A.* 97, 14444–14448.
- Kaneko, Y., 1996. Age variation of the third upper molar in *Eothenomys smithii*. *Mammal Stud.* 21, 1–13.
- Koenigswald, W.v., 1982. Zum Verständnis der Morphologie der Wühlmausmolaren (Arvicolidae, Rodentia Mammalia). *Z. Geol. Wiss.* Berlin 10 (7), 951–962.
- Kochev, V.A., 1983. The quantitative assessment of variation in the occlusal surface of molars in collared lemmings. In: Manziy, S.V. (Ed.), *Morphological Peculiarities of the Vertebrates of the Ukraine*. Naukova Dumka, Kiev, pp. 59–62 (in Russian).
- Koshkina, T.V., 1955. Metod opredeleniya vozrasta ryzhikh polevok i opyt ego primeneniya (A method for age determination in the bank vole and its application experience). *Rus. J. Zool.* 34 (3), 631–639 (in Russian).
- Kourová, T.P., 1985. Vozrastnaya izmenchivost' formy i razmera zhevatelnoy poverkhnosti M3 i m1 polevki Middendorfa [Age variation in shape and size of the occlusal surface of M3 and m1 in Middendorff's vole]. In: *Investigation of Small Mammals in the Urals (Theriological Problems in the Urals)*. The Urals Scientific Center of the USSR Academy of Sciences, Sverdlovsk, 22–24 (in Russian).
- Kourová, T.P., 1986. Izmnenie risunka zhevatelnoy poverkhnosti zubov trekh vidov polevok na postyuvlenilnykh stadiyakh razvitiya. In: *Proc. IV Congress of Russian Theriological Society*. Vol. 2, Moscow, p. 65 (in Russian).
- Kovaleva, V.Y., Pozdnyakov, A.A., Efimov, V.M., 2002. Investigation of variability structure of root vole (*Microtus oeconomus* Pallas) molar morphotypes using bilateral asymmetry. *Rus. J. Zool.* 81 (1), 111–117.
- Ledevin, R., Quéré, J.-P., Renaud, S., 2010. Morphometrics as an insight into processes beyond tooth shape variation in a bank vole population. *PLoS One* 5 (11), e15470.
- Maleeva, A.G., 1982. Simmetriya i asimmetriya stroeniya zhevatelnoy poverkhnosti korennykh zubov u polevok na primere stepnoy pestrushki (*Lagurus lagurus* Pall.). In: *Proc. of the Zool. Institute of the USSR Academy of Sciences*, Saint-Petersburg, 115, pp. 23–37 (in Russian).

- Markova, E.A., 2006. Interspecific differentiation and intraspecific variation in odontological characters in sibling species *Microtus arvalis* and *M. rossiaemeridionalis*, extended abstract of Cand. Sci. (Biol.). Dissertation. Institute of Plant and Animal Ecology, the Ural Branch of RAS, Ekaterinburg (in Russian).
- Markova, E.A., Malygin, V.M., Montuire, S., Nadachowski, A., Quéré, J.-P., Ochman, K., 2010. Dental variation in sibling species *Microtus arvalis* and *M. rossiaemeridionalis* (Arvicolinae Rodentia): between-species comparisons and geography of morphotype dental patterns. *J. Mol. Evol.* 17 (2), 121–139.
- Maul, L.C., Masini, F., Abbazzi, L., Turner, A., 1998. The use of different morphometric data for absolute age calibration of some South- and Middle European arvicolid populations. *Palaeontograph. Italica* 85, 111–151.
- McGuire, J.L., 2010. Geometric morphometrics of vole (*Microtus californicus*) dentition as a new paleoclimate proxy: Shape change along geographic and climatic clines. *Quatern. Int.* 212 (2), 198–205.
- Nadachowski, A., 1982. Late Quaternary Rodents of Poland with Special Reference to Morphotype Dentition Analysis of Voles. Państwowe Wydawn. Nauk, Warszawa-Kraków.
- Navarro, N., Zatarian, X., Montuire, S., 2004. Effects of morphometric descriptor changes on statistical classification and morphospaces. *Biol. J. Linnean Soc.* 83, 243–260.
- Ognev, S.I., 1950. The Mammals of USSR and Adjacent Countries. Vol. VII (Rodentia). Akad. Nauk SSSR, Moscow-Leningrad (in Russian).
- Olenev, G.V., 1980. Vital determination of taxonomic status of murid rodents by tooth-prints. *Rus. J. Zool.* 59 (2), 294–295.
- Olenev, G.V., 2009. Determining the age of cyclomorphic rodents: functional-ontogenetic determination, ecological aspects. *Rus. J. Ecol.* 40 (2), 93–104.
- Oppenheimer, J.R., 1965. Molar cusp pattern variations and their interrelationships in the meadow vole, *Microtus p. pennsylvanicus* (Ord.). *Am. Midl. Nat.* 74 (1), 39–49.
- Palmer, A.R., 1994. Fluctuating asymmetry analyses: a primer. In: Markov, T. (Ed.), *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer, Dordrecht, pp. 335–364.
- Pankakoski, E., 1980. An improved method for age determination in the muskrat, *Ondatra zibethica* (L.). *Ann. Zool. Fenn.* 17, 113–121.
- Perrin, M.R., 1988. Molar root-length as an indicator of age in *Clethrionomys gapperi*. *Acta Theriol.* 23 (f.19–30), 423–434.
- Piras, P., Marcolini, F., Raia, P., Curcio, M., Kotsakis, T., 2010. Ecophenotypic variation and phylogenetic inheritance in first lower molar shape of extant Italian populations of *Microtus (Terricola) savii* (Rodentia). *Biol. J. Linn. Soc.* 99 (3), 632–647.
- Polly, P.D., Killick, L., Ruddy, M., 2011. Using left-right asymmetry to estimate nongenetic variability in vole teeth (Arvicolinae, Muridae Rodentia). *Palaeontol. Electron* 14 (3), 41A.
- Pozdnyakov, A.A., 2011. The structure of morphological variability (with the masticatory surface morphotypes of the lower first molar in voles as an example). *Zh. Obshch. Biol.* 72 (2), 127–139.
- Pozdnyakov, A.A., 2007. The structure of morphological variability of common voles (*Microtus: Rodentia Arvicolidae*) in the light of epigenetic theory of evolution. *Usp. Sovrem. Biol.* 127 (4), 416–424.
- Rörig, G., Börner, C., 1905. Studien über dass Gebiss mitteleuropäischer recenter Mäuse. *Arb. Kais. Biol. Anst. Land. Forstw.* 5, 37–89.
- Ruiz Bustos, A., 1996. Parametrization of the distribution of arvicolid tooth enamel. *Acta Zool. Cracov.* 39 (1), 435–446.
- Salazar-Ciudad, I., Jernvall, J., 2002. A gene network model accounting for development and evolution of mammalian teeth. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8116–8120.
- Schmidt-Kittler, N., 1986. Evaluation of occlusal patterns of hypsodont rodent dentitions by shape parameters. *N. Jb. Geol. Palaont., Abh.* 173 (1), 75–98.
- Smirnov, N.G., Fedorov, V.B., 2003. Holarctic collared lemmings: traces of their spread as related to the history of Arctic biota. *Rus. J. Ecol.* 34 (5), 332–338.
- Smirnov, N.G., Bolshakov, V.N., Borodin, A.V., 1986. Pleistocene Rodents of the North of Western Siberia. Nauka Publishers, Moscow (in Russian).
- Sokal, R.R., Rohlf, F.J., 1994. *Biometry*, 3rd ed. W.H. Freeman, New York.
- Sözen, M., Çolak, E., Yigit, N., Özkurt, S., 1999. The age variations of *Microtus guentheri* Danford and Alston, 1880 (Mammalia: Rodentia) in Turkey. *Tr. J. Zool.* 23, 145–155.
- StatSoft Inc. 2001. *Statistica* (data analysis software system), version 6. www.statsoft.com
- Stohl, G., 1984. Über einige Besonderheiten “künstlicher” Feldmaus-Populationen (*Microtus arvalis* [Pallas]) (Mammalia, Rodentia). *Vertebrata Hung.* 22, 51–63.
- Tupikova, N.V., Sidorova, G.A., Konovalova, Z.A., 1970. A method of age determination in *Clethrionomys*. *Acta Theriol.* 13 (8), 99–115.
- Van der Meulen, A.J., 1973. Middle Pleistocene smaller mammals from the Monte Peglia (Orvieto, Italy) with special reference to the phylogeny of *Microtus* (Arvicolidae, Rodentia). *Quaternaria, Roma* 17, 1–144.
- Vasilyev, A.G., Vasilyeva, I.A., 2009. Homological Variability of Morphological Structures and Epigenetic Divergence among Taxa: Principles of Population Meroonomy. KMK Scientific Press, Moscow (in Russian with English abstr).
- Viriot, L., Chaline, J., Schaaf, A., Le Boulengé, E., 1993. Ontogenetic change of *Ondatra zibethica* (Arvicolidae, Rodentia) cheek teeth analyzed by digital image processing. In: Martin, R.A., Barnosky, A. (Eds.), *Morphological Change in Quaternary Mammals of North America*. University Press, Cambridge, pp. 373–391.
- Yeremina, I.V., 1974. Polyimorfism of occlusal pattern of cheek teeth in common vole. In: *Fiziologicheskaya i Populyatsionnaya Ecologiya Zhivotnykh* (Physiological and Population Ecology of Animals). Saratov University Press, Saratov, 77–91 (in Russian).
- Zejda, J., Zukal, J., Steiner, H.M., 1997. Symmetric pattern in M₁ and M³ molars in *Clethrionomys glareolus*. *Folia Zool.* 46 (1), 89–90.
- Zimmermann, K., 1935. Zur Rassenanalyse der Mitteleuropäischen Feldmaus. *Arch. Naturgesch.* NF 4, 258–273.
- Zimmermann, K., 1958. Selektionswert der simplex-Zahnform bei der Feldmaus (Eine Entgegnung). *Zool. Jb. Syst.* 86, 35–40.