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GENERAL BIOLOGY

Ontogenetic and Evolutionary Trends in the Tooth Enamel Features in Craseomys Voles (Arvicolinae, Rodentia)

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Abstract—In *Craseomys rufocanus* and *Craseomys rex*, the age-related and species differences in thickness and microstructure of the first lower molars (ml) have been identified and studied. The results suggest that the enamel dimensional and microstructural features may serve as additional indicators of the vole tooth evolutionary stage within a single phyletic lineage.

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In voles (Arvicolinae), the cheek tooth enamel that forms cutting edges of prisms has undergone dimensional and structural changes during the evolution of the dental system, which, along with changes in hypsodonty and configuration features of the tooth occlusal surface allow to consider the enamel complexity as a parameter characterizing the evolutionary level of taxa [1, 2]. In voles, the enamel band thickness on the leading and trailing edges of the molar prisms is traditionally used to differentiate five types of enamel development in the following evolutionary stages: the primary undifferentiated type → "*Mimomys*" type → secondary undifferentiated type → "Microtus" type → "Dicrostonyx" type. The microstructure features are used equally with changes of enamel thickness and identification of three main types of enamel microstructure, radial, lamellar or uniserial HSB, and tangential [2], make it possible to use them both in morphological studies of the vole molars and for evolutionary and biostratigraphic reconstructions [2–6].

The objective of this study was to determine the dimensional and structural features of the molar enamel in two vole species of the genus *Craseomys* (*C. rufocanus* and *C. rex*), which differ in the hypsodonty and variants of the occlusal surface of molar complexity on different ontogenetic stages.

The enamel features of the first lower molars (m1) (dimensions and microstructure) were studied in *C. rufocanus* ($n = 12$) and *C. rex* ($n = 13$) from Shikotan Island (the Southern Kuril Islands).

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The taxonomical status of each individual vole was determined based on the morphotypic and morphometric characters of the third upper and first lower molars [7]. To evaluate age-related variation, the material was divided into groups according to the ontogenetic stages, which were determined from the degree of the molar crown and root development [8].

The types of enamel microstructure were characterized according to W. von Koenigswald [2]. After grinding and polishing in transversal (over the occlusal surface) and longitudinal (along the side edges of the molar prisms) sections of the occlusal surface, teeth were treated with 2 N HCl for 3 s and copiously washed with water. Enamel examination was conducted using a TESCAN VEGA3 microscope (Tescan, Czech Republic).

To evaluate the molar enamel differentiation according to thickness, the enamel thickness quotient (SDQ index ± *SD*) [9] were calculated for the enamel band of the T1 prism of the first lower molar (m1). Interspecific comparisons were performed using Student's *t*-test and Pearson chi-square tests (χ^2) and the Statistica 6.0 software (StatSoft, 1984–2001, United States).

The age-related differences in enamel thickness were determined in *C. rufocanus* and *C. rex* by studying young animal molars with still undeveloped roots (stage 4) and old animal molars, in which the tooth root height exceeded already the crown height (stage 9). We have found that, in old animals of both species, the m1 enamel was thicker ($p < 0.01$) than in young ones (table). This is well explained by the results of enamel analysis of the longitudinal sections of the m1 crown in *C. rufocanus* at the ontogenetic stage 5 (when the occlusal surface and crown of molar are completely formed, while the formation of the cervical part of root

Total enamel thickness on the leading and trailing edges of T1-T2 prisms and the enamel thickness quotients (SDQ indices ± SD) of T1 prism of the first lower molar (m1) at ontogenetic stages 4 and 9 in *C. rufocanus* and *C. rex*

No.	Species	Ontogenetic stages of m1	Total enamel thickness, μm		SDQ index
			leading edge of m1	trailing edge of m1	
1	*C. rufocanus*	4	41.49 ± 6.82 $n = 6$ $p_{1,3} < 0.01$ $p_{1,2} < 0.01$ $p_{1,4} < 0.01$	54.24 ± 6.75 $n = 6$ $p_{1,3} < 0.01$ $p_{1,2} > 0.05$	126.68 ± 19.48 $n = 6$
2	*C. rex*	4	59.56 ± 8.96 $n = 7$ $p_{2,4} < 0.01$ $p_{2,3} < 0.01$	54.69 ± 8.13 $n = 7$ $p_{2,4} < 0.01$	87.68 ± 6.10 $n = 7$
3	*C. rufocanus*	9	65.07 ± 6.80 $n = 6$ $p_{3,4} < 0.01$	93.17 ± 9.65 $n = 6$ $p_{3,4} > 0.05$	139.60 ± 5.76 $n = 6$
4	*C. rex*	9	83.16 ± 7.72 $n = 6$	81.80 ± 7.02 $n = 6$	102.54 ± 13.44 $n = 6$

$M \pm SD$; n is the number of m1 examined. Significance of differences was estimated using Student's t test.

begins): at the top of the molar crown, enamel is thinner (the thickness of the T1 leading edge is 56.02 μm and the thickness of the T1 trailing edge is 57.51 μm); at the same time, nearer to the bases of the prisms, enamel becomes thicker (the thickness of the T1 leading edge is 71.13 μm and the thickness of the T1 trailing edge is 87.70 μm).

Regarding the differentiation types, according to the enamel thickness, the molar enamel of *C. rufocanus* is differentiated by the "Mimomys" type: the enamel of the convex edges of prisms (trailing edges on the lower molars) are thicker than the enamel of the concave ones (leading edges on the lower molars). Enamel of *C. rex* molars can be assigned to the secondary undifferentiated type, because at all ontogenetic stages, it is equally thick on the concave and convex edges of the prisms, though becoming slightly thinner in the reentrant angles. In some cases, on the concave edges, enamel is somewhat thicker. The results of the enamel thickness measurements are shown in the table. In *C. rufocanus*, the enamel thickness reentrant angles has proved to be higher than in *C. rex*. Hence, the enamel differentiation type is more archaic in *C. rufocanus*.

Three types of the m1 enamel microstructure are distinguished: radial and lamellar enamel on the leading edges and radial and tangential enamel on the trailing edges of the molar prisms (Fig. 1). Both *C. rufocanus* and *C. rex* have typical lamellar enamel, a more advanced type of structure than the discrete lamellar enamel identified in *Clethrionomys glareolus* [2].

Studying of the molar longitudinal sections showed that, at ontogenetic stages 5 and 9, enamel was differentiated almost over the entire dental crown in both species; nearer to the bases of prisms, only radial enamel was observed, while the lamellar and tangential enamels disappeared. In the basal part of the molar, enamel was already poorly structured and it was impossible to determine the enamel microstructure based on the prisms orientation.

An analysis of the dependence of the enamel thickness and its microstructure (expression of a particular enamel type) showed distinctions between *C. rex* and *C. rufocanus*. In *C. rex*, the total enamel thickness was greater on the leading edges of the m1 in young specimens at stage 4 and in old ones at stage 9 (table). Both the radial and typical lamellar enamels were thicker than in *C. rufocanus* (Figs. 1, 2). In both species, these enamel types were expressed throughout the edge of the molar prisms; however, the lamellar enamel usually did not reach the top of the reentrant angles at stages 4 ($\chi^2 = 1.8$, $df = 2$, $p = 0.41$) and 9 ($\chi^2 = 2.04$, $df = 2$, $p = 0.36$). Typical lamellar enamel was observed along with the radial enamel on the tops of reentrant angles only in three *C. rex* specimens (m1 at stages 4 and 9) and in one *C. rufocanus* specimen (m1 at stage 9).

On the m1 trailing edges, we detected both the radial and tangential enamel, total thickness of which was the same in the young specimens of both species (differences were insignificant, table). In old voles, the enamel of the trailing edges of the rooted m1 was significantly thicker in *C. rufocanus* than in *C. rex* and it equally consisted of the radial and tangential enamel (Fig. 1, 2). It should be noted that in *C. rufocanus*, formation of the developed tangential enamel on the trailing edges of m1 was observed at all ontogenetic

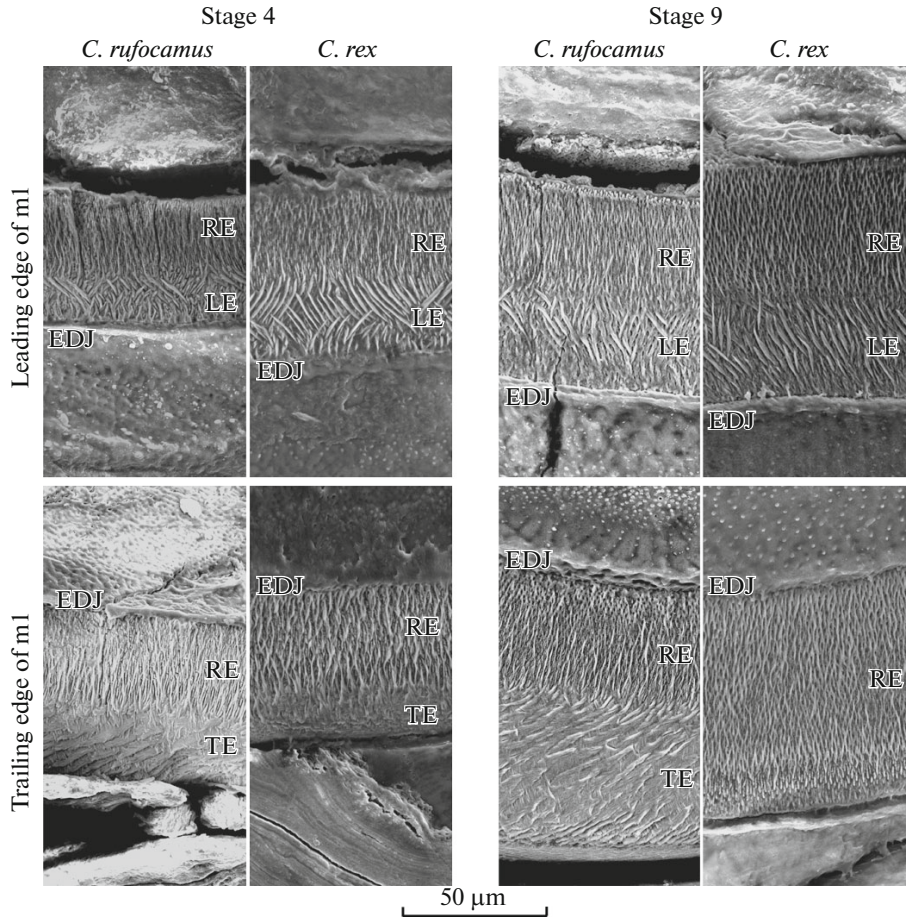

Fig. 1. Microphotographs of the enamel structure on the leading and trailing edges of m1 at the ontogenetic stages 4 and 9 of *C. rufocanus* and *C. rex*. Enamel types: RE, radial; LE, lamellar; TE, tangential; EDJ, enamel-dentine junction.

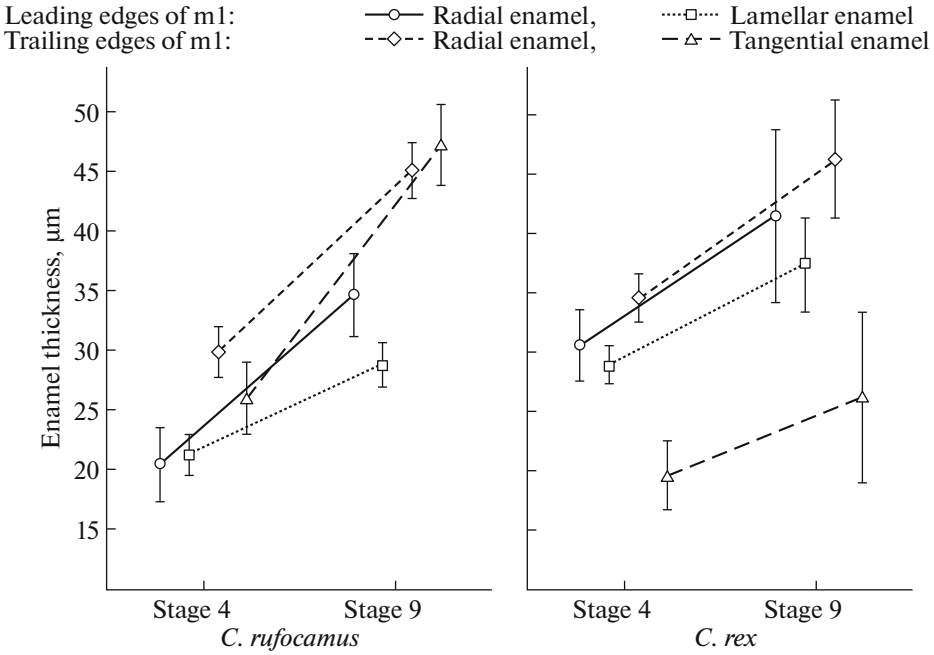

Fig. 2. The radial and lamellar enamel thickness on the leading edges of m1 and the radial and tangential enamel thickness on the trailing edges of m1 in *C. rufocanus* and *C. rex*. $\bar{X} \pm 95\%$ confidence intervals, $n = 12$ (*C. rufocanus*) and 13 (*C. rex*).

stages. In *C. rex*, the trailing edges of m1 consisted of the radial enamel almost completely, while the tangential enamel was represented only slightly, fragmentary, or not met on the molars at stages 4 ($\chi^2 = 8.78$, $df = 3$, $p = 0.03$) and 9 ($\chi^2 = 10$, $df = 4$, $p = 0.04$). In almost all old *C. rex* specimens, there was no tangential enamel with the exception of a single m1 with enamel fragments on the trailing edges of T2–T4 triangles and posterior unpaired lobe.

Thus, with growing of m1 crown in *C. rex*, tangential enamel disappears gradually, and this is accompanied by a relative diminishing of the enamel thickness, while at the early ontogenetic stages, this enamel type is represented by the trailing edges of the prisms in both species. In *C. rufocanus*, the enamel thickness on the trailing edges of the m1 increases with crown formation, and the molars have well-developed tangential enamel at all ontogenetic stages.

The fact that gradual diminishing of the tangential type of enamel structure is related to a decrease in the total enamel thickness on the convex (trailing) edges of molars is characteristic of most phyletic lineages of the vole genera *Mimomys–Allophaiomys–Microtus* and *Borsodia–Lagurus* [2, 5, 10, 11] and was, probably, a result of transition from rhizodont to hypselodont molars. According to all of the parameters, the *C. rex* molars were at the final rhizodont stages [7]; therefore, we observed thinning of the trailing edges of the m1, as well as reduction of the tangential enamel of this species.

Thus, the enamel features can serve as additional indicators of the vole tooth evolutionary stage within a single phyletic lineage. However, because of the parallel evolution of different species, they may have similar enamel structures of the molars. Hence, enamel microstructure cannot serve the main or independent diagnostic feature of a taxon.

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