Mammal Study 21 : 1-13 (1996)

Age variation of the third upper molar in *Eothenomys smithii*

Yukibumi KANEKO

Biological Laboratory, Faculty of Education, Kagawa University, Takamatsu 760, Japan
Fax. 0878-36-1652

Abstract. A study was made of age variation in the size and enamel patterns of the third upper molar of 99 *Eothenomys smithii* specimens from Japan. No significant age variation was found in either the frequency of the patterns, or the width of the dentine confluent space between the second and the third triangles. Deep lingual reentrant folds, on the posterior loop, appear in specimens where the condylobasal length (CBL) is of 22-24 mm, then the pattern with a shallow reentrant fold increases in frequency in larger CBL classes. The depth of the inner fold showed the same tendency as the changes in the patterns. A significant association, however, between five enamel patterns and age classes, depends on classification according to CBL or body weight. This proved insignificant in five CBL classes, but significant in three CBL or body weight classes. A gradual transition in the age variation of the posterior loop patterns was found among *Eothenomys* species which have rootless molars throughout life. The simple enamel pattern form significantly increased in frequency with advancing age in *E. andersoni* and *E. shanseius*, resembling *Clethrionomys glareolus* and *C. rufocanus* ; on the other hand, in *E. regulus, E. inez, E. eva, E. chinensis, E. wardi, E. custos* and *E. proditor* no age variation was found on the posterior loop, thus resembling *Microtus pennsylvanicus*. *E. smithii* shows a little age variation in the enamel patterns, the variation of which is of an intermediate type.

Key words : age variation, enamel pattern, *Eothenomys smithii*, size of molar, third upper molar.

With regard to the phylogeny of the Arvicolidae, Bauchau and Chaline (1987), and Chaline and Graf (1988), considered that, based on a comparison of molar structures, the occlusal enamel patterns of the third upper molar tended to vary from simple to more complex forms. The genus *Clethrionomys* develops molar roots with advancing age, whereas the genus *Eothenomys* develops no roots. The two genera, however, resemble each other in many other characters of the skull and dental morphology (Hinton 1926, Kaneko 1990, 1992), and in their karyotypes (Yoshida *et al.* 1989). Through the ontogenetic process of *C. glareolus* (Zejda 1960), *C. rufocanus* (Abe 1982) and *E. andersoni* (Miyao 1966, Kitahara 1995), a large proportion of molars changes from complex enamel patterns to simpler forms. No age variation was found, however, on the same molar in *E. smithii* (Tanaka 1971). Tanaka's (1971) results for *E. smithii* may have been biased because of his relatively small sample group of specimens collected during just one period of the year, when fully adult animals may have been absent.

The purpose of this study, therefore, was to reexamine the age variation in both size and enamel pattern of the third upper molars of *E. smithii*, and to compare the results with those of other *Eothenomys* species.

Materials and Methos

A total of 99 specimens of *E. smithii* were collected at Minoura, Toyohama District, Kagawa Prefecture, Japan, (34°02′30″N, 133°37′30″E). Specimens in each of the 12 months were sampled at one period during the years 1977-80 (Kaneko 1989). The collecting site for this study was less than 50 km from Tanaka's (1971) site on the same island, Shikoku. Five measurements of the third upper molar and the condylobasal length (CBL) were taken from cleaned skulls, these were: total length (TM3L), anterior length (AM3L), posterior length (PM3L), the width of dentine confluent spaces between the first and second triangles (WDC) and the depth of the third lingual reentrant fold or the posterior loop (DRF, Fig. 1). Tooth dimensions were measured to the nearest

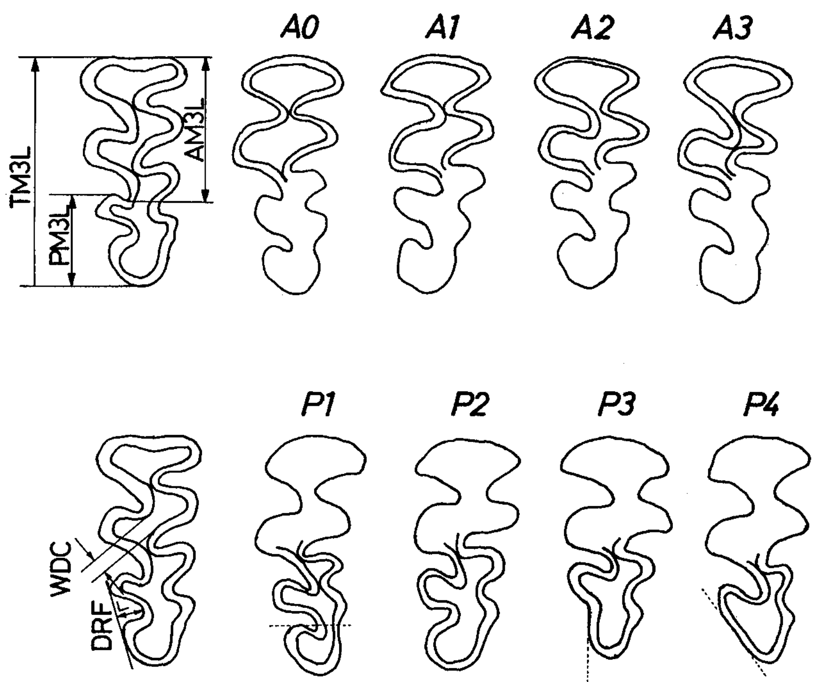

Fig. 1. Measurements taken (left), and enamel patterns (A0-A3 and P1-P4) on the third upper molar according to Tanaka (1971). TM3L, AM3L, PM3L, WDC and DRF are explained in text.

0.01 mm using a stereo-microscope (Nikon, SMZ-10) with an objective micrometer (Kogaku, minimum interval=0.05 mm). The CBL was measured to the nearest 0.1 mm with a dial caliper (minimum interval=0.05 mm).

Tanaka (1971) defined the enamel patterns formed on the occlusal surface by the enamel lamellae only in figures; however, in this study more precise criteria have been used. Four patterns (A0-A3) in the shape of the dentine confluent spaces between the second and third triangles were recognized. In A0, the lamellae do not form two triangles, but a wide dentine confluent space instead. A1 and A2 are intermediate patterns between A0 and A3 (A1 shows a smaller protrusion of the enamel lamella, and A2 a larger protrusion). In A3 the lamellae form two complete triangles. Four other patterns (P1-P4) were observed on the posterior loop, or on the fourth salient angle. In P1 the pattern is complex, with three reentrant folds on the lingual side, with the third fold exceeding the transverse line at the anterior edge of the salient angle of the posterior loop. P2 is intermediate between patterns P1 and P3. P3 has three salient angles with a straight-sided posterior loop on the lingual side. In P4 the pattern is simple with two reentrant folds on the lingual side and without concavity on the posterior loop. Enamel patterns were observed on the right or left molar under a stereo-microscope with a ×20 lens.

In this study, CBL was used as an approximate indicator of age, because it correlates positively with age as defined by root development in *Clethrionomys rufocanus* (Kaneko 1990). As there have been no reports indicating sexual differences in either size or enamel patterns, both males and females were combined for analysis.

RESULTS

As CBL increased, both total length (TM3L) and anterior length (AM3L) increased significantly ($r=0.661$, $p<0.001$ and $d.f.=97$ in TM3L; $r=0.676$, $p<0.001$, $d.f.=97$ in AM3L). As CBL increased, the posterior length (PM3L) increased until CBL reached 22.5 mm where it reached asymptote, though a significant regression coefficient was calculated throughout the size of CBL ($r=0.305$, $0.001<p<0.01$, $d.f.=97$, Fig. 2).

The depth of the reentrant fold of the posterior loop (DRF) was nearly constant against CBL=22-25 mm and decreased slightly in CBLs larger than 25 mm. With the increase of CBL, width of the dentine confluent spaces (WDC) remained almost constant. Regression coefficients between CBL and DRF, and between CBL and WDC were insignificant ($r=-0.198$, *n. s.*, $d.f.=97$ in DRF; $r=-0.057$, *n. s.*, $d.f.=97$ in WDC, Fig. 3).

The average length (X), standard deviation (SD), and coefficient of variation (CV) of these five measurements are tabulated for five size classes of CBL. Average TM3L and AM3L increased continuously as CBL increased, whereas PM3L ceased to increase from the CBL=22.0 mm class onwards. The coefficient of variation is greater in PM3L than in TM3L and AM3L for each size class. Average DRF decreased from CBL=25 mm onwards, whereas average

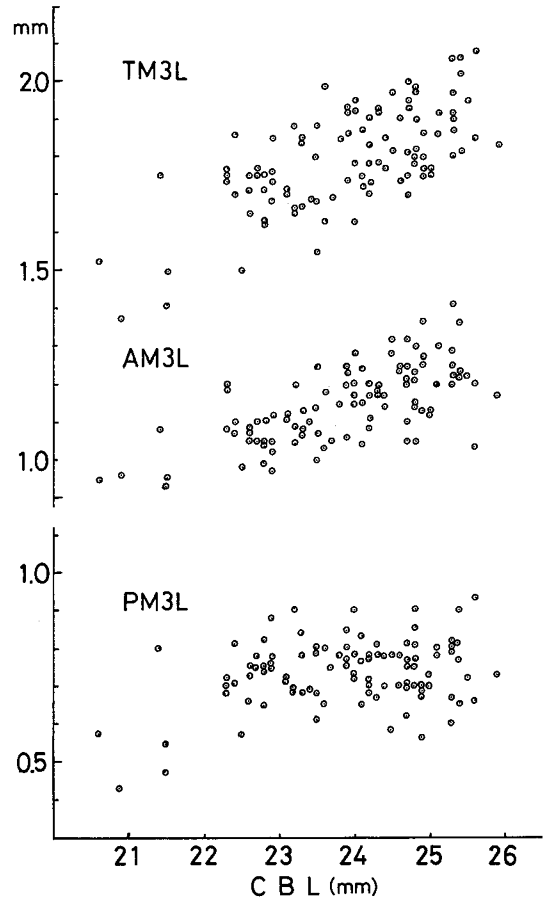

Fig. 2. Plots of TM3L, AM3L and PM3L against the condylobasal length (CBL).

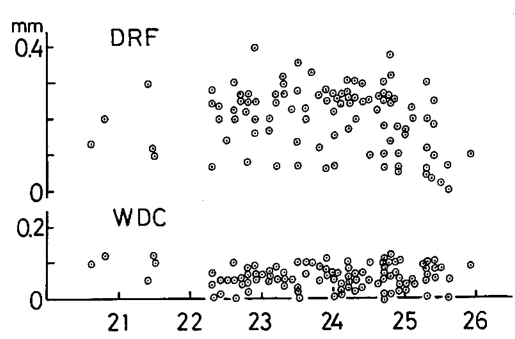

Fig. 3. Plots of WDC and DRF against the condylobasal length (CBL).

WDC was slightly longer in the 20 mm CBL class. The coefficient of variation was greater was DRF in the 25 mm CBL class (Table 1).

The association of enamel patterns between left and right third upper molars was tested using the G-test with Williams' adjustment (Sokal and Rohlf 1973). No independence was shown between right and left molars in the patterns of the dentine spaces between the second and third triangles (A0-A3 ; $G_{adj}=35.66$, $p<0.005$, *d. f.*$=9$, Table 2), or between right and left patterns of the posterior loop (P1-P4 ; $G_{adj}=43.88$, $p<0.005$, *d. f.*$=9$, Table 2). Similarly no association was shown between the patterns of the dentine confluent spaces and those of the posterior loop (A0-A3 and P1-P4, $G_{adj}=6.56$, *n.s.*, *d. f.*$=9$, Table 3). Consequently, the left third upper molar was used for further studies.

In an analysis of the width of dentine spaces (WDC) and the enamel patterns (A0-A3) between the second and third triangles for each of the five size classes of CBL, the 0.05-0.10 mm WDC class and pattern A2 predominated in every CBL size class over 22.0 mm, and average WDC remained almost constant throughout the size classes (Fig. 4). No association was found between the enamel patterns and the five CBL size classes ($G_{adj}=18.73$, *n.s.*, *d. f.*$=12$, Table 4). When the frequency of the patterns was divided into three body weight classes, as defined by Tanaka (1971), or by CBL (20.5-, 23.0-, and 25.0- mm

Table 1. Five measurements of the third upper molars of *Eothenomys smithii*.

		Five CBL classes (mm)				
		20.5-	22.0-	23.0-	24.0-	25.0-
N		5	19	21	37	17
Total	*X*	1.508	1.720	1.769	1.832	1.908
length	*SD*	0.150	0.082	0.119	0.093	0.102
(TM3L)	*CV*	9.946	4.757	6.661	5.064	5.368
(mm)	Min.	1.37	1.50	1.55	1.63	1.76
	Max.	1.75	1.86	1.99	2.00	2.08
Anterior	*X*	0.974	1.068	1.122	1.190	1.215
length	*SD*	0.060	0.060	0.073	0.080	0.087
(AM3L)	*CV*	6.181	5.572	6.534	6.679	7.170
(mm)	Min.	0.93	0.97	1.00	1.04	1.03
	Max.	1.08	1.20	1.25	1.36	1.41
Posterior	*X*	0.564	0.730	0.748	0.739	0.752
length	*SD*	0.144	0.071	0.073	0.076	0.087
(PM3L)	*CV*	25.497	9.760	9.818	10.303	11.510
(mm)	Min.	0.43	0.57	0.61	0.56	0.60
	Max.	0.80	0.88	0.90	0.90	0.93
Confluent	*X*	0.094	0.050	0.064	0.056	0.059
width	*SD*	0.026	0.028	0.029	0.032	0.032
(WDC)	*CV*	27.766	55.800	44.479	57.271	54.422
(mm)	Min.	0.05	0.00	0.00	0.00	0.00
	Max.	0.12	0.10	0.11	0.12	0.10
Depth of	*X*	0.170	0.223	0.221	0.217	0.130
reentrant	*SD*	0.082	0.076	0.088	0.083	0.087
fold	*CV*	48.177	34.187	39.638	38.199	67.154
(DRF)	Min.	0.10	0.07	0.06	0.05	0.00
(mm)	Max.	0.30	0.40	0.36	0.38	0.30

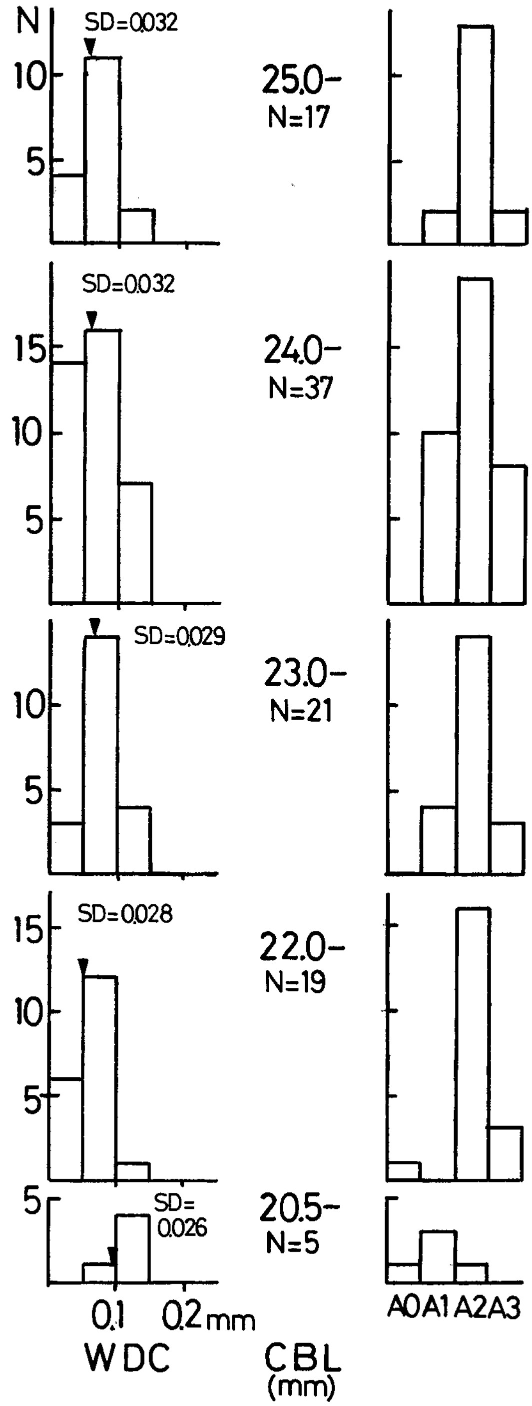

Fig. 4. Frequency distributions of WDC measurements and enamel patterns A0–A3 of the dentine space for five CBL classes. A closed triangle shows the average, and *SD* indicates the standard deviation of the average.

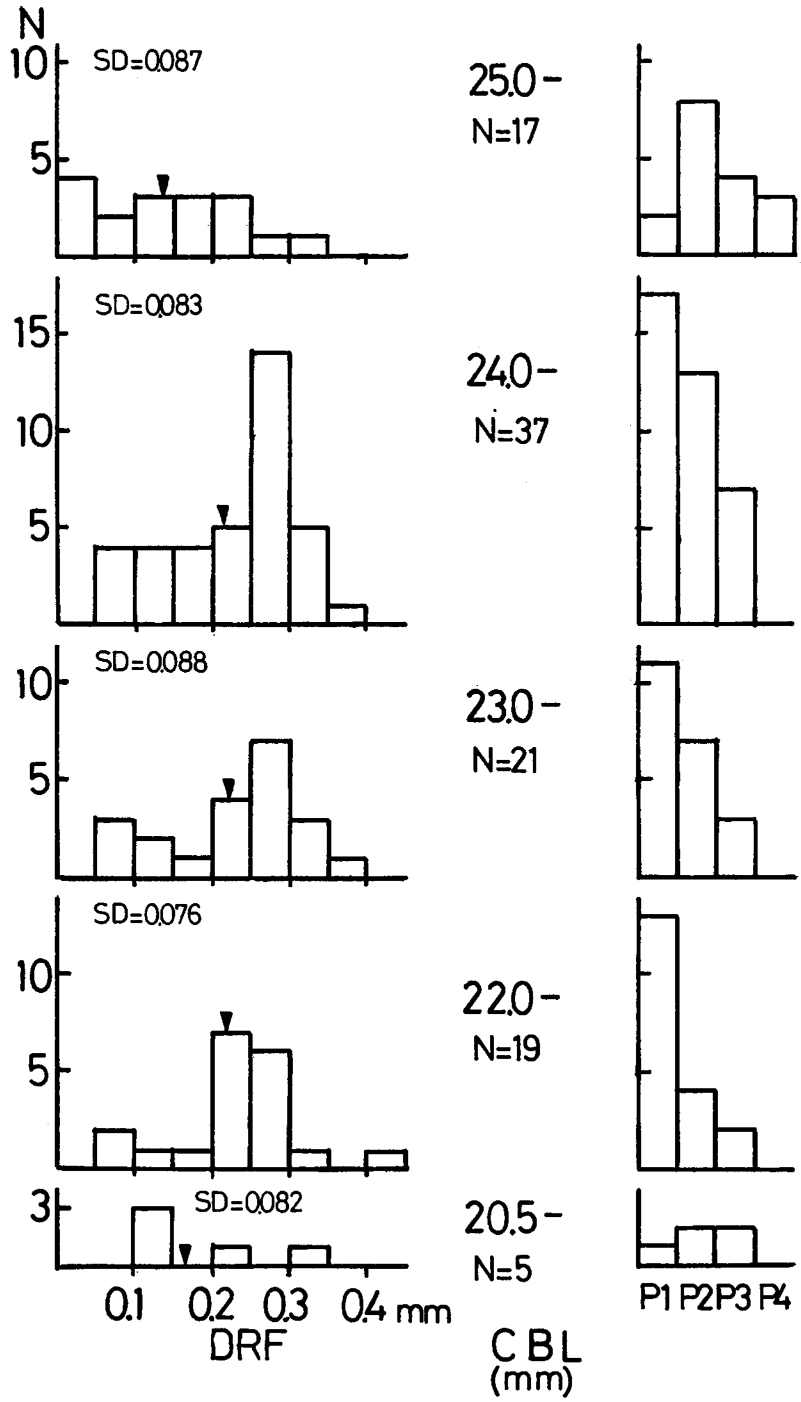

Fig. 5. Frequency distributions of DRF measurements and enamel patterns P1–P4 for five CBL classes. A closed triangle shows the average, and *SD* indicates the standard deviation of the average.

Table 2. A test of independence for frequencies of the enamel patterns (A0–A3 and P1–P4) between the right and left molars of *Eothenomys smithii*.

		The left				
		Confluent patterns of the 2nd and 3rd spaces				
		A0	A1	A2	A3	Total
The right	A0	1	2	0	0	3
	A1	1	11	2	0	14
	A2	0	6	59	4	69
	A3	0	0	1	12	13
	Total	2	19	62	16	99
		Patterns of posterior loop				
		P1	P2	P3	P4	Total
The right	P1	34	7	0	1	42
	P2	10	24	5	0	39
	P3	0	3	12	0	15
	P4	0	0	1	2	3
	Total	54	34	18	3	99

Table 3. A test of independence for frequencies between the enamel patterns (A0–A3 and P1–P4) of *Eothenomys smithii*.

		Confluent patterns between the 2nd and 3rd spaces				
		A0	A1	A2	A3	Total
Posterior loop patterns	P1	1	5	28	10	44
	P2	0	7	24	3	34
	P3	1	6	9	2	18
	P4	0	1	1	1	3
	Total	2	19	62	16	99

based on Table 4), no significant association was shown between the two dimensions ($G_{adj}=8.13$, *n.s.*, *d.f.*=9 for CBL ; $G_{adj}=4.83$, *n.s.*, *d.f.*=6 for body weight, Table 5). Thus, the variation of the pattern of the dentine confluent spaces (A0–A3) is independent of age.

In an analysis of the depth of the reentrant fold (DRF) and of the enamel patterns of the posterior loop (P1–P4) for each CBL size class, patterns P1–P3 appeared with similar frequencies in CBL classes from 22 mm to 24 mm, as did DRF, with almost the same average, though within a wide range between 0.05 and 0.45 mm (Fig. 5). In the 25 mm CBL class, average DRF became slightly shallower, and pattern P4 appeared for the first time, and P2 became the most frequent pattern. No association was found between the patterns of the posterior loop and the five CBL size classes at the 5% level ($G_{adj}=19.36$, $0.05<p<0.1$, *d.f.*=12, Table 4). However, when the patterns of the posterior loop were classified into three body weight classes, as defined by Tanaka (1971), and

Table 4. A test of independence for frequencies between the enamel patterns (A0–A3 and P1–P4) and five CBL size classes of *Eothenomys smithii*.

		Confluent patterns between the 2nd and 3rd spaces				
		A0	A1	A2	A3	Total
Five CBL classes (mm)	20.5-	1	3	1	0	5
	22.0-	1	0	15	3	19
	23.0-	0	4	14	3	21
	24.0-	0	10	19	8	37
	25.0-	0	2	13	2	17
	Total	2	19	62	16	99
		Posterior loop patterns				
		P1	P2	P3	P4	Total
Five CBL classes (mm)	20.5-	1	2	2	0	5
	22.0-	13	4	2	0	19
	23.0-	11	7	3	0	21
	24.0-	17	13	7	0	37
	25.0-	2	8	4	3	17
	Total	44	34	18	3	99

Table 5. A test of independence for frequencies between the enamel patterns (A0–A3 and P1–P4) and three body weight classes of *Eothenomys smithii*.

		Confluent patterns between the 2nd and 3rd spaces				
		A0	A1	A2	A3	Total
Body weight (g)	10.0-	1	3	3	2	9
	20.0-	1	12	41	11	65
	30.0-	0	4	18	3	25
	Total	2	19	62	16	99
		Posterior loop patterns				
		P1	P2	P3	P4	Total
Body weight (g)	10.0-	3	3	3	0	9
	20.0-	36	21	8	0	65
	30.0-	5	10	7	3	25
	Total	44	34	18	3	99

by CBL (20.5-, 23.0- and 25.0- mm based on Table 4), significant associations were found for both dimensions ($G_{adj}=17.69$, $p<0.01$, *d. f.*$=6$, for CBL; $G_{adj}=15.34$, $p<0.025$, *d. f.*$=6$, for body weight, Table 5).

When the total (TM3L), anterior (AM3L), and posterior (PM3L) lengths of the third upper molar were examined in relation to the four enamel patterns on the posterior loop (P1–P4) for each of the four CBL size classes (Fig. 6), it was found that average PM3Ls tend to decrease from P1 to P4 at the 24 and 25 mm CBL classes, whereas average AM3L increased slightly.

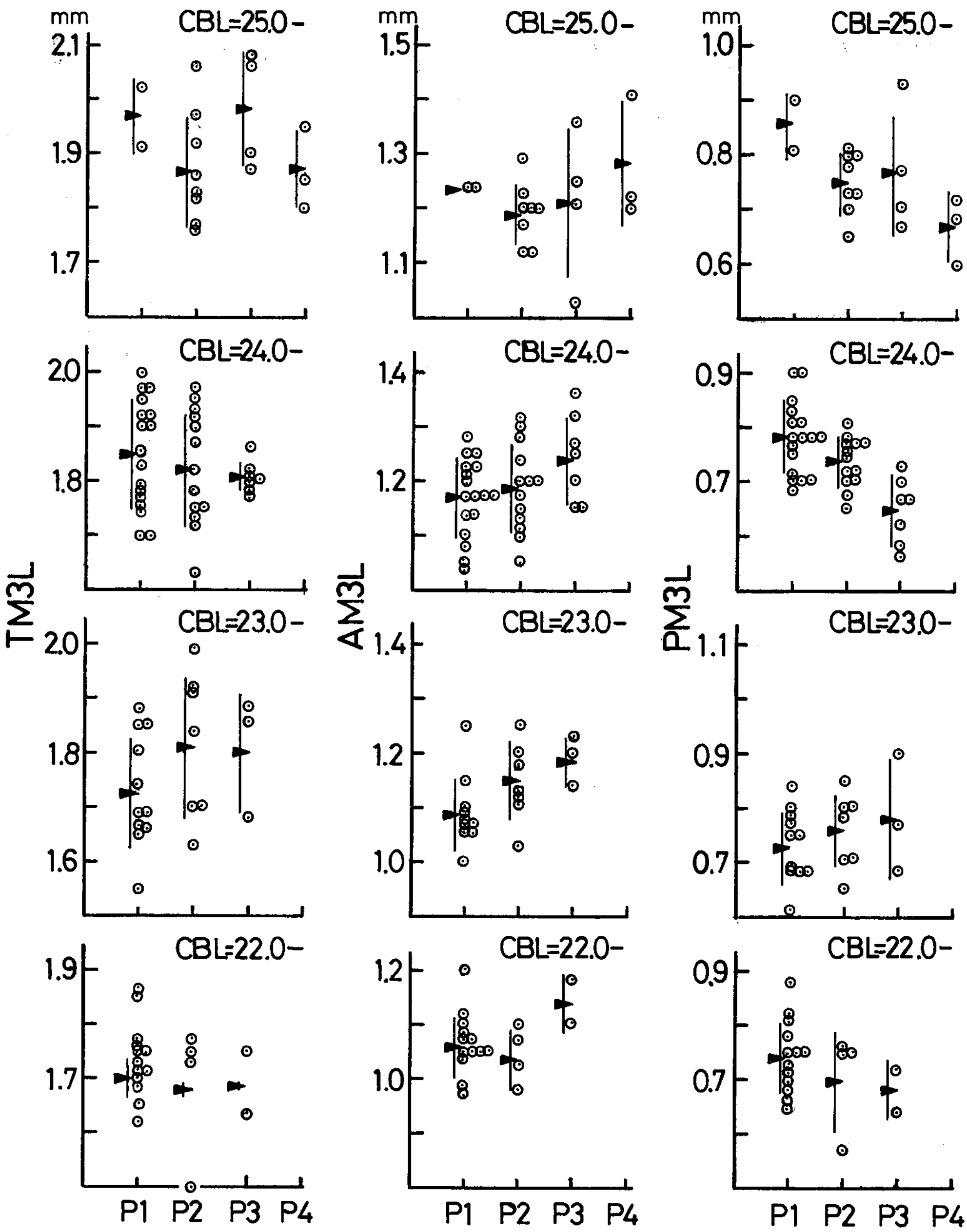

Fig. 6. Measurements of TM3L, AM3L and PM3L against enamel patterns P1-P4 for four CBL classes. A closed triangle shows the average, and a vertical line indicates the standard deviation of the average.

Discussion

In revising the taxonomic position of *Eothenomys smithii*, Tanaka (1971) first showed that no age variation was found on the enamel patterns of the dentine confluence between the second and third triangles and the enamel patterns of posterior loop on the third upper molar. Sixty-six specimens used by Tanaka (1971) were collected at the end of July at 1700 m on Mt. Tsurugi, Tokushima Prefecture, Shikoku, Japan. The breeding season of this vole is at its peak during July when Tanaka (1971) collected specimens, and fully adult voles represented only a small proportion of the population (Kaneko and Morii 1976). In this study 99 specimens collected throughout a year were examined, and these included a larger proportion (26%) of old adults with body weight heavier than 30 g, than in Tanaka's (1971) sample (16%). In the specimens collected for this study, however, the posterior loop of the third molar showed some age variation, as shown by the increase of the frequency of P2, the appearence of P4 (the simple form) and the shallower reentrant fold (DRF) in the largest CBL size class (Figs. 3 and 5, and Table 1).

Table 6. The relationship between enamel patterns and skull sizes in species of *Eothenomys* (data from Kaneko 1990, 1992).

Species	Size (mm)	Posterior loop patterns*				
		Type 6	Type P2	Type P3	Type P4	Total
E. shanseius	12.2-	5	0	1	0	6
(I-M3)	13.1-	6	2	3	2	13
$G_{adj}=52.02$	14.0-	1	6	9	3	19
d. f. = 12	15.2-	0	2	8	16	26
$p<0.05$	16.1-	0	1	6	10	17
	Total	12	11	27	31	81
E. inez	11.3-	1	0	0	0	1
(I-M3)	12.2-	1	2	4	0	7
$G_{adj}=6.28$	13.1-	0	8	12	0	20
d. f. = 9, *n. s.*	14.0-	0	2	3	0	5
	Total	2	12	19	0	33
E. eva	11.3-	0	0	0	3	3
(I-M3)	12.2-	0	3	2	12	17
$G_{adj}=5.74$	13.1-	0	0	9	20	29
d. f. = 6, *n. s.*	14.0-	0	0	0	1	1
	Total	0	3	11	36	50
E. regulus	21.0-	1	2	0	0	3
(CBL)	22.0-	1	3	0	0	4
$G_{adj}=8.94$	23.0-	0	5	1	0	6
d. f. = 15, *n. s.*	24.0-	0	6	2	0	8
	25.0-	1	5	0	0	6
	26.0-	0	6	2	0	8
	Total	3	27	5	0	35

* Type 6 has three salient angles on the lingual side, a short posterior loop, and a confluent dental isthmus between triangles. Except for Type 6, all types appearing in Kaneko (1990, 1992) were followed in the present classification.
n.s. : non-significant.

A statistically significant association was found between the posterior loop patterns (P1-P4) and three CBL classes (20.5-, 23.0- and 25.0- mm, Table 4), because the DRF was nearly constant throughout the 22-24 mm CBL classes but decreased only in the largest 25 mm CBL class (Fig. 3 and Table 1). Furthermore, a test between patterns P1-P4 and the three body weight classes would be significant (Table 5), because body weight correlates siginificantly with CBL ($r=0.911$, $p<0.001$, $d.f.=97$) and individuals with a CBL of more than 25 mm correspond with those of body weights of over 30 g.

As age increases, the pattern of the posterior loop tends to become simple in *Clethrionomys glareolus* (Zejda 1960) and *C. rufocanus* (Abe 1982), which have rooted molars in older individuals. The simple form increases in frequency from the root ratio exceeding 63% in *C. glareolus* and 32% in *C. rufocanus* (Zejda 1960, Abe 1982). Due to the loss of the third reentrant fold with age, the proportion of the simple form increases in *C. glareolus* and *C. rufocanus*. In contrast, age variation was not observed on the loop in *Microtus pennsylvanicus*, which remains rootless throughout life (Oppenheimer 1965).

Among ten species of *Eothenomys* having rootless molars throughout life, a gradual transition is found in the age variation of the posterior loop pattern: in *E. andersoni* the ratio of the simple form of the posterior loop increases with advancing age (Miyao 1966, Kitahara 1995), and in *E. shanseius* the proportion of the simple form increases with increasing CBL, though samples from different populations were pooled (Table 6), resembling in this respect *Clethrionomys glareolus* and *C. rufocanus*. In contrast, age variation has not been observed in either *E. inez, eva, regulus, chinensis, wardi, custos*, or *proditor*, they thus resemble *Microtus pennsylvanicus*, though samples from different populations were pooled (Table 6 and unpublished data). Thus, *E. smithii* is an intermediate type between these two groups showing a little age variation.

In *E. smithii*, the posterior length of the third upper molar (PM3L) ceased to grow with age, though both the total molar length (TM3L) and the anterior length (AM3L) increased with age (Table 1 and Fig. 2). The growth of TM3L, therefore, is related to that of the anterior length. However, when PM3L was plotted against the four posterior loop patterns (P1-P4, Fig. 6), the posterior length tended to become relatively shorter from P1 to P4 as CBL increases. In *C. rufocanus*, the simple form (P4) increases greatly in frequency (Abe 1982), as the length decreases with advancing age (Abe 1973). It is suggested, therefore, that the increase in the frequency of P4 is due to a shortening of PM3L with advancing age. Furthermore, in *E. smithii* it appears that PM3L does not decrease prominently with advancing age (Fig. 2), because the increase in the frequency of the simple P4 pattern is relatively lower than in *C. rufocanus*.

Acknowledgements: My special thanks are due to Dr. K. Maeda, Nara University of Education, for reading a draft, and to anonymous referees for reading and improving the final manuscript.

References

Abe, H. 1973. Growth and development in two forms of *Clethrionomys*. II. Tooth characters, with special reference to phylogenetic relationships. J. Fac. Agr. Hokkaido Univ. 57 : 229−254.

Abe, H. 1982. Age and seasonal variations of molar patterns in a red-backed vole population. J. Mammal. Soc. Japan 9 : 9−13.

Bauchau, V. and J. Chaline. 1987. Variabilité de la troisième molaire supérieure de *Clethrionomys glareolus* (Arvicolidae, Rodentia) et sa signification évolutive. Mammalia 51 : 587−598.

Chaline, J. and J.-D. Graf. 1988. Phylogeny of the Arvicolidae (Rodentia) : biochemical and paleontological evidence. J. Mammal. 69 : 22−33.

Hinton, M. A. C. 1926. Monograph of the Voles and Lemmings (Microtinae), Living and Extinct. British Museum (Natural History), London, 488 pp.

Kaneko, Y. 1989. Seasonal changes of the number collected and reproduction in *Eothenomys smithii* at the foot of a lower mountain, Minoura, Kagawa Prefecture, Japan. Kagawa Seibutsu (15/16) : 67−74 (in Japanese with English abstract).

Kaneko, Y. 1990. Identification and some morphological characters of *Clethrionomys rufocanus* and *Eothenomys regulus* from USSR, northeast China, and Korea in comparison with *C. rufocanus* from Finland. J. Mammal. Soc. Japan 14 : 129−148.

Kaneko, Y. 1992. Identification and morphological characteristics of *Clethrionomys rufocanus, Eothenomys shanseius, E. inez* and *E. eva* from the USSR, Mongolia, and northern and central China. J. Mammal. Soc. Japan 16 : 71−95.

Kaneko, Y. and R. Morii. 1976. Altitudinal survey of small rodents in Mt. Tsurugi, Tokushima Prefecture. Mem. Fac. Educ., Kagawa Univ. II 26 : 43−52 (in Japanese with English abstract).

Kitahara, E. 1995. Taxonomic status of Anderson's red-backed vole on the Kii Peninsula, Japan, based on skull and dental characters. J. Mammal. Soc. Japan 20 : 9−28.

Miyao, T. 1966. Small mammals on Mt. Yatsugatake in Honshu. V. Variation of the third upper molar pattern in *Clethrionomys andersoni*. J. Growth 5 : 7−12 (in Japanese with English summary).

Oppenheimer, J. R. 1965. Molar cusp pattern variations and their interrelationships in the meadow vole, *Microtus p. pennsylvanicus* (Ord). Am. Midl. Nat. 74 : 39−49.

Sokal, R. R. and J. Rohlf. 1973. Introduction to Biostatistics. W. H. Freeman, San Franciso and London, 368 pp.

Tanaka, R. 1971. A research into variation in molar and external features among a population of the Smith's red-backed vole for elucidation of its systematic rank. Japanese J. Zool. 16 : 163−176.

Yoshida, I., Y. Obara and N. Matsuoka. 1989. Phylogenetic relationships among seven taxa of the Japanese Microtine voles revealed by karyological and biochemical techniques. Zool. Science 6 : 409−420.

Zejda, J. 1960. The influence of age on the formation of the third upper molar in the bank vole *Clethrionomys glareolus* (Schreber, 1780) (Mamm.: Rodentia). Zool. Listy 9 : 159−166.

(accepted 3 November 1995)