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Determining the Age of Cyclomorphic Rodents: Functional–Ontogenetic Determination, Ecological Aspects

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Abstract—Functional–ontogenetic determination of age-dependent dental changes (ADCs) in voles has been demonstrated. The relationship between ADCs and the functional state of animals has been analyzed. A scheme and a table for determining individual chronological and biological ages in the bank vole (*Clethrionomys glareolus* Sch.) are proposed, which markedly improve the accuracy of results. Cases of rodent maturation and breeding in winter are considered.

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Key words: rodents, types of ontogeny, age-dependent dental changes, individual age determination.

My previous studies (Olenev, 1989, 2002) deal with functional determination of ontogenetic changes in age markers of rodents and their practical applications. The analysis of these data has made it possible to improve the method for determining the age of voles with rooted molars (the genus *Clethrionomys*), making it almost twice more accurate. However, the proposed method has not gained wide recognition, which can be explained by its certain technical awkwardness, sensitivity to some objective ecological factors operating in the continuously changing environment, and the reluctance of researchers to depart from traditional approaches to age determination. Theoretical and experimental results obtained since then have provided a basis for this paper.

As follows from major reviews dealing with age determination in mammals (Karaseva and Telitsina, 1996; Klevezal, 2007), the majority of biologists still determine the age of small mammals by the methods proposed in the 1970s. Meanwhile, newly discovered trends in the functioning of age markers offer a new approach to this problem.

TECHNICAL AND METHODOLOGICAL FEATURES OF THE STUDY

The results of long-term studies (1975–2004) involving large-scale individual marking of bank voles (*Clethrionomys glareolus* Sch.) Southern Urals provided comprehensive, objective information on the dynamics of abundance and age structure of their population, which made it possible to follow the life course of individual animals during the entire postnatal period.

In this study, I widely used the original functional–ontogenetic approach based on the concept of multiversality of individual development in small rodents (Olenev, 2002). The essence of this approach is that the main criterion for identifying structural units within a population is the functional unity of individuals in groups corresponding to two types of ontogeny. In other words, these groups are identified on the basis of the functional status of individuals (with respect to specific features of growth, development, and reproductive conditions) and the sequence of its changes in time, with each group consisting of individuals united by their functional role in reproduction of the population.

The theoretical and methodological foundations of the functional–ontogenetic approach were developed in the course of studies on populations of cyclomorphic mammals. They provided a basis for interpreting a broad spectrum of phenomena related to various aspects of interactions between animals and their environment under conditions of exposure to a variety of environmental factors. Evidence for functional determination of numerous biological characteristics (demographic and spatial structure; ontogenetic, morphological, and morphophysiological parameters; unconventional interior indices; natural resistance; etc.) allowed successful application of this approach to the age markers of rodents (Olenev, 2002). It should be noted that correct age determination in a given species is possible only on the basis of a detailed and complete description of its ontogeny. The rate of age-dependent dental changes (ADCs) in this study was performed with

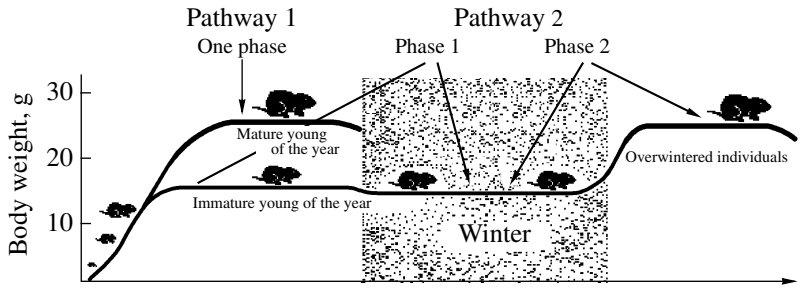


Fig. 1. Scheme of two alternative ontogenetic pathways in murine rodents.

regard to the functional state of individuals related to the type of their ontogeny.

Although the functional–ontogenetic approach has been discussed repeatedly (Olenev, 1989, 2002, 2004), it is expedient to consider again its essence, i.e., the types of ontogeny; otherwise, discussing trends in the dynamics of age markers would be meaningless.

Cyclomorphic mammals, including most species of small rodents, are characterized by cyclic changes in most biological parameters over a period of approximately one year, mass breeding, and generation overlap in the presence of two alternative ontogenetic pathways. Both types of ontogeny fully manifest themselves in rodent populations inhabiting the temperate zone of the Northern Hemisphere and its Arctic periphery, where the climate is sharply continental. Studies were performed in a region where seasonal manifestations of animal living activities are fairly contrasting, but comparison of our results with published data allows us to exclude the effect of regionality.

Below, the basic parameters of the two types of ontogeny are defined and characterized using the example of bank vole, a widespread rodent species (Fig. 1).

The First Ontogenetic Pathway

Mature young of the year. These animal are characterized by monophasic growth. Most of them (usually 70–90%) belong to the first cohorts. They rapidly grow, mature, and enter reproduction upon reaching the definitive body size and weight characteristic of overwintered individuals. These animals have a high metabolic rate, rapidly grow old, and usually die in the year of birth, with their life span ranging from 3 to 6 months. They are stress-reactive and aggressive. The function of this group is to increase population size.

The Second Ontogenetic Pathway

The growth is biphasic. Most animals are of the last cohorts, but there is always a considerable proportion of first-cohort animals remaining immature in the year of birth. Life span is 12–15 months. The main function is to preserve this part of the population with minimal losses until next spring and to begin the cycle of its renewal.

Phase 1. Immature young of the year. The phase covers the period from birth to the spring peak of growth and maturation in the next year. At the age of approximately 1.5 months, irrespective of the date of birth, body weight ceases to increase (Fig. 1). The metabolic rate and stress reactivity of these animals are low, hierarchical relationships in this group are poorly manifested. The rate of aging is almost half that in mature young of the year. The animals of this group are most resistant to a broad spectrum of adverse influences and serve as a kind of population reserve, especially in critical periods.

Phase 2. Overwintered animals (for convenience, all animals living through December 31 are included in this group). Almost all these animals resume growing in early spring, after the "conservation" period, and mature within a short time. Their body weight stabilizes again upon reaching the definitive value for the species (Fig. 1). The rates of metabolism and senescence are similar to those in the group of mature young of the year, although the absolute age of overwintered animals is much greater. Upon sexual maturation, they become highly stress-reactive and aggressive, with dominance in the group being well manifested.

One more feature of this study was that a *reference animal pool* was created by releasing bank voles with precisely determined dates of birth in an isolated plot (an island 2.2 ha in area). Indigenous voles with known dates of birth were also taken into account on the island, while individuals of the same functional status (ontogeny type) but of unknown age were removed in order to preserve the natural age structure of the population. This structure was compared with that "on the continent" (Olenev, 1989). Regular live-trap censuses were taken every month. All trapped animals were weighed and examined to record their functional state, the onset of maturation, involvement in breeding, number of pregnancies, etc. This allowed us to determine the exact period during which each individual remained in a certain functional group (i.e., its functional status was known). If necessary, reference animals of known age and functional status could be taken from the population. In addition to individually marked animals, we

used collections of voles from similar biotopes that were processed by the method of morphophysiological indicators (Shvarts et al., 1968).

To study specific features of the dynamics of body weight and ADCs in voles breeding in captivity, a laboratory colony was established (its founders were taken from the same natural population) in which approximately 20 litters were monitored beginning from birth. ADCs were analyzed using the original scale of age classes (Olenev, 1989) based on consistent changes in the pattern of the M^2 proximal surface hidden in the alveolus (classes 1–6) and then on the tooth root index determined as the ratio of root length to the total tooth length (class 7). It appears that M_1 used in some studies is less suitable as an indicator, since it is more variable and has a characteristic lateral bend.

AGE MARKERS

Age markers consistently changing in the course of mammalian ontogeny are described comprehensively in the monograph by Klevezal (2007). In particular, they include adhesion lines between layers (zones or rings) formed in mineralized recording structures (teeth and bones); the degrees of skull structure development, fusion of cranial sutures, and ossification of epiphyses in the limb bones; and the weight of the eye lens. Unfortunately, these markers are ineffective in studies on short-live species such as small rodents.

In *Clethrionomys* voles, age-dependent changes in tooth structure are often used as such an indicator (Koshkina, 1955; Pokrovskii and Lobanova, 1970; Tupikova et al., 1970; Bashenina, 1981; Prychodko, 1951; Wasilewski, 1952; Zejda, 1971; Mazak, 1962; Haitlinger, 1965; Gruber and Kahman, 1968; Claude, 1970; Klevezal, 2007; etc.). To minimize errors in the original techniques proposed by different authors and to unify the results of analysis, the stage of true root formation (the borderline state between age class 6 and class 7 with a tooth root index of 0.01) was taken as the nodal point of ADC. The data reported by these authors proved to be contradictory, with the estimated ADC rate and the age of true root formation in the bank vole ranging from 0.05 to 0.5 mm per month and from 2 to 8 months, respectively.

It was found that the main factor responsible for these contradictions is the lack of knowledge of relationships between the rate of ADC and the types of ontogeny as well as between a certain degree of ADC and the exact age of an individual. These relationships can be revealed only as a result of long-term individual marking in natural populations. It will be shown below that the degree of ADC is the most objective age marker only slightly subject to random fluctuations (provided it is used correctly). The irreversible, unidirectional course of ADC is also an advantage of this marker. After taking into account trends and nuances of changes in the rate of ADCs, it became possible to propose an adequate method of individual age determination that is effective in a wide range of ecological conditions.

RESULTS OF STUDIES ON AGE-DEPENDENT DENTAL CHANGES

Functional–Ontogenetic Determination of ADCs (Unmarked Animals, Natural Population)

Consider the course of ADCs in a typical sample of bank voles from a natural population (Fig. 2). The lines showing transitions from one age class to another were plotted with regard to the functional status of animals. The rate of ADCs in voles of the first ontogeny type (mature young of the year) proved to be much higher than in those of the second ontogeny type (immature young of the year). The period of transition from age class 1 to class 6 proved to be almost half shorter in the former than in the latter. As follows from characteristics of the two ontogenetic pathways (see above), animals with the first and second types of ontogeny markedly differ in a number of parameters and play different roles in population reproduction. Thus, there is *a distinct relationship between ADCs and the functional status of animals, with the rate of ADCs being determined functionally (as are many other parameters).* Changes in individual values of the tooth root index in the group of overwintered individuals are shown in Fig. 2 with a thick line.

Data on overwintered voles provides the possibility of illustrating certain general trends in ADC variation. As an example, consider two biotopic groups differing in certain features, including shifts in the dates of breeding and age structure (Olenev, 1982) (Fig. 3). It can be seen in this figure that the rate of ADCs (the slope of plots) barely differs from year to year and does not depend on specific biotope conditions, being in essence a species-specific parameter. Conversely, interannual and biotope-related differences in the degree of ADCs (the relative locations of the plots) are considerable. The average level of ADC values in any year depends on the age composition of voles at the onset of wintering. When these animals are mainly of the first cohorts born in the previous year, the wintering population is older (the plots are displaced upward); when they are mainly of the last cohorts, the population is younger (the plots are displaced downward). Four points in circles refer to cases of last-year breeding under snow. According to the degree of ADCs, these animals are markedly older than others: they were born in the previous winter season, prior to the onset of mass spring maturation.

Trends in ADCs (Reference Animals, Natural Population)

The finding that the rate of ADCs depends on the type of ontogeny and the use of precisely dated material (marked voles from the natural reference population)

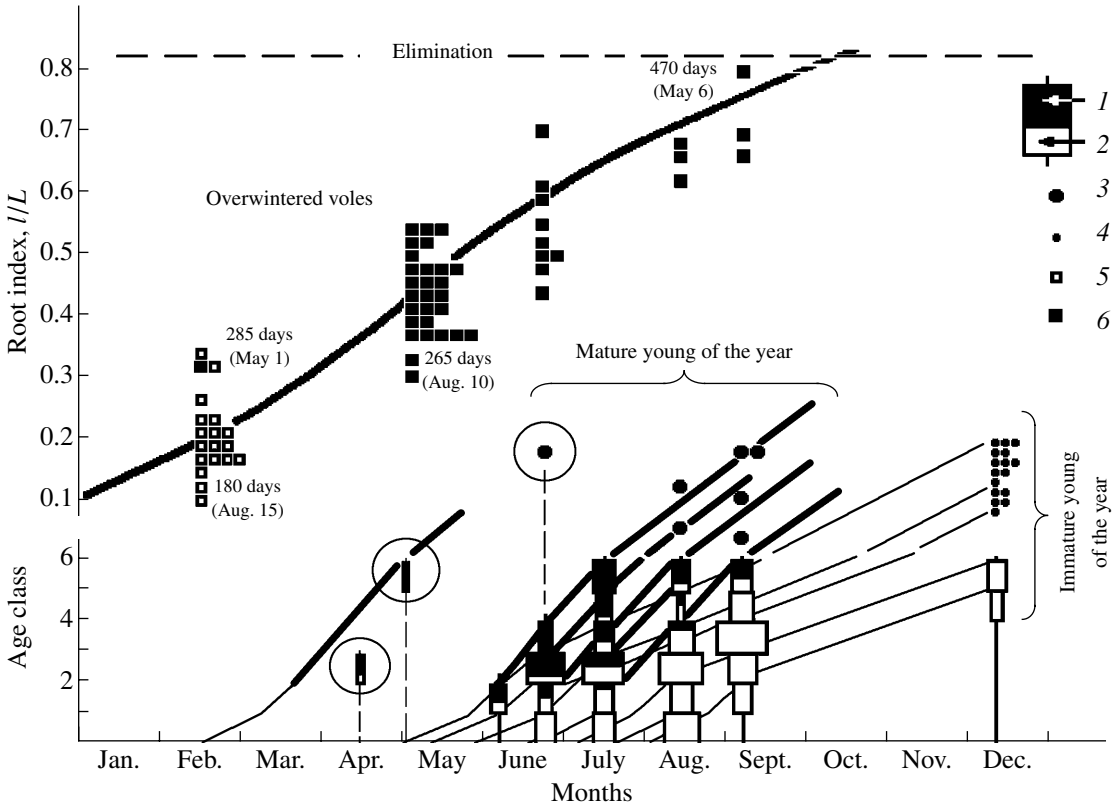


Fig. 2. Patterns of age-dependent dental changes in bank voles with different types of ontogeny. *Medium thick lines* show age-dependent dental changes (transitions from one age class to another) in mature young of the year (**ontogeny type 1**); *thin lines* (up to age class 3) show these changes in juveniles and immature young of the year (**ontogeny type 2, phase 1**); and the *thick line* shows age-dependent changes in average values of the tooth root index in overwintered voles (**ontogeny type 2, phase 2**), with the ages of some of the oldest and youngest individuals in different samples and calculated dates of their birth in the previous year being indicated near the line. Encircled positions refer to winter breeding; (*1*) the proportion of mature young of the year in a given age class, (*2*) the proportion of immature young of the year in a given age class; (*3–6*) tooth root indices for (*3*) mature young of the year, (*4*) immature young of the year, (*5*) immature overwintered voles, and (*6*) reproductive overwintered voles.

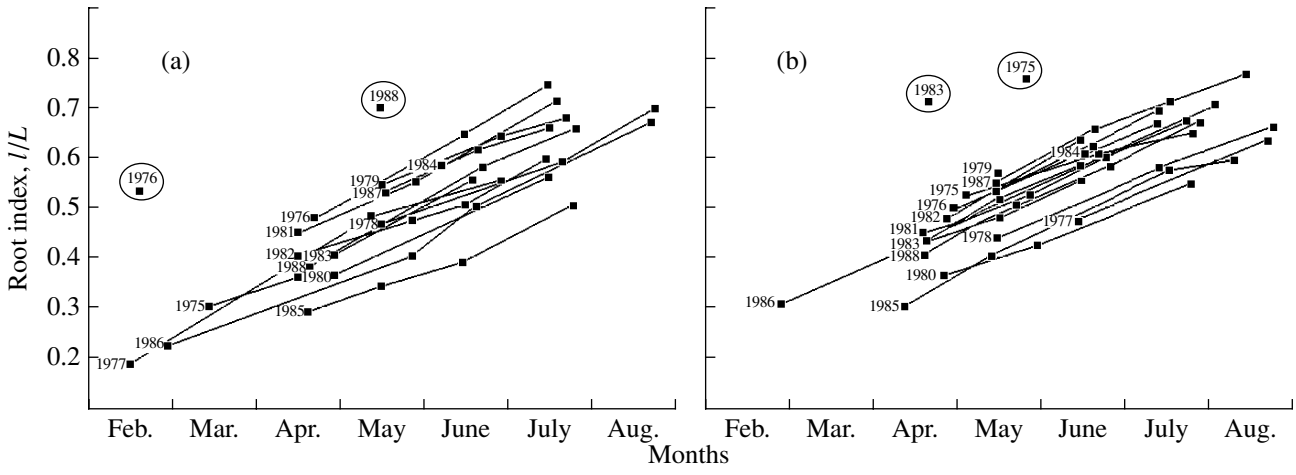


Fig. 3. Interannual and biotopic differences in age-dependent dental changes (average values) within the group of overwintered voles: (a) moist biotopes and (b) dry biotopes. Data recorded in each year are shown as an individual line. Values indicative of breeding under snow are encircled.

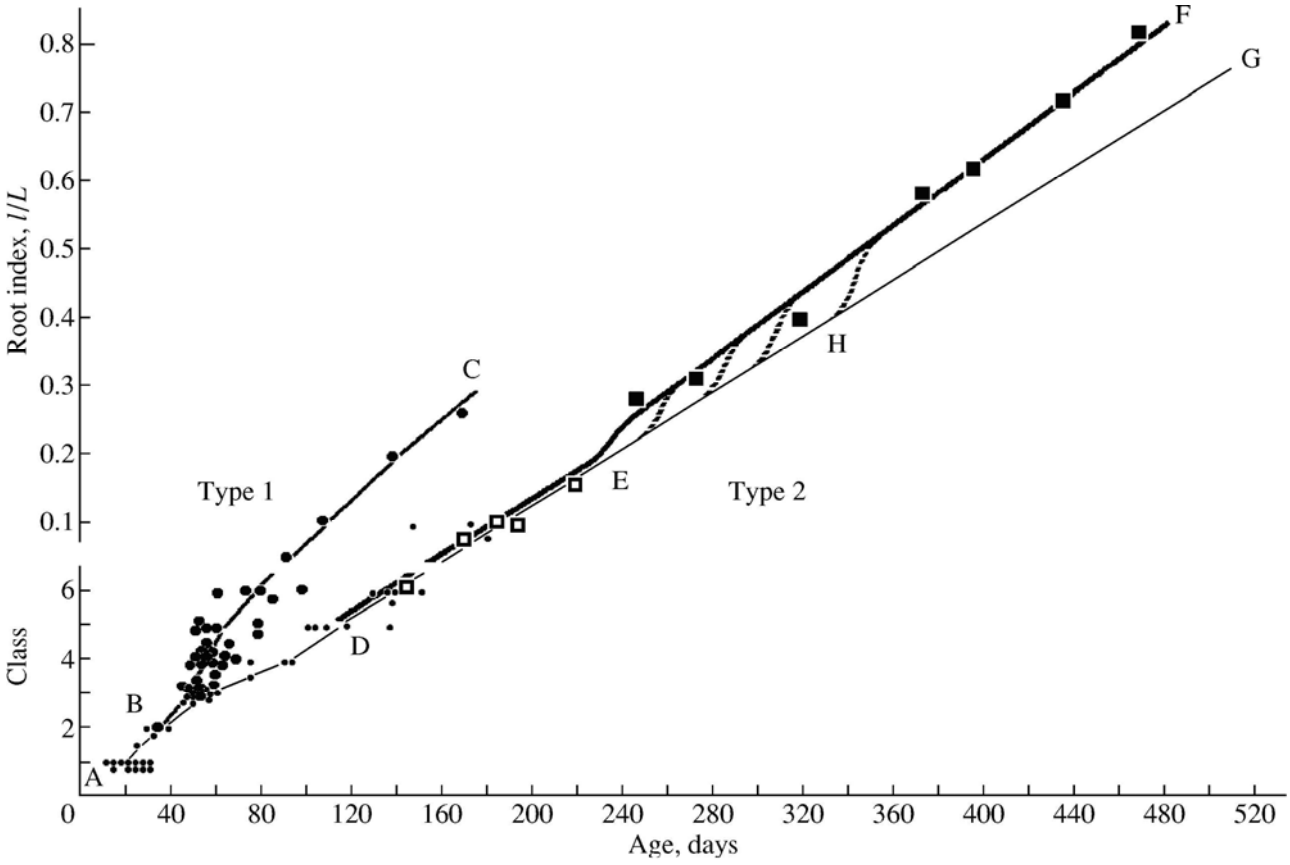


Fig. 4. Age-related changes in teeth (M^2) of marked bank voles from the reference animal pool (natural population) with different types of ontogeny: **AB**, juveniles (the type of ontogeny is not yet manifested); **BC**, mature young of the year, ontogeny type 1 (•); **BDE**, immature young of the year, ontogeny type 2, phase 1 (·); **DEF-DEHF**, overwintered voles, ontogeny type 2, phase 2 (□ immature and ■ mature individuals); **DE**, common segment (both immature young of the year and overwintered individuals); segment **EH** is the range of ages (220-340 days) at which overwintered voles usually begin to reproduce in spring (broken lines reflect variation in the age of maturity); and **BDEHG** is the segment suitable for calculating the biological age of individuals.

provided the possibility of representing corresponding relationships graphically (Fig. 4). Despite a long observation period (12 years), the numbers of reference animals were small, especially in the overwintered group, which is explained primarily by high natural mortality and emigration from the marking plot.

In general, the plot of the ADC rate for individuals with different types of ontogeny is forked and has two branches, ABC and ABDEF (Fig. 4). The pattern of their divergence resembles that of the two ontogenetic pathways (see Fig. 1).

J u v e n i l e s. The corresponding segment of the plot (AB) is common to voles with different types of ontogeny and refers to actively growing and developing young (immature) individuals prior to their possible divergence in the type of ontogeny (at point B).

Diagnostic characters. Although the morphophysiological parameters of these animals may be indicative of the onset of sexual maturation (enlarged testes or uterus), they cannot yet be attributed to the first ontogeny type by formal criteria. Attributing them to the second type would also be erroneous, although their immaturity formally corresponds to this type. Such individuals have a high metabolic rate, and their growth and development still continue. Thus, they form a special group that should be analyzed separately. The degrees of ADCs in most of these animals are of age classes 1 and 2, which corresponds to the period from their emergence from the nest to the age of 30–45 days. As a rule, their body weight does not exceed 12 g. Individuals captured in nature usually weigh no less than 8 g (rarely, 6 g). Their age is determined as shown in Table 2.

Causes of errors: individual differences in body weight at birth, incorrect attribution to the functional group, and individual variability.

M a t u r e y o u n g o f t h e y e a r, o n t o g e n y t y p e 1 (Fig. 4, plot segment BC). The intensive growth and development of these individuals continue, as in the group of juveniles.

Diagnostic characters. Body weight upon maturation usually exceeds 14 g, ranging from 17 to 30 g (Olenev, 1989, 2004). Males have well-developed testes weighing more than 150 mg (Olenev, 1983), with sperm-filled epididymides. Their weight may decrease

in autumn, after the breeding season (Olenev et al., 1980). Males with "collapsed" (turgorless) testes and epididymides should also be included in this group (Olenev, 1979).[1] Females have suckling spots, an open vagina or a vaginal plug, a thickened uterus, embryos, corpora lutea, placental spots, and enlarged adrenals. These animals are of the first ontogeny type and should be analyzed as a separate group. The rate of ADC is maximum for the species, with the root index changing by up to 0.09 per month. The age is determined by the key shown in Fig. 5.

Causes of errors: individual differences in the absolute age at the onset of maturation (a manifestation of the first ontogeny type), incorrect diagnosis of the group, or inaccuracy in determining the degree of ADC (by the key shown in Fig. 5).

Immature young of the year; ontogeny type 2, phase 1 (Fig. 4, plot segment BDE). When these individuals exit the juvenile group, their growth and development slow down and they fail to mature in the year of birth.

Diagnostic characters. Body weight stabilizes at 14–20 g; in winter, this range usually narrows to 15–18 g due to the "gate effect" (Olenev, 1979). Males have underdeveloped but turgid testes (weighing less than 10 mg) and epididymides; in females, the vagina is closed and the uterus is threadlike. The rate of ADCs is minimum for the species, with the root index changing by about 0.06 per month (i.e., this rate is 1.5 times lower than in mature young of the year). The age is determined by the key shown in Fig. 5.

Causes of errors: inaccuracy in determining the degree of ADCs.

Overwintered animals; ontogeny type 2, phase 2 (Fig. 4, plot segments DEF and DEHF). In practice, it has proved convenient to date this group from January 1. These animals usually remain immature until spring (segment DE).

Diagnostic characters. Body weight upon maturation usually ranges from 21 to 34 g. Males have well-developed testes weighing more than 150 mg, with sperm-filled epididymides; females have an open vagina or a vaginal plug, suckling spots, a thickened uterus, embryos, placental spots, corpora lutea, and enlarged adrenals. These animals are of the second ontogeny type and should be analyzed as a separate group. The rate of ADCs temporarily increases with the onset of maturation and then becomes similar to that in mature young of the year.

Causes of errors: inaccuracy in determining the degree of ADCs, individual differences in the absolute age at the onset of spring maturation. The possibility of incorrect diagnosis of the group is practically excluded: overwintered voles can hardly be mistaken for mature young of the year by the degree of ADCs, since they are at least 8 months older than the latter group (the tooth root index in them usually exceeds 0.2 by spring, whereas the degree of ADCs in mature young of the year is only of the first classes).

Although different individuals mature and enter reproduction almost simultaneously and are similar in their functional status, they may differ in absolute age. In Fig. 4, the DE segment of the plot refers to both immature young of the year and overwintered individuals. This is due to differences in the dates of their birth in the previous year. By the end of this year, the age of spring-born animals (from the first cohorts) reaches 240 days, while that of autumn-born animals (from the last cohorts) is only 120 days; adding 100 more days elapsed between January 1 and April, we obtain that they enter reproduction at ages of about 340 and 220 days, respectively. In the EH segment, broken lines show differences in the age at which overwintered individuals become reproductive. A certain increase in the rate of ADCs in this period coincides with the spring growth peak (i.e., the degree of ADCs and body weight increase almost synchronously).

An important fact is that the highest values of the tooth root index recorded in nature never exceeded 0.87, which corresponded to an absolute age of 510 days. Apparently, further tooth wear leads to tooth crown breakage, which makes the animal incapable of normal feeding and is therefore lethal. Thus, such individuals have completed their function in the population and are eliminated from it, with ADCs being a factor limiting the period of their presence in the population.

The factual material (data on the reference animal pool) analyzed using the functional–ontogenetic approach and subsequent logical considerations allowed us to proposed a practical key for determining the age of bank voles (Fig. 5). Attention should be paid to differences in the time required for transition from one age class to another. It is clear that they do not result from sharp changes in the rate of ADCs but are an inevitable consequence of arbitrary division into classes. It should be noted, however, that the rate of ADCs increases temporarily in the periods of rapid growth and maturation accompanied by intensification of metabolism (See Fig. 4, the region of point B and segment EH).

Practical Algorithm of Age Determination with Regard to the Type of Ontogeny

(1) The tooth is extracted from the alveolus with forceps immediately after cleaning the skull by boiling. If a dry skull is used, it is necessary to separate the alveolar bone tissue from the tooth (on its buccal side) with a thin injection needle and, rocking the tooth, extract it

[1] In natural bank vole populations, reproductive male young of the year perish after breeding and disappear by spring. However, such reproductive males caught in autumn and kept in a vivarium separately from each other sometimes return to immaturity (their testes and epididymides collapse, body and adrenal weights decrease), overwinter, and show signs of rematuration, making attempts to enter reproduction in spring.

by pushing toward the lingual side, taking care not to damage the proximal tooth surface used for diagnosis.

(2) The degree of ADCs (age class) is estimated under a binocular microscope with reference to the key (Fig. 5). If tooth roots are detectable, measurements are made to calculate the root index.

(3) Thereafter, the type of ontogeny characteristic of a given individual is determined. Initially, the animal is attributed either to the overwintered group (ontogeny type 2, phase 2) or to young of the year (both groups of the latter are described in detail above). Errors in this task are practically excluded due to major differences in the degree of ADCs (the tooth root index in the overwintered group exceeds 0.2 by spring, whereas young of the year show only the first classes of ADCs) (Fig. 2). Among young of the year, differentiation between reproductive (type 1) and immature (type 2, phase 1) is made on the basis of functional diagnostic characters described in detail above (see also Fig. 1). As a result, all animals in the sample are divided into groups corresponding to ontogeny type 1 and two phases of ontogeny type 2; the group of juveniles is analyzed separately.[2]

(4) After determining the class of ADCs (or root index) and the type of ontogeny, the age of an individual is estimated using the key (Fig. 5).

(5) If necessary, the date of birth and biological age of the individual are calculated.

Retrospective Analysis of the Results of Age Determination by Different Authors in Light of the Functional–Ontogenetic Approach: Causes of Inconsistencies

Many authors have noted seasonal differences in the rate of ADCs in rodents from natural populations without making an attempt to explain them. The causes of inconsistencies in published data on the ADC rate become clear when these data are analyzed in terms of the functional–ontogenetic approach, due to which the age of rodents can be determined almost two times more accurately. The point is that the majority of researchers performed their studies without regard to specific features of ADCs in animals differing in their functional status, which were represented in the samples in different proportions. For example, if the samples were taken in autumn, they consisted mainly of individuals with the second type of ontogeny (immature young of the year) and, therefore, with the lowest ADC rates. Conversely, if the samples were from laboratory colonies (animals bred in a vivarium), the authors dealt mainly with individuals of the first ontogeny type characterized by the highest ADC rates. Importantly, the lowest and highest ADC rates reported by different authors proved to be close to the rates recorded in this study for individuals with different types of ontogeny. Thus, the causes of inconsistencies in published data are now apparent.

Most authors tacitly link seasonal differences in the ADC rate with the time of birth (at best, with seasonal generations), considering that virtually all animals born in spring are involved in breeding.[3] Unfortunately, they ignore the important fact that, although spring-born animals are usually of the first ontogeny type (mature young of the year), there is always a considerable proportion of individuals with the second type of ontogeny (young of the year remaining immature in the year of birth), which differ specifically in the rate of ADCs. This proportion is no less than 10–30% but may reach 100% in certain years (Olenev, 2002, 2004). The results of our 30-year observations on marked animals show that the date of birth itself has no decisive significance. The main factor is the ontogenetic pathway followed by an animal: spring- and autumn-born individuals with the same type of ontogeny do not differ in the rate of ADCs. Therefore, an analysis of ADCs in each individual should be performed taking into account its type of ontogeny.

As an example, consider the method of age determination in *Clethrionomys* voles proposed by Tupikova et al. (1970), which has been justly regarded as efficient and enjoyed wide use until recently. However, a comparison of the results obtained by this method with our data on the reference population (Table 1) reveals errors that are often systematic. Thus, according to these authors, the initial stage of tooth root formation takes place at the age of 60 days, but our data show that this applies only to individuals with the first type of ontogeny, whereas the corresponding age for those with the second type is almost twice as great. Errors are also observed when the ratio of root length to tooth length is used. Apparently, the samples they analyzed were mixed and consisted of individuals with either type of ontogeny, which accounts for certain coincidences with our data as well as for significant differences from them. According to some authors who indicated the extreme ages of tooth root formation (even when the lower molar M_1 was used), *the earliest age* corresponding to this degree of ADC was 2 months, which is characteristic of individuals with the first type of ontogeny (they apparently occurred in the samples). The data concerning *the latest age* are somewhat ambiguous, although agree fairly well with those for individuals with the second type of ontogeny. A probable explanation of this ambiguity is that true root formation at a relatively late age is characteristic of individuals from laboratory colonies, which were used by the majority of researchers.

[2] Individual age within the group of juveniles is determined by body weight, according to Table 2.

[3] An alternative to analysis of seasonal generations or weight groups is to take account of individuals with different types of ontogeny and to perform separate analysis of samples at the level of functional groups. This allows the study of "pure" samples, with all consequent advantages.

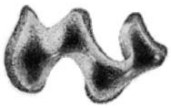
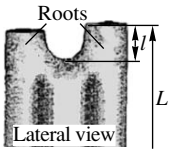
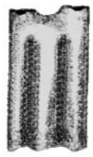
Age class	View of M^2 alveolar tooth end	Description	Age (variation range), days	
			ontogeny type 1, mature young of the year	ontogeny type 2, immature young of the year and overwintered voles
1		The alveolar tooth end (especially on the buccal side) looks as if it were made up of triangular prisms with acute edges, each containing a closed pulp cavity	25 (–30)	25 (–30)
2		The edges of all prisms are slightly blunted, pulp cavities remain closed. A bone film subsequently forming bone vesicles appears at reentrant angles	35 (25–45)	35 (35–45)
3		The edges of prisms at the alveolar tooth end are blunted, and their basal parts are bent toward the tooth center. The tooth has a single (merged) pulp cavity, in which bone vesicles are visible	50 (40–60)	55 (45–65)
4		The edges of prisms at the alveolar tooth end are blunted, their basal parts on the buccal and lingual sides are strongly bent toward the tooth center. The pulp cavity is 8-shaped and contains bone vesicles	55 (45–70)	90 (75–100)
5		Central parts of the buccal and lingual tooth edges are almost closed on each other so that the pulp cavity is dumbbell-shaped	65 (50–80)	115 (100–130)
6		The tooth has two separate, almost circular pulp cavities. This is the first stage in the formation of roots, which are as yet impossible to measure	75 (60–95)	135 (120–155)
7		The tooth has distinct roots, which can be measured from the lateral view. The tooth root index is calculated as the ratio of the maximum root length (l) to the maximum tooth length (L)		
		Root index 0.1	105 (90–120)	180 (155–200)
		Root index 0.2	135 (120–150)	225 (210–245)
		Root index 0.3	175 (160–190)	265 (245–285)
		Root index 0.4	210 (no data)	305 (285–325)
		Root index 0.5		345 (325–365)
		Root index 0.6		390 (370–410)
		Root index 0.7		430 (410–450)
		Root index 0.8		470 (no data)

Fig. 5. Illustrated table for determining the age of voles with rooted molars (*C. glareolus*) from natural populations by the pattern of proximal (alveolar) M^2 tooth end and root index. Teeth of age class 7 are shown from the buccal side, masticatory surface down.

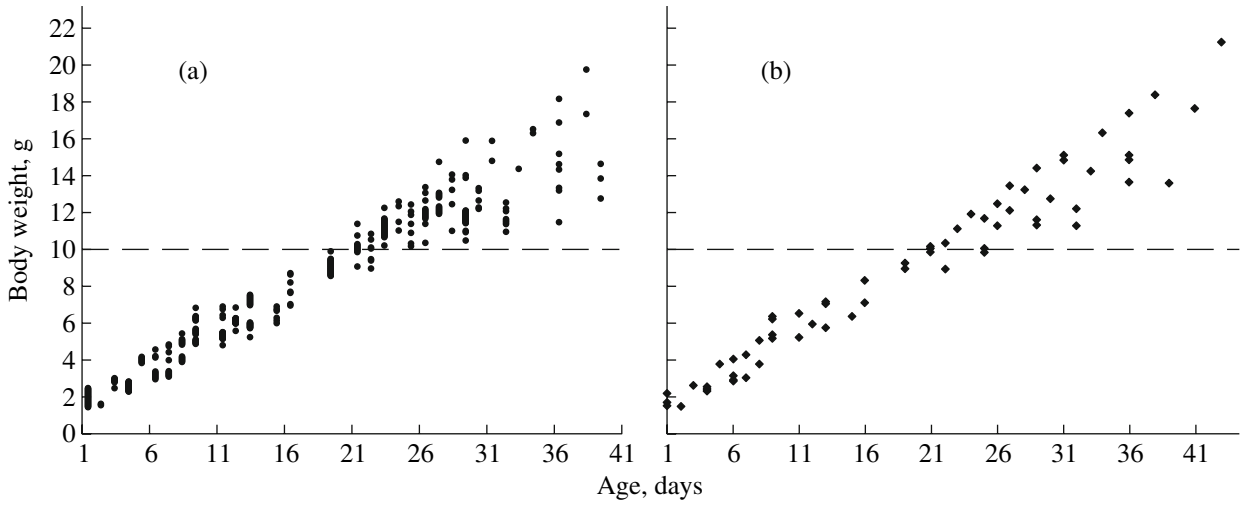


Fig. 6. Dynamics of body weight-to-age ratio in bank voles from the laboratory colony: (a) individual values and (b) average values for individual litters. The broken line delimits the range of body weight values suitable for correct age determination.

As shown previously (Olenev, 1989), the data on ADC obtained with animals from laboratory colonies cannot be properly extrapolated to rodents from natural populations. However, the rate of ADC in immature young of the year (ontogeny type 2, phase 1) has proved to be equal both in laboratory colonies and in natural populations, with the tooth root index increasing by 0.06 per month. Hence, it has been assumed that this is the minimum possible ADC rate that may be regarded as a species-specific character. As a rule, samples from natural populations taken in spring and summer consist of individuals with either type of ontogeny, each characterized by a specific ADC rate. This fact should always be taken into account; otherwise, errors in age determination will inevitably increase almost twofold.

ADDITIONAL PARAMETERS THAT MAY BE REQUIRED FOR AGE DETERMINATION (TRENDS AND NUANCES)

Body weight. In the initial period of postnatal ontogeny (in the juvenile group), body weight changes linearly (Fig. 6) and, therefore, can serve as a reliable indicator of individual age. The degree of ADCs in this period corresponds to class 1. In the sample plotted in Fig. 6, almost all individuals reached maturity at the age of 1.5 months (ontogeny type 1). Maturation of summer-born young of the year in vivarium colonies is an established fact (Pokrovskii and Bol'shakov, 1979). It is noteworthy that the degree of point scattering in the plot remains almost the same in the segment corresponding to the period from birth to the time when body weight reaches 10–11 g (Fig. 6, broken line). This is so

Table 1. Comparative table showing systematic errors in age determination that are inevitable when no attention is paid to specific features of age-dependent dental changes related to the type of ontogeny

Data by Tupikova et al. (1970)		Data on the reference population		
Conditional age group	Age, days	Age class and root index	Age, days	
			ontogeny type 1	ontogeny type 2
I	30	1	25 (up to 30)	25 (up to 30)
II	60	3	50 (40–60)	55 (45–65)
III	60	5	65 (50–80)	115 (100–130)
III	60	6	75 (60–95)	135 (120–155)
Root index <1/4	90–120	0.1	105 (90–120)	180 (155–200)
1/4	150–180	0.25	155 (140–170)	245 (230–260)
1/3	210–240	0.33	187 (175–200)	278 (260–300)
1/2	270–300	0.5	Absent	345 (325–365)
2/3	330–360	0.7	″	430 (410–450)
XI, XII	360–480	0.8	″	470 (450–)

because individual differences in body weight are retained for a relatively long time despite the possibility of compensatory growth, on the one hand, and keeping conditions are also relatively stable, on the other hand. Differences in the average body weight of individuals from different litters indirectly confirm this conclusion (Fig. 6).

A generally similar picture, but with certain nuances, is also characteristic of young of the year from the natural population. A large proportion of these animals fail to mature in the year of birth (ontogeny type 2), and the period of their maturation is extended to at least 25 days, averaging 30 days (Olenev, 2002). This is due mainly to the diversity of variable environmental conditions to which the animals are exposed during the period of transition to adult life. In small rodents, it begins when the young are weaned and start leaving the nest. This is when environmental conditions determine the ontogenetic pathway that the animal will follow. The scattering of individual body weight values increases significantly during this period, which imposes limitations on the use of weight as an indicator of age. In animals from laboratory colonies, this takes place when the weight exceeds 10 g, compared to 9 g in natural populations. Moreover, the growth of certain individuals in natural populations sometimes becomes retarded after exceeding 9 g, with their body weight stabilizing temporarily. This may be a source of additional errors in analysis.

Consideration of these features and limitations provides for a rational approach to the use of individual body weight for calculating individual age (Table 2).

Chronological (absolute) and biological (functional) ages. In research on rodents, attention is basically paid to the *absolute age* of an individual, or the period (days) from its birth to capture. However, it is sometimes necessary to determine the *biological age* of an individual, which reflects the level of its morphofunctional development, parameters of life activities, the type of ontogeny, and the period of being at a certain phase of ontogeny.

The rate of change in age markers (in our case, ADCs) depends on the intensity of metabolic processes and correlates with the rate of senescence. To determine the biological age of an individual, it is necessary to estimate the time of its stay within a certain group (Figs. 1, 4), provided the rate of ADCs is known for each group. In practice, the plot shown in Fig. 4 is used for this purpose. The biological age is determined by substituting the recorded value of the tooth root index or age class in the segment BDEHG; i.e., it is temporarily assumed that the second type of ontogeny is characteristic of a given individual. The possibility of determining both chronological and biological ages provides a basis for rationally composing samples for analysis and solving specific research problems.

Winter maturation and breeding have a special place in the biology of cyclomorphic rodents. In the Ural region, for example, cases of breeding under snow have been described for a number of rodent species, including the steppe lemming (*Lagurus lagurus* Pall.), field vole (*Microtus agrestis* L.), northern red-backed vole (*Clethrionomys rutilus* Pall.), mice of the genera *Apodemus* (*Sylvaemus*) and *Mus*, root vole (*M. oeconomus* Pall.), common vole (*M. arvalis* Pall.), and narrow-skulled vole (*M. gregalis* Pall.) (Nikiforov, 1956; Shubin and Suchkova, 1973; Rusakov and Starikov, 2001; etc.). However, as noted by these authors themselves, more detailed studies have shown that breeding under snow in most cases is accounted for by anthropogenic factors, in particular, easily available food in the form of afterharvest crop residues, straw, haystacks, nearby granaries, etc. In fact, this is an artificially stimulated type of breeding.

Winter breeding is a common phenomenon in regions with mild winters, e.g., in Ukraine (as follows from our observations in Zhitomir oblast and data obtained by L.G. Vinogradskaya). On the other hand, mass breeding in lemming populations at the phase of population growth in the Subarctic may begin early, before snow melts. This is true winter breeding, which gives rise to the additional (third) generation, with the number of cohorts sometimes exceeding five (Sutton and Hamilton, 1932; Koshkina and Khalanskii, 1961; Krebs, 1970; Chernyavskii, 1979; Chernyavskii and Tkachev, 1982; etc.).

In addition, it is necessary to distinguish true winter breeding in natural habitats—untimely maturation in winter followed by mating (i.e., premature phase 2 of the second type of ontogeny)—from prolonged breeding in late autumn, which may be retrospectively regarded as winter breeding. As a rule, *true winter* breeding takes place in the second half of winter and,

Table 2. Individual ages calculated from body weights of bank voles in the period of early postnatal ontogeny (samples from a natural population and from a laboratory colony derived from the same population)

Body weight, g	Age, days	
	laboratory colony	natural population
2	1–4	1–4
4	7 ± 3	7 ± 3
5	10 ± 3	10 ± 3
6	12 ± 3	12 ± 3
7	15 ± 4	15 ± 4
8	17 ± 4	17 ± 4
9	20 ± 4	21 ± 5
10	23 ± 5	**25 (18–32)**
11	**27 (20–34)**	**29 (20–38)**
12	**29 (21–40)**	**33 (21–53)**

Note: Values shown in boldface are unreliable because of drastically increased error.

according to our observations, only under conditions of increasing daylight period (in the Northern Hemisphere, after December 22). It should be noted that this response depends not on the length of the daylight period but specifically on its consistent increase. One more precondition is sufficient food supply, which stimulates maturation to some extent and is necessary for satisfying the high energy requirements of breeding animals. Snow depth and, to a lesser extent, ambient temperature are also important. Some trends are confirmed in the course of vole breeding under laboratory conditions. Reproduction of mature young of the year brought to the vivarium from natural populations and kept in pairs continue breeding in winter, while immature young of the year brought in the same period and kept under the same conditions fail to mature. As soon as February, however, a diet supplemented with green food stimulates their maturation and subsequent breeding, although no such effect is observed against the background of a decreasing daylight period (Pokrovskii and Lobanova, 1970). By analogy with plants, such individuals should undergo "vernalization."

True winter breeding in regions with distinct winters is a very rare phenomenon that has been recorded only a few times over the whole period of our observations. An illustrative example is the case of bank vole breeding under snow in February 1986 (Olenev, 1988): it took place in only one biotope type (the floodplain of a small forest river) where the yield of bird cherry fruits in autumn was unusually abundant. The stomach contents of voles were rich in these fruits, and "feeding stations" with bird cherry stones gnawed through by rodents were found after snow melted. It should be noted that the survival rate of winter-born young was extremely low, and only single individuals managed to reach maturity. Mass mortality was observed in the spring–summer period characterized by unstable environmental conditions. In some situations, however, the ecological reserve provided by winter breeding may be relevant for the population.

Specific features of ADC analysis in the case of winter breeding. Before determining the type of winter breeding, it is necessary to make sure that it actually took place. Primary evidence comes from the presence of young individuals in spring catches made before the onset of mass breeding and from the interior parameters of these animals. In *Clethrionomys* voles, the fact of winter breeding is confirmed by the presence of individuals with unusually high degrees of ADCs, which deviate from the common plot (encircled points in Figs. 2 and 3). Once recorded, such unusually high values are usually observed in subsequent samples as well, with the range of their deviation from the plot remaining unchanged until the animals die. The dates of birth calculated by the table fall on the past winter period (see Fig. 2) or, in some cases, on the winter of the previous year (see Fig. 3).

CONCLUSIONS

The trends and reference parameters of ADCs revealed in this study appear to be fully valid for the bank vole. Moreover, they can be successfully used for age determination in other voles of the genus *Clethrionomys*, e.g., the northern red-backed vole (*Cl. rutilus*). There are reasons to believe that the functional–ontogenetic approach is also applicable to studies on other age markers in most species of cyclomorphic rodents. The most important factor is selection of a valid marker. For example, Kolcheva (1992) used as a marked the degree of cusp wear in the molars of pygmy wood mice (*Apodemus* (*S.*) *uralensis* Pall.). The results of her study confirmed functional determination of age-dependent changes and provided for significant improvement in the accuracy of individual age determination in mice.

The rate of ADCs is almost invariable from year to year and independent of specific conditions in natural habitats and phases of the population cycle. Therefore, it can reasonably be regarded as a species-specific character. This rate in the group of immature young of the year is minimal for the species, being 1.5 times lower than in mature young of the year (the tooth root index increases by about 0.06 vs. 0.09 per month). Teeth with extreme values of this index are prone to crown breakage, which is fatal to the animal. Thus, ADCs are a factor limiting the period of an individual's presence in the population.

It should be emphasized that trends in ADCs characteristic of individuals from natural populations should not be extrapolated to animals from laboratory colonies, and vice versa.

Trends in functional determination of ontogenetic changes in the age markers of rodents deserve due attention; otherwise, errors in determining individual age will inevitably increase almost twofold.

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