

FUNCTIONAL DETERMINATION OF ONTOGENETIC CHANGES IN AGE MARKERS IN RODENTS AND THEIR PRACTICAL UTILIZATION IN POPULATIONAL INVESTIGATIONS

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The dynamics of basic intrapopulation parameters were studied in a natural population of the bank vole (*Clethrionomys glareolus*) in field conditions using individual live tagging (1975-1987). The possibility was analyzed of two pathways of ontogenetic development which are realized in nature (manifestation of polyvariant development); this was reflected in the functional approach presented by us and used during analysis of the age structure of populations at the level of physiological functional groupings (PFG). At the level of PFG, patterns of functioning of age markers were studied in detail in rodents. The functional approach permitted reasons for basic contradictions and difficulties to be removed and explained when determining the age of animals according to degree of age changes in teeth. Accuracy increased and errors were reduced by two times when determining age of vole molars.

In the 1970s Academician S. S. Shvarts (1980), examining concepts concerning pathways of evolutionary transformation of populations, wrote: "The primary task in evolutionary ecology is to establish how the populational structure of a species influences the course of the evolutionary process, or expressed differently, what the interassociation is between the ecological and genetic structure of a population, how change in ecological structure of a population affects its genetic composition" (p. 164). He considered that one of the important aspects when resolving this question is investigation of the specifics of seasonal generations of rodents, animals which are a convenient object for such purposes. Having a short life cycle, being "ephemeral animals," they are also simultaneously cyclomorphic animals; almost complete replacement of the population occurs during a year.

The question of the specifics of seasonal generations was raised by S. S. Shvarts in the following way: "We do not always know what the specific reasons are which determine the morphophysiological features of specific generations of rodents, but we know precisely that all of them can be reduced to two fundamentally different factors:

1. Morphological specifics of seasonal generations, the result of direct reaction of an organism to change in environmental conditions.
2. Morphological specifics of seasonal generations, the result of reorganization of the genetic structure of populations" (p. 165).

S. S. Shvarts arrived at the conclusion that change in ecological (in this case, age) structure of a population is inseparably associated with change in its genetic structure. Change in ecological structure of populations must, therefore, be regarded as a most important factor in the microevolutionary process.

This permits discussion of the importance of age structure in dynamics of populations, but in rodent populations, which are distinguished by a high degree of complexity in age structure, it is the basic factor for a broad spectrum of populational transformations. This position was reflected by us in a functional approach developed by us, which has been successfully used during analysis of the age structure of populations.

During 1975-1987, field investigations were conducted in the V. I. Lenin Il'menskii Preserve (a band of pine-birch forests in the forest-steppe zone of the Southern Urals). Mass individual tagging of animals produced complete and objective data concerning dynamics of numbers and age structure and, primarily, the fate of individual animals could be tracked

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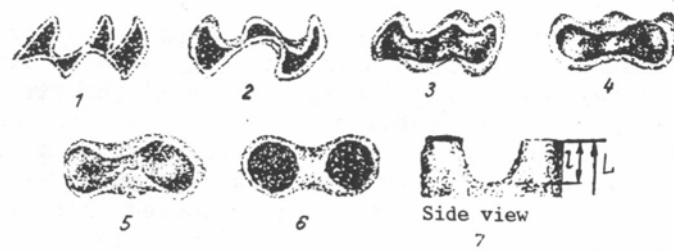


Fig. 1. Age classes of bank vole teeth distinguished according to pattern of the socket (root) surface of M2. Description of classes: 1) socket surface has the appearance of sharply expressed prisms with sharp angles; 2) angles are slightly flattened, the edges are somewhat concave inward, but there is no oval yet; 3) there is an oval, the central part is somewhat narrower in width, the edges are slightly concave inward; 4) appearance somewhat reminiscent of a figure eight, the central section is considerably narrower, but there are no junctions yet, edges are strongly concave inward; 5) central sections are closed, there is a bridge (in the form of a closed figure eight); 6) the bridge has just disappeared, two closed spaces are distinguished, the first stage of formation of the root; 7) root is clearly expressed (the root index is the measure of: ratio of length of the root to length of the tooth).

during the entire period of postnatal development. In addition, in an isolated section (an island with an area of 2.2 ha) a "bank of reference animals" was created as a result of the release of rodents with a precisely known birth date; aboriginal animals have also been preserved on the island with precisely known ages. At the same times, animals with close, but not defined ages, were removed from the site in order to preserve the natural age structure, which was controlled by comparison with that on the mainland.

During regular monthly captures of animals, they were weighed and the condition of their reproductive systems was recorded according to external features: buildup of the epididymis, nipples, vaginal plugs, et al., the onset of maturation was noted, as well as entry into breeding and number of pregnancies*. This permitted the time of placement of each animal into a specific **physiological functional grouping (PFG)** to be determined adequately precisely and its functional status to be known.

In addition to tagging (more than 1000 animals), materials were used in the work concerning death of animals caught in similar biotopes and processed by morphophysiological indicators (Shvarts et al., 1968). Data analyzed in the work relate to a population of bank voles, which is a background species in the region.

During analysis of the degree of age changes in teeth (ACT), the scale of classes suggested by us previously was used (Fig. 1), based on regular changes in the pattern of the socket surface of the M2 enclosed within the alveolar cavity (first through sixth class) and then according to the root index (seventh class): the ratio of the length of the root to length of the tooth.

During study of intrapopulation structure, the task inevitably arises of distinguishing structural age units and determining that level of such units which is most useful for specific purposes in the investigations. Usually, by tradition and even now when analyzing intrapopulation structure, "weight groups" are used. It is completely normal that with such a mechanistic approach, animals will fall into the same groups which differ considerably in age (error may constitute from several months to half a year). The fallibility of such an approach is so obvious that we shall not dwell further on a discussion of it.

Another level at which analysis of age structure is conducted is the "cohort" level (in the majority of domestic works, including our previous works, the terminology "generation"

* In order to determine number of groups (up to four) of placental spots, the uterus was clamed between two microscope slides.

was used, which is less accurate). Successive mass appearances of litters are usually understood as cohorts in rodents and other animals producing several litters in a row, starting with the beginning of spring breeding. The appearance of the first cohorts occurs during a comparatively short interval of time, equal approximately to the duration of pregnancy (20 days), while in subsequent cohorts this period is prolonged considerably. Cohorts can be fully and substantively called elementary structural-age units of the population. If there is no individual or other monthly (mass) tagging conducted (for example, with tetracycline, radionuclides, etc.), which in turn have their own limitations, then distinct separation of them is accompanied by substantial difficulties caused basically by temporal overlapping of periods of emergence of various cohorts, governed by the noncorrespondence of the onset of pregnancies and maturation of animals. The complex method of identifying cohorts permits this problem to be handled to a certain degree (Olenev, 1979), but is very laborious.

Identification of cohorts would be substantially simplified if a clearly workable age marker were present which is suitable for individual determination of age and subsequent calculation of the birth date.

Thanks to the works of ecologists, and primarily Ural ecologists, the terminology "seasonal generation" was introduced into the literature (Olenev, 1964; Shvarts et al., 1957, 1964; Pokrovskii, 1967; Amstislavskaya, 1975; et al.). Seasonal generations are those groups of individuals (of the year's litters) born in different seasons of the year, developing in different environmental conditions, and possessing specific biological properties (Shvarts, 1969, 1980). Work with seasonal generations is complicated by the fact that, for example, the spring generation not only includes the year's young which participate in breeding and create individuals of a given seasonal generation, but also a considerable number of them which do not breed during the year of birth, which is characteristic of almost all individuals except the autumn generation. As our observations of tagged animals showed, the proportion of nonbreeding animals in the spring generation can fluctuate from 10 to 30% in normal conditions and reaches 100% in extreme conditions of drought (Olenev, 1981a) or under the influence of increased density (Shilov et al., 1977). By autumn, the identification of a "pure" seasonal generation is even more complicated.

It is obvious that weight groups and seasonal generations are not always suitable for the performance of a qualitative analysis of age structure. As has already been discussed, the identification of cohorts, permitting a detailed analysis to be conducted of age structure, is impeded by the determination of the birth date of individuals. On the other hand, knowing the absolute age of an animal and the date of its birth, it is easily possible to convert to any of the age units being considered. To determine absolute age successfully would be a clearly useful age marker and would permit successful determination of absolute age.

As age markers which change regularly during ontogenesis, body weight and relative weight of the thymus are usually used at initial stages of ontogenesis; during the entire lifespan, weight of the crystalline lens of the eye, the bone suture line, age changes in teeth, et al. are used (Olenev, 1964, 1967; Shvarts et al., 1968; Tupikova et al., 1970; Bashenina, 1975; Akhmetov, 1978; Olenev et al., 1980; Malafeeva, 1987; Wasilewski, 1952; Zejda, 1961; Haitlinger, 1965; Adamczewska-Andrzejewskay, 1973). However, the majority of authors when suggesting methods of determining absolute age, indicate at the same time the substantial seasonal variations in rate of age changes in indices recommended by them as indicators of age. For example, the rate of age changes in teeth (ACT) can vary from 0.05 mm per month in autumn animals to 0.5 mm per month in animals born in the summer (Lowe, 1971). Other data available in the literature concerning rate of ACT are also contradictory. The magnitude of these contradictions can be judged according to the limits [boundaries] of development periods presented by various investigators for true roots in the bank vole: from 2,5 (Prichodko, 1951) to 6 months (Lowe, 1971).

We shall attempt to indicate that for vole molars the degree of ACT is a better age marker, and with correct utilization the most objective index, subject little to random changes. The irreversible, unidirectional character of ACT determines the advantages of this index.

We set as our primary task the detailed study of reasons for changes in the rate of ACT and then to formulate an objective system for estimating ACT as an index of age. A distinguishing feature of the work, and its basis, was the fact that when estimating the rate of ACT, physiological and functional conditions of animals were considered, associated with rate, sexual maturity, and time of entry into breeding.

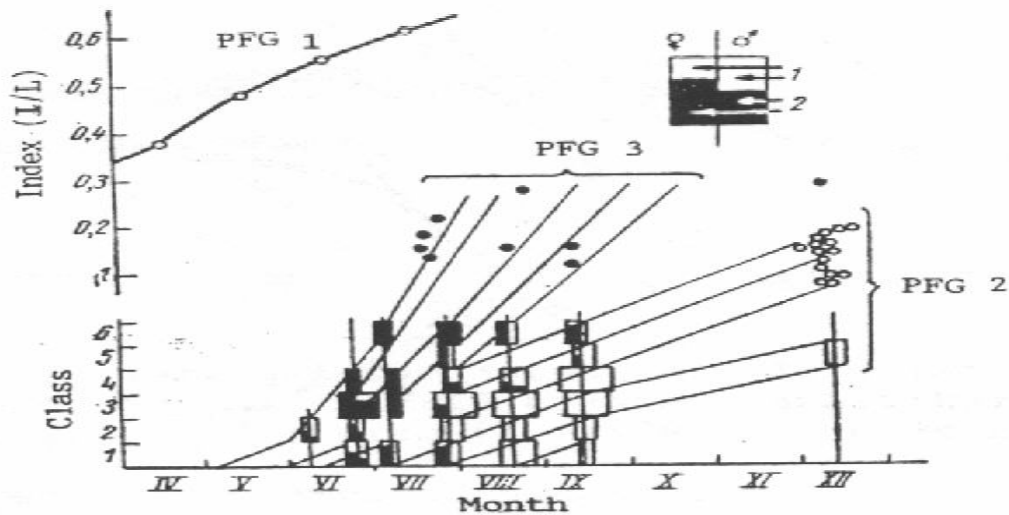


Fig. 2. Dynamics of age changes in teeth of bank voles of different groupings using the example of the 1977 sample (curve for **PFG I** is multiyear): 1) percentage of immature not entering breeding (in a given class) of individuals (**PFG 2**); 2) percentage of mature (in a given class) of individuals (**PFG 3**).

TABLE 1. Some **Morphophysiological** characteristics of age groups of the year's young (2.5 months) in Bank voles (untagged animals)

| Trait | Sex | Spring grouping (PFG 3) | | Autumn grouping (PFG 2) | |
|----------------------|---------|-------------------------|------------------|-------------------------|------------------|
| | | n | $x \pm m$ | n | $x \pm m$ |
| Body weight, g | Males | 31 | 19.2 ± 0.57 | 41 | 15.9 ± 0.25 |
| | Females | 39 | 25.5 ± 1.12 | 15 | 15.6 ± 0.31 |
| Kidney index | Males | 30 | 7.95 ± 0.15 | 37 | 6.95 ± 1.81 |
| | Females | 37 | 7.34 ± 0.24 | 15 | 7.46 ± 0.22 |
| Adrenal index | Males | 30 | 0.17 ± 0.007 | 40 | 0.17 ± 0.006 |
| | Females | 39 | 0.40 ± 0.02 | 14 | 0.17 ± 0.008 |
| Thymus index | Males | 24 | 1.47 ± 0.23 | 40 | 3.43 ± 0.15 |
| | Females | 29 | 1.33 ± 0.22 | 14 | 3.45 ± 0.21 |
| Wright of Testes, mg | Males | 31 | 317.4 ± 15.0 | 41 | 28.4 ± 3.97 |

FUNCTIONAL APPROACH, ACT (UNTAGGED ANIMALS)

The sample of 1977 (untagged animals) is characterized in Fig. 2 according to degree of ACT. Lines reflecting transitions from class to class according to degree of ACT were constructed with regard for physiological condition of animals associated with sexual maturity. The rate of ACT in voles born in the spring and entering breeding proved to be markedly higher than in those born in the second half of the summer and not breeding. The transition time from the first class to the sixth proved to be always two times shorter in spring animals which bred than in nonbreeding **autum** animals. Qualitative analysis of these two groups of animals showed that a high rate of ACT is characteristic of rapidly growing and rapidly developing animals which mature early and without any delay in entry into breeding. The complex of **morphophysiological** indices (Table 1) corroborates this, attesting to high intensity of metabolic processes associated with the physiological condition of animals of this group. Therefore, a distinct association is noted between rate of ACT and functional role of groups in reproduction of the population: the breeding proportion of individuals (the majority of

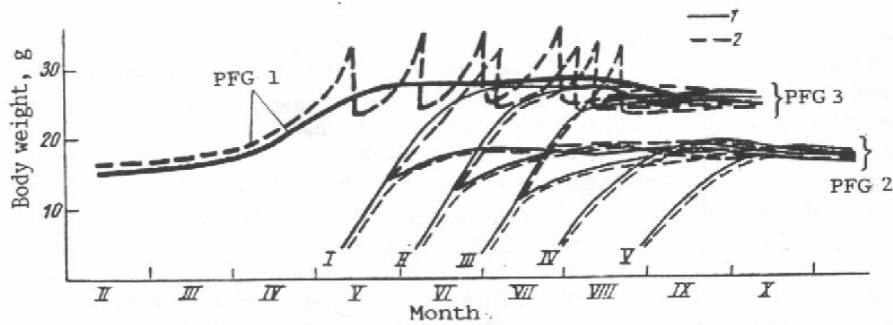


Fig. 3. Dynamics of body weight of individuals (averaged values) of individual cohorts (I-V). Tagged animals of natural population in 1977: 1) males; 2) females.

those born in the spring) provides for increase in numbers in the current year, while the nonbreeding part (primarily the autumn group) is responsible in the next year. A result of the different functions is also **morphophysiological** specifics of the group.

Having adopted as the basis for separation of groups the functional status of the animals included in them, their functional condition associated with specifics of growth, development and reproductive conditions, it has been suggested (Olenev, 1981b, 1983a) that three physiological functional groupings (**PFG**) be distinguished. Each grouping constitutes individuals which were produced, as a rule, from several mixed cohorts, connected by functional unity in reproduction of the population.

PFG 3: The Year's Young Which Breed during the Year of Birth. The basic group, from the first cohorts (70-90%), grow rapidly, reaching an average body weight of 25 g, and often enter breeding (usually at 25-45 days of age) having not finished growth. This grouping is characterized by monophasic growth (Fig. 3). The initial stage of formation of true roots is noted in them at 65-75 days of age. The complex of morphophysiological traits (see Table 1) attests to the high stress of metabolic processes. Rapid aging is characteristic of these

Animals, **life span is 3-5 months.** Their function is increase in population numbers.

PFG 2: The Year's Young Which do not Breed during the Year of Birth. The basic group are representatives of later cohorts, but there are also always nonbreeding individuals from initial cohorts (10-30%). At the first stage of ontogenesis, growth proceeds in the same way as in **PFG 3**, but at approximately one month of age, at a body weight of 16-18 g, growth is suspended, and the first phase of growth is concluded (see Fig. 3). Animals thus do not mature during the year of birth. The initial stage of formation of true roots in them is noted at an age of 120-130 days. Activity of metabolic processes is reduced (see Table 1). Aging is delayed as compared with **PFG 3** by almost two times, life span (with regard for further continuance in **PFG 1**) is 13-14 months. Their function is survival during the winter period in a state of "preserved youth" (term suggested by S. S. Shvarts) with the least numerical losses; sexual maturity is deferred to the spring of the next year. This grouping is adapted in the best way to survival of the winter period (Olenev et al., 1980).

PFG 1: Animals Which Winter (Formerly PFG 2). The spring period of conservation is concluded by short-term growth (second phase of growth) and maturation within the course of two-three weeks, and body weight reaches 25-27 g during this (see Fig. 3). Almost all animals which wintered mature. Absolute age in individuals differs. Activated processes of metabolism are similar in level to those for individuals in **PFG 3**, although according to absolute age animals are much older than representatives of **PFG 3**

Individuals composing each specific grouping, despite differing absolute (calendar) age, are physiologically identical; this identity is governed by functions accomplished in the population. It can be concluded from this that the rate of ACT is found in direct associatio with processes of ontogenetic development, growth, maturation, and breeding of animals. As is known, these processes are rather energy consuming

There is interest in a comparative analysis of the rate of ACT in individuals of **PFG 3** and 2: both groupings are represented only by the year's young, animals close in absolute and physiological age can be easily distinguished, and each grouping reflects an alternative pathway of ontogenetic development. The two types of development determine two types of rate of ACT, which are a reflection of physiological and functional conditions of the organism.

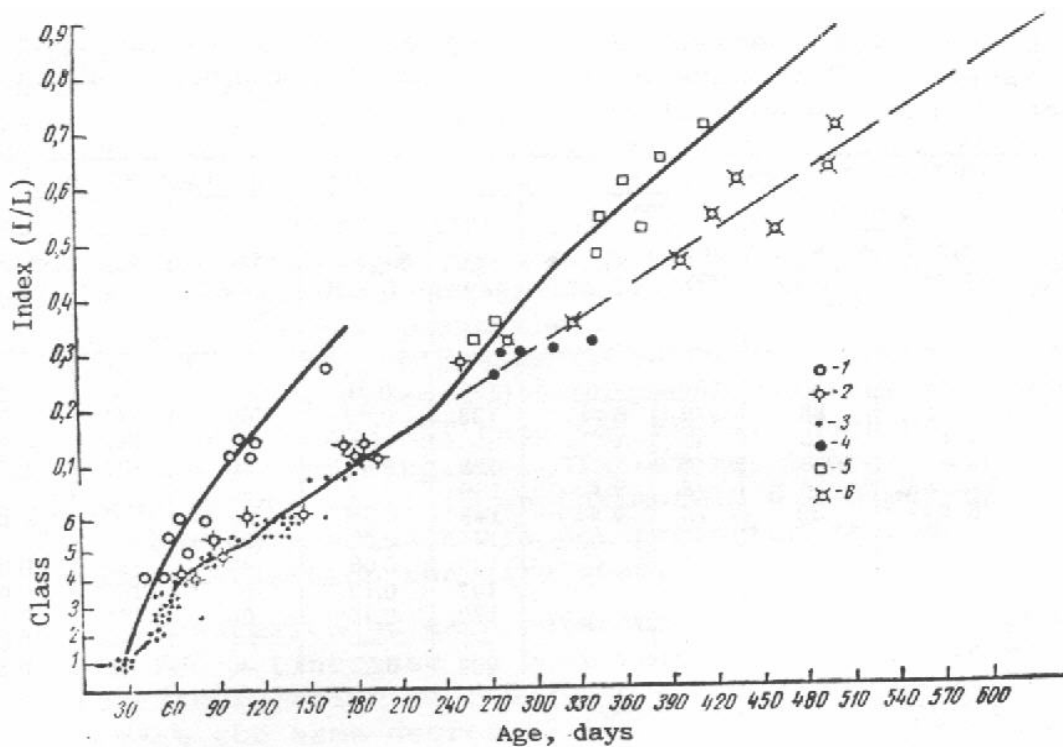


Fig. 4. Dynamics of age changes in animal teeth (bank voles) of different functional condition. Natural population of tagged "reference" animals; 1) **PFG 3**; 2) **PEG 3** (recomputed); 3) **PFG 2**; 4) **PFG I** (nonbreeding, before spring leap in growth; 5) **PFG I** (breeding); 6) **PFG I** (recomputed). Broken line **PFG 2** → **I** potential.

The presence of two pathways of development undoubtedly bears an adaptive character in the population. There is a basis to consider that some nonspecific "trigger" mechanism exists for switching on the rate of growth and development which operates under certain changes in **abiotic** and biotic factors which play the role of provoking factors, a "trigger-effect" (Olenev, 1987). The divergence of animals along two pathways of development was also noted by us for single individuals from one litter. Of independent interest are the reasons determining a specific pathway of development (**PFG 3** and **2**). Preliminary results of an analysis conducted concerning epigenetic threshold traits with the use of **Festing's** genetic **mandibular** test indicated an absence of significant differences between **PFG** (Olenev, 1988). All of this permits genetic uniqueness of individuals proceeding along two pathways of **ontogenetic** development to be regarded as of low probability.

ADDITIONAL CHARACTERISTICS OF PFG (TWO TYPES OF DEVELOPMENT)

The **PFG** are characterized not only by specifics in rate of ACT, levels of metabolism (according to **morphophysiological** indices), and rates of aging and **lifespan** (Olenev, 1982). Biochemical changes have also been noted (Gulyaeva and Oienev, 1979), in addition to differences at the tissue level (according to **mitotic** index) (Olenev et al., 1983), substantial differences in **radioresistance** (Grigorkina and Olenev, 1987), accumulation of heavy metals in bone tissue (Bezel' and Olenev, 1988), and **technogenic** pollutants are manifested differently in rate of cellular division (Gatiyatullina et al., 1988). At the **populational** level differences in **PFG** have been demonstrated according to reactions to extreme influences: drought (Olenev, 1981a) and population density (Olenev and Kolcheva, 1987), which bear an adaptive character.

Consequently, the functional structure of a population and the specifics of two types of development exert an influence on a wide spectrum of indices used in populational investigations, including age markers, and this cannot be ignored.

From this not only did the reasons for divergence of literature data according to ACT rate become understood (investigators worked without regard for the specifics of ACT for **PFG**), but the possibility also appeared for use of the patterns established when determining age.

TABLE 2. Comparative degree of Age Changes in Teeth (ACT) of Bank voles (PFG 2 and 3) in animals of comparable absolute age (reference animals)

| Natural population | | | | | Laboratory population | | | | |
|--------------------|---------------|---------------------|-----------|---------------|-----------------------|---------------|---------------------|-----------|---------------|
| PFG 3 | | | PFG 2 | | PFG 3 | | | PFG 2 | |
| Age, days | Degree of ACT | Time in PFG 3, days | Age, days | Degree of ACT | Age, days | Degree of ACT | Time in PFG 3, days | Age, days | Degree of ACT |
| 163 | 0.27 | 130 | 151 | 0.08 | 138 | 0.01 | 45 | 136 | 6 cl |
| 112 | 0.14 | 85 | 116 | 6 cl | 138 | 0.09 | 80 | 143 | 5 cl |
| 40 | 4 cl | 15 | 47 | 2 cl | - | - | - | 142 | 5 cl |
| - | - | - | 48 | 2 cl | 138 | 0.09 | 80 | 133 | 0.07 |
| 54 | 5-6 cl | 25 | 48 | 2 cl | 139 | 0.12 | 90 | - | - |
| 76 | 6 cl | 30 | 76 | 4 cl | 145 | 0.08 | 60 | 143 | 5 cl |
| | | | | | 145 | 0.06 | 60 | - | - |
| | | | | | 146 | 0.06 | 40 | 153 | 0.08 |
| | | | | | 147 | 0.13 | 65 | 156 | 0.09 |
| | | | | | 170 | 0.15 | 60 | 179 | 0.13 |
| | | | | | - | - | - | 163 | 0.09 |
| | | | | | 204 | 0.23 | - | 193 | 0.10 |

POSSIBILITIES OF DETERMINING AGE BY ACT (REFERENCE ANIMALS)

Precisely dated material obtained on tagged "reference" animals from nature permits a demonstration of the precision achievable in practice in determining age of individuals. It should be noted that out of the "reference" animals with a precisely known date of birth, 84 individuals were chosen during all years of investigation, and of them very few of them survived the winter as a result of natural mortality and emigration, as represented in Fig. 4.

In general form the ACT rate for different PFG looks like a fork; for the year's young of **PFG 3** and **2**, divergence in rate (trigger-effect) begins to be manifested at an age of 30-40 days. It should be noted that because of differences in absolute age, during which maturation of separate individuals occurs (those entering **PFG 3**), when the ACT rate increases in them, points of the curve (**PFG 3**, see Fig. 4) are more dispersed. The different ACT rates for various PFG is traced especially clearly in animals of comparable age (Table 2, "naturals"). The time for transition from class to class differs. For example, for individuals of **PFG 2**, transition from the first class (from birth to 30 days of age) to the second occupies 20 days, while the period from the second to the fourth was short, 15 days. It is understood that the reason for this is not in abrupt changes in ACT rate, but in the artificial subdivision into classes.

Differences computed in specific ACT rate for **PFG 3** and **2** attest to a certain reduction in it with age in the **PFG 3**: from the first to the sixth classes, the difference in rate is almost twofold, while during formation of roots, 1.8 times. The average specific rate of ACT computed according to change in root index constitutes 0.003 for **PFG 3** per day or 0.091 per month (from 0.07 to 0.11), and 0.0017 per day and 0.050 per month for **PFG 2**.

Analogous data (see Table 2) for animals from a laboratory colony (founders were taken from the same population) attest to the fact that rates of ACT for the **PFG 3** is lower in them, while periods for entry into breeding are more prolonged than in natural populations. This also explains the reasons for the contradiction of literature data.

For grouping of **PFG I** animals which had wintered (natural), an increase in ACT rate is noted from the time of the growth spurt almost synchronously with increase in body weight (usually in March in the Urals), preceding onset in breeding, usually 1.5 months before its start. The first part of the fork (Fig. 4, dotted line) is usually absent in association with the fact that almost all animals which wintered participate in breeding**. Maturation and entry into breeding occur in unison and during a rather a short time; by this time body weight (see Fig. 3) and absolute weight of the majority of internal organs increased.

**Single individuals were noted (untagged animals) born in the autumn of 1974 which had not bred in extreme conditions of drought (1975) and entered into breeding only in the spring of 1976.

Despite the almost simultaneous maturation, entry into spring breeding, similar functional state, and close physiological age, calendar age of individual animals differed strongly: up to several months (in this grouping all cohorts of the past year of birth were represented; Olenov, 1982). The absolute age by the start of the spring growth spurt (from 200 to 320 days) was predetermined by periods of birth of animals. In this regard, they also had a differing degree of ACT, which is responsible for the substantial dispersion of points on Fig. 4. In addition, data obtained by us indicate that the ACT rate of animals which wintered, which increases from the time of the spring growth spurt, at first exceeds the average value (0.003 per day) for this grouping, and then, after the completion of growth and maturation, when animals continue to breed, decreases to below the average value. Graphically, this has the appearance of a weakly expressed S-shaped curve (see Fig.4, PFG 2 → 1, left part of fork) and reflects the average ACT rate for PFG 1. Such nonlinearity in ACT rate I also increases the error when determining age. Therefore, average specific ACT rate in them from the time of the spring growth spurt is approximately 0.003 per day, i.e., almost identical to PFG 3. Data for laboratory animals are not presented because of substantial fluctuations in periods of spring maturation and life span.

Knowing the start of maturation of each reference animal (of the year's young), i.e., the age at its entry into PFG 3 (increase in body weight continued in animals at this time), and the difference in rate in relation to PFG 2, recomputation for age can be made, during which the animal would reach the same degree of ACT as if it had remained in PFG 2, i.e., in the case that it had not matured. After recomputation (PFG 3 by PFG 2), points proved to be shifted to the right by the number of days that an animal remained in PFG 3 with regard for the difference in rate and were easily superimposed on the curve for rate for PFG 2 (see Fig. 4). This is all the more persuasive since with recomputation the time for placement of an animal in a PFG is reduced to nothing. Analogous recomputation for grouping of animals which wintered, despite considerable dispersion of original points because of a difference in age at the start of the spring growth spurt, permitted construction of the potential line for nonbreeding animals which wintered (dotted line), which was the natural continuation of PFG 2.

Data presented above not only corroborate the validity of computation of the ACT rate and objective reality of existence of two ACT rates as a reflection of two pathways of development, but also substantiate the functional approach during analysis of the dynamics of age markers.

The use of the functional approach into practice when determining age is applicable to some degree to the majority of representatives of the Muridae family (cyclomorphic ephemeral animals) living in territories with rather pronounced seasonal changes in living conditions. The affinity of an animal to a PFG is first established. For this, body weight is reliably established and the condition of the reproductive system is determined, which is a good indicator of the functional condition of an individual. At the first stage, according to degree of ACT (Fig. 1), we can easily distinguish (Fig. 4) the animals which wintered (PFG 1) from the year's young (3 and PFG 2). Differences in degree of ACT between those which wintered and the year's young are so great (root index of those which wintered is already more than 0.2 by spring) that errors are eliminated***. At the second stage separation into PFG is made within the group of the year's young (3 and PFG 2). The following traits are referred to the traits of maturation or breeding in females; with continuing increase in body weight (approximately 20 g), thickening of the uterus is noted, the vagina opens, and with rising body weight (more than 20 g), embryos or fetuses are noted, there are placental spots and lactation; in males body weight increases (more than or approximately 20 g), development of testes is noted (more than 50 mg), and with increased body weight (more than 20 g), there are testes with pronounced epididymides. In this case we classified the year's young to PFG 3. If there were no spots or embryos in females, if the uterus was threadlike, the vagina closed, and body weight, or if pronounced appendages**** were absent in males, weight of testes was less than 50 mg, and body weight low (less than 20 g), we classified them to the PFG 2. Practice showed the reliability of these elementary indices for division into PFG.

***If the spring sample according to ACT degree is unusually diverse (there is a substantial proportion of individuals with indices less than 0.2 and of even the first six classes), it can be maintained that winter breeding occurred.

****Males with reduced epididymides and testes were classified to those which had participated in breeding, i.e., to PFG 3.

For the year's young (**PFG 3** and **2**) after division into groupings, we found the absolute age of an individual according to Fig. 4 with regard for degree of ACT (see Fig. 1). Having reckoned this number from the date of the capture of an individual, we can figure the actual date of birth. Errors in the interval from the second class to index 0.3 constitute from 5 to 20 days (except for the fifth and sixth classes, where there are considerably more errors). Within the limits of the first ACT class (period from birth to approximately one month of age), it is advisable to use body weight to determine age, since at this time it changes linearly.

Determination of the age of animals which had wintered (**PEG 1**), which as we have already discussed differed substantially by the onset of maturation, was made in the following way: if the spring growth spurt had already begun in an animal (increased body weight noted, close to 20 g; see Fig. 3), the left part of the fork was used to determine age (**PFG I**, those breeding, solid line, see Fig. 4); if the spring growth spurt had not yet begun, i.e., if body weight did not exceed 20 g, age was determined according to the right part of the fork (see Fig. 4, **PFG 2**→**1**, dotted line).

MARKER INDICES OF DYNAMICS OF AGE CHANGES IN MOLARS OF VOLES (BANK VOLE), SOUTHERN URALS

Natural Population (reference animals)

Breeding Year's Young (**PFG 3**). Average age at start of maturation of the year's young is 30 days, minimal is 25 days (first ACT class), the maximally recorded is 90 days (fifth class), which are single cases. Average life span is 140 days (root index of 0.2), maximally recorded is 160 days (index of 0.27). Average ACT rate is equal to 0.003 per day, 0.091 per month.

Nonbreeding Year's Young (**PFG 2**). Average (general) life span (also considering residence in the grouping of those which wintered, **PFG 1**) constitutes 390 days. Maximally recorded life span is 420 days with a root index of 0.7*****. Average ACT rate is equal to 0.0017 per day and 0.050 per month.

Those Which Wintered (**PFG 1**). A unified start of spring growth spurt and entry into breeding is noted, but not connected with time of birth of individuals from different cohorts (their calendar age) and probably determined by the combined influence of factors of exogenous and endogenous nature.

Minimally recorded absolute age at onset of spring growth spurt is 200 days, 240 for maturation, and maximals are 320 and 360 days, respectively. The ACT rate, starting at the time of the spring growth spurt, is identical to that for **PFG 3** and equal to 0.003 per day (average in this grouping). Individuals with a root index of more than 0.25 are classified to those which had wintered.

Comparative analysis of the seasonal ACT rate for nonbreeding animals from autumn and spring cohorts (**PFG 2**) did not reveal significant differences according to this index.

Laboratory Colony (founders from the same population)

PFG 3. For vivarium animals, maturation of the year's young during longer periods is characteristic; minimally recorded age is 40 days (first class), average is 65 (fourth class), and maximal is 170 (index of 0.07). On the whole, periods for onset of maturation are extended.

PFG 2. Rate of ACT for both natural and vivarium animals is the same. This is probably the minimally possible ACT rate for this species.

The difference in ACT rate between **PFG 3** and **2** in vivarium conditions is manifested less distinctly (by 1.4 times) as compared with natural animals (1.8 times).

The maximally recorded life span in vivarium conditions is substantially longer than in nature, and reaches two years or more.

What has been presented above attests to the invalidity of using data of laboratory investigations concerning ACT for animals from natural populations.

***** *It must be noted that absolute life span of animals is specific for individual years and there are considerable differences between years (Olenev, 1982). For untagged animals from nature, the maximal root index noted was 0.87.*

The application of the functional approach permits substantial reduction in the errors I which are inevitable when working with age markers and provides a more precise **methodological** basis for analyzing a broad spectrum of features in the **ontogenetic** development of **cyclomorphic** rodents.

CONCLUSIONS

Using the example of a natural bank vole population (Southern **Urals**), the possibility of two alternative pathways of growth and development regularly realized during ontogenesis was demonstrated (single-phase and **bi-phase** growth), which in our opinion are a manifestation of polyvariant development of animals. We are probably dealing here with the phenomenon of polyvalency, i.e., the inheritance of not a single program of ontogenetic development. On the basis of one genotype, a specific ontogenetic program can be realized, one pathway of development expressed in **PFG 2** and **3**. Two pathways of development are inherent to the majority of species of **cyclomorphic** rodents, which are characterized by a broad spectrum of biological features, and in particular by differences in intensity of metabolism, growth rate, time of maturation, entry into breeding, life span, *et al.*

The essence of the functional approach consists of the fact that functions fulfilled by animal groups (and also their results) are used as the basis for separating **intrapopulation** structural units. Populations are characterized by functional structurization, while functions, and participation in reproduction first of all, are the binding force determining the specifics of individuals in groupings. Such an approach contributes to clarification of mechanisms which direct physiological processes of separate individuals along a pathway adaptive for the population.

It is suggested that three physiological functional groupings (**PFG**) be distinguished. Individuals of groupings composing the population are qualitatively of unequal value in time according to the contribution they make to general functions of the population; the birth time of individuals (their calendar age) does not play a special role during this.

Therefore, the two variants of ontogenetic development should be classified with the important adaptive mechanisms at the **populational** level which are strictly associated with dynamics of the age structure and, therefore, with the dynamics of numbers.

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