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# Ecological Analysis of Spleen Hypertrophy in Cyclomorphic Rodents Taking into Account the Type of Ontogeny

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**Abstract**—The functional approach at the level of physiological functional groups (PFGs) reflecting two types of ontogeny was used to analyze spleen hypertrophy in five species of rodents. In addition to a wide variation of spleen weight, its hypertrophy was observed: in bank voles (*Clethrionomys glareolus*), the spleen weight was as great as 3.5 g versus the "normal" value of 100–150 mg. The variation range of the relative spleen weight was estimated. For example, in the dominant species, *C. glareolus*, this range was considerable (from 2 to 125‰). A distinct relationship with the types of animal ontogeny was revealed. The genus specificity of the phenomenon—its occurrence in three species of *Clethrionomys* voles and absence in *Microtus* voles and mice—was determined in the study region. The animals with normal and hypertrophied spleens did not differ from each other significantly in their vital activity (at least, with respect to the parameters studied). The voles with hypertrophied spleens normally reproduced, and the hypertrophy was asymptomatic. These findings suggest that the populations have adapted to a damaging factor (or factors) in the course of a prolonged coevolution. This parameter is regarded as the indicator of the presence of a damaging factor in the population.

*Key words*: rodent populations, ontogenetic types, spleen hypertrophy, genus specificity, adaptation, damaging factor.

At the current stage of the development of ecology, populations are viewed as dynamic biological systems that have certain internal mechanisms ensuring their existence in a varying environment. All forms of the interaction between populations and the environment are mediated by physiological responses of individuals. Analysis of the dynamics of the weights of individual organs serves as the basis for the method of morphological and physiological indicators, the introduction of which was an important step forward in studying rodent populations (Shvarts *et al.*, 1968). The morphological and physiological parameters of the spleen turned out to be good indicators. The spleen is a very variable organ, which is probably accounted for by its multiple functions (Ivanter, 1985). In addition, the spleen is subject to hypertrophy, which increases the variation range of its parameters by an order of magnitude. However, the researchers that dealt with the spleen hypertrophy merely reported the presence of giant spleens at best, without attempting to analyze the phenomenon.

Earlier, the results of analysis of many biological parameters, including morphological and physiological, were used to demonstrate that their dynamics can be estimated more correctly if the researcher takes into account the ontogenetic types of the animals studied (the concept of the functional approach) (Olenev, 1989). We regarded the functional approach as instrumental in estimating the contribution of the ontogenetic component to spleen hypertrophy and the possibility of using this parameter in research. In addition, we attempted to demonstrate new prospects and advantages of the functional approach using the ecological analysis of this phenomenon as an example. In the future, this would permit more correct use of the traditional interior parameters.

The numerous functions of the spleen have not been studied comprehensively to date. The sensitivity of the spleen to toxic substances, radiation, infectious diseases, helminthiases, and other factors is well known. However, the words of the outstanding researcher Barcroft (1926), who once said that the cynicism marking the habitual attitude to this organ comes to the point of not mentioning it in textbooks at all, are still true to a certain degree.

In this study, we performed an integrated ecological analysis of spleen hypertrophy—an extremely sensitive and, hence, variable interior parameter—based on the analysis of changes in the main population parameters with regard to the functional structuring of populations (on the basis of two ontogenetic types).

## SPLEEN VARIABILITY AND THE PHENOMENON OF SPLEEN HYPERTROPHY

In contrast to most traditional interior parameters, the spleen index is not likely to directly depend on body weight and is therefore an exception to Hesse's rule of series. The absence of this relationship is explained not only by the exceptional individual variability of the spleen in general (Rench, 1948; Kalabukhov, 1950; Shvarts, 1953; Yablokov, 1966; Ivanter *et al.*, 1985), but also by the high level of sensitivity of this organ to various external factors. The high sensitivity level of the spleen to various environmental factors (hence the wide variation of its size) is also confirmed by the lack of negative feedback between the weight and the degree of variation in the character, whereas most other interior parameters display this feedback. Moreover, the spleen index displays a distinct positive feedback: the variation of the parameter is the widest in the months when the index is relatively high and decreases with the subsequent decrease in the relative weight of the organ. This determines the importance of the spleen as an indicator of body state and makes it an interesting object to study.

Earlier, Bashenina (1981) mentioned the high variability of the spleen and pathological cases when the organ weight sometimes exceeded 1 g. Ivanter *et al.* (1985) described the time course of the spleen relative weight in detail; they also noted the exceptionally high variability of the organ, including the cases of its hypertrophy. The pattern of seasonal changes in the spleen index is characterized by a winter depression, which is characteristic of morphological and physiological indicators. Unfortunately, the analysis did not take into account the functional status of the animals. Therefore, the samples studied were actually heterogeneous, which undoubtedly affected the interpretation of the results. Currently, we and other researchers sometimes find animals with hypertrophied spleens whose indices are greater than 100‰ and the absolute weight is as high as 3.5 g versus the normal value of 100–150 mg in bank voles (Fig. 1). Note that we distinguish increased (no larger than 10‰) and hypertrophied (11–180‰ or even larger) spleens.

The abnormally great weight of the organ in these cases, which is not characteristic of any other traditional parameter, allows us to regard the spleen hypertrophy as an individual phenomenon. The term hypertrophy may not be quite correct; however, we have now no grounds to use another term. Note that, in this study, it was not our task to determine the specific cause of this phenomenon or analyze its medical aspects.

## THE SCALE OF THE PHENOMENON AND SPLEEN SENSITIVITY TO VARIOUS FACTORS: ANALYSIS OF POSSIBLE CAUSES

According to published data, the hypertrophy of the spleen is a common occurrence. For example, we earlier summarized numerous data on the prevalence of this phenomenon in the Ural region (Olenev and Pasichnik, 1999). Spleen hypertrophy was frequent in the Southern Urals (Il'menskii State Reserve) and almost the entire Orenburg oblast, the Middle Urals


**Fig. 1.** A dissected bank vole (female, PFG 1; body weight 30 g, spleen weight 2g, spleen index 66.7‰) with a hypertrophied spleen (S). A normal spleen is shown in the inset.

(the Shigaevo station in the Visimskii State Reserve). Spleen hypertrophy in voles from the Urals was also mentioned in earlier studies (Ponomarev, 1974; Bashkirtsev *et al.*, 1965; Bol'shakov *et al.*, 1965). Being unable to perform virological, parasitological, and other necessary studies, we attempted to analyze available published data on the increased size of the spleen in voles, even if the increase is too small to be regarded as hypertrophy.

**Industrial factors.** Most sources report on changes in the relative spleen weight (usually, within the normal range, i.e., no more than by 10‰). Let us illustrate this by some examples.

Ignatova (1998) performed studies in the Rudnaya River basin (Primorskii krai). The main industrial pollutants in that region are dustlike small particles, solutions and sublimates of heavy metals (tin, lead, zinc, cadmium, copper, manganese, nickel, and iron) and nonmetals associated with them (arsenic, sulfur, boron, and fluorine) in the form of soluble compounds. Almost all rodents in this river basin died in the fall, and new

**Table 1.** Mean spleen indices (‰) in animals from natural and industrially affected ecosystems (data from Ignatova, 1998)

| Sample | Ecosystems | |
|---|---|---|
| | undisturbed | industrially affected |
| Genus *Apodemus* | 1.1–2.0 | 5.1–8.1 |
| Genus *Clethrionomys* | 1.3–3.1 | 3.4–7.4 |

temporary populations were formed by animals migrating from neighboring regions in the spring.

The increase in spleen index, which was common to all rodent species studied (Table 1), is also related to leukemia (Yakimenko, 1993). This conclusion is based on the increased (by a factor of 3) chromosome aberration rate in somatic cells in all animals living in the region.

A similar increase in the spleen weight of rodents was observed in the Tomsk oblast (Paderov and Prochan, 1998). In addition, a comparison of spleen morphological characteristics in two vole species (*Clethrionomys glareolus* and *Cl. rutilus*) in the buffer zones around industrial enterprises and in control areas permitted the estimation of the variability of the spleen and its functional ability to respond to environmental factors. Therefore, the spleen can be used as a marker of exposure to harmful environmental factors. In the polluted area, the spleen weight was increased and the capsule was thickened, compared to the control parameters.

A significant increase in the spleen index of the bank vole was found in the oil fields of the Kuibyshev oblast (Shaposhnikov, 1971). The variation in spleen weight after exposure to ionizing radiation is well known (Brues and Stround, 1964). However, numerous data on the role of the spleen in the cases of radiation damage are ambiguous and often contradictory (Belousova *et al.*, 1979).

**Natural infections and parasites.** Approximately 20 species of protozoans, 40 species of parasitic worms, and 600 species of ectoparasites, in addition to infectious agents (viruses, rickettsiae, and bacteria) have been found to parasitize European bank voles. All of them may somehow affect the vital activity of the animals.

Some authors relate the increase in spleen size and spleen hypertrophy to various infections, because animals with hypertrophied spleens are often found in the natural foci of infectious diseases. Let us consider the ambiguity of this relationship using *hemorrhagic fever with renal syndrome* (HFRS), a viral infection, as an examples.

The bank and northern red-backed voles and two species of shrews are the main reservoirs of HFRS in the Urals; they are characterized by the highest prevalence and species diversity of gamasid mites (as many as 14 species). There is evidence (Bashkirtsev *et al.*, 1965; Bol'shakov *et al.*, 1965; Ponomarev, 1974) that animals with considerably increased spleens are often found in the HFRS foci of the Cisural and Middle Volga regions. The authors cited above related this phenomenon to the effect of HFRS virus. On the other hand, a chronic, asymptomatic form of HFRS is known in the bank vole (Bernshtein *et al.*, 1971; Demina, 1991; Apekina *et al.*, 1991; Demina *et al.*, 1997; Mikhailova, 1999). The authors of later studies conclude that a considerably increased spleen cannot be the indicator of HFRS, that lifelong (15 months) circulation of the HFRS hantavirus in the animal blood is possible, and the epizootic has no noticeable effect on the animals infected by this hantavirus.

Our analysis of the laboratory data kindly provided by V.A. Demina (Institute of Poliomyelitis and Viral Encephalitis, Russian Academy of Medical Sciences) yielded similar results. The animals kept in a vivarium were infected with HFRS virus. The mean spleen index in infected animals was nonsignificantly higher than in control animals ($t$ = 0.73, $p$ = 0.46; $f$ = 1.025, $p$ = 0.932). The spleens with indices higher than 10‰ (i.e., hypertrophied) were found in only two animals (17.3 and 22.0‰), both from the control group. No cases of spleen hypertrophy were observed among infected animals. Thus, we excluded the relationship between spleen hypertrophy and HFRS virus infection.

It has been demonstrated (Olenev and Kolcheva, 1983) that changes in the size and structure of bank vole population largely determine the course of the epizootic process in forest foci of encephalitis; however, no relationship with spleen hypertrophy was found. Infections such as tularemia, leptospirosis, and plague may cause considerable changes in the spleen of rodents, including its hypertrophy.

Several types of responses to the *presence of blood parasites* are known in animals. For example, anemia is related to infection by *Babesia*, *Trypanosoma*, *Hepatozoon*, and *Grahamella*, which is accompanied by changes in the spleen (Wiger, 1978). The bank vole is most frequently the host of *Trypanosoma* and/or *Grahamella* (Wiger, 1979). The spleen index usually ranges from 1.5 to 5.0‰. Unfortunately, the variation range of spleen index is often not indicated in available publications; however, the increase of the spleen upon parasitic infection is noted.

*Helminthic infestation of animals* may also cause a similar increase of the spleen. For example, the relationship between infestation by helminths and animal age has been found (Tenora and Wiger, 1979; Bashenina, 1981; Chechulin, 1998; Moskvitina *et al.*, 1998). The low infestation rate of young animals is usually explained by passive immunity. In adult, reproducing animals, immunity and, hence, resistance decrease with age; in addition, the number of contacts with infection carriers increased. We would like to note in advance that, in our opinion, the functional state of the animals, which determines the metabolic rate, number of con-

**Table 2.** Mean values and variation limits of relative spleen weight (‰) in rodents from three genera as related to the types of their ontogeny (PFGs). Most informative values are boldface

| Species | Group | Mean, ‰ | Maximum, ‰ | Minimum, ‰ | Standard deviation | *n* |
|---|---|---|---|---|---|---|
| *Clethrionomys glareolus* | PFGs 1–3 | **36.5** | **125.0** | 1.5 | 30.3 | 587 |
| | PFG 2 | 4.5 | 111.5 | 1.7 | 2.04 | 501 |
| *Clethrionomys rutilus* | PFGs 1–3 | **12.2** | **34.2** | 1.6 | 10.3 | 19 |
| | PFG 2 | 5.6 | 7.7 | 2.2 | 1.7 | 7 |
| *Clethrionomys rufocanus* | PFGs 1–3 | **25.3** | **64.5** | 2.43 | 16.9 | 17 |
| | PFG 2 | 4.3 | 6.7 | 1.9 | 2.3 | 4 |
| *Apodemus uralensis* | PFGs 1–3 | 4.8 | 20.6 (single cases) | 1.4 | 2.8 | 49 |
| | PFG 2 | 4.5 | 8.4 | 0.99 | 1.8 | 33 |
| *Microtus agrestis* | PFGs 1–3 | 4.5 | 13.4 (single cases) | 1.2 | 3.3 | 22 |
| | PFG 2 | 3.9 | 6.6 | 1.8 | 1.5 | 10 |
| *Microtus oeconomus* | PFGs 1–3 | 5.6 | 7.7 | 2.8 | 2.1 | 3 |

tacts, and many other characteristics, is the main factor responsible for a high infestation rate in a part of the population (physiological functional groups 1 and 3, see below).

**Spleen hypertrophy is strictly localized.** The very fact that there are regions where all animals in rodent populations have spleens of normal size and the cases of spleen hypertrophy are absent, as, e.g., in the Primorskii krai (Ignatova, 1999), is of considerable interest. During the studies performed in HFRS foci (Tula oblast and southern Udmurtia and Bashkortostan), where the bank vole is the main reservoir of the infection, hypertrophied spleens were not found in the Tula population and were found in only 2.1% of the animals in the Ufa population (Bashkortostan).

All the above data, including the ambiguity of possible interpretations of the mechanisms of spleen hypertrophy, indicate that further studies and new, original approaches (including ecological) are necessary. The analysis described below allowed us to regard the spleen as an indicator of the presence of deleterious factors in the population. Identification of these factors was beyond the scope of our study, which was in fact purely ecological.

## MATERIAL AND METHODS

Most of the material was collected in Il'menskii State Reserve (the Southern Urals) in two stationary plots located in biotopes of different types and in the marking plot. In this work, we analyzed our own material obtained during 26 years (1975–2000) of studies on rodents and generalizations made in that period. We used data on seven species of murine rodents belonging to the genera *Clethrionomys*, including *Cl. glareolus* (Schreber, 1780), *Cl. rutilus* (Pallas, 1779), and *Cl. rufocanus* (Sundevall, 1847); *Microtus*, including *M. oeconomus* (Pallas, 1776), *M. agrestis* (Linnaeus, 1761), and *M. arvalis* (Pallas, 1779); and *Apodemus*, represented by *A. (Sylvaemus) uralensis* (Pallas, 1758). We used both conventional and new approaches and methods of field and experimental studies: the methods of mark—recapture and irreversible removal, an improved method for determining the absolute age of voles (Olenev, 1989), and the method of morphological and physiological indicators (Shvarts *et al.*, 1968).

*The functional approach* (Olenev, 1981, 1989, 2002) served as the methodological basis of the study. The essence of this approach is the use of the functional similarity of individuals in the groups corresponding to two types of ontogeny as the main criterion for distinguishing intrapopulation structural units. The grouping is based on the functional status of animals (the functional state determined by specific features of growth, development, and reproductive state) and the synchronism of its changes with time.

For convenience, we divide the animals into three physiological functional groups (PFGs). *PFG 3 (reproductive young of the year)* comprises the animals that do not cease growing and developing (monophasic growth, the first type of ontogeny) before they mature and enter reproduction. They usually die before the winter of the current year. *PFG 2 (nonreproductive young of the year)* comprises the animals that, after a short period of growth and development (phase 1, the second type of ontogeny), cease growing and remain frozen, with the reduced rates of all metabolic processes, until the next spring. *PFG 1 (overwintered animals, formerly PFG 2)* comprises the animals most of which began reproduction after a short period of growth and maturation (phase 2, the second type of ontogeny). Each PFG includes animals from different cohorts that are connected with one another by the common functional role in the maintenance of the pop-

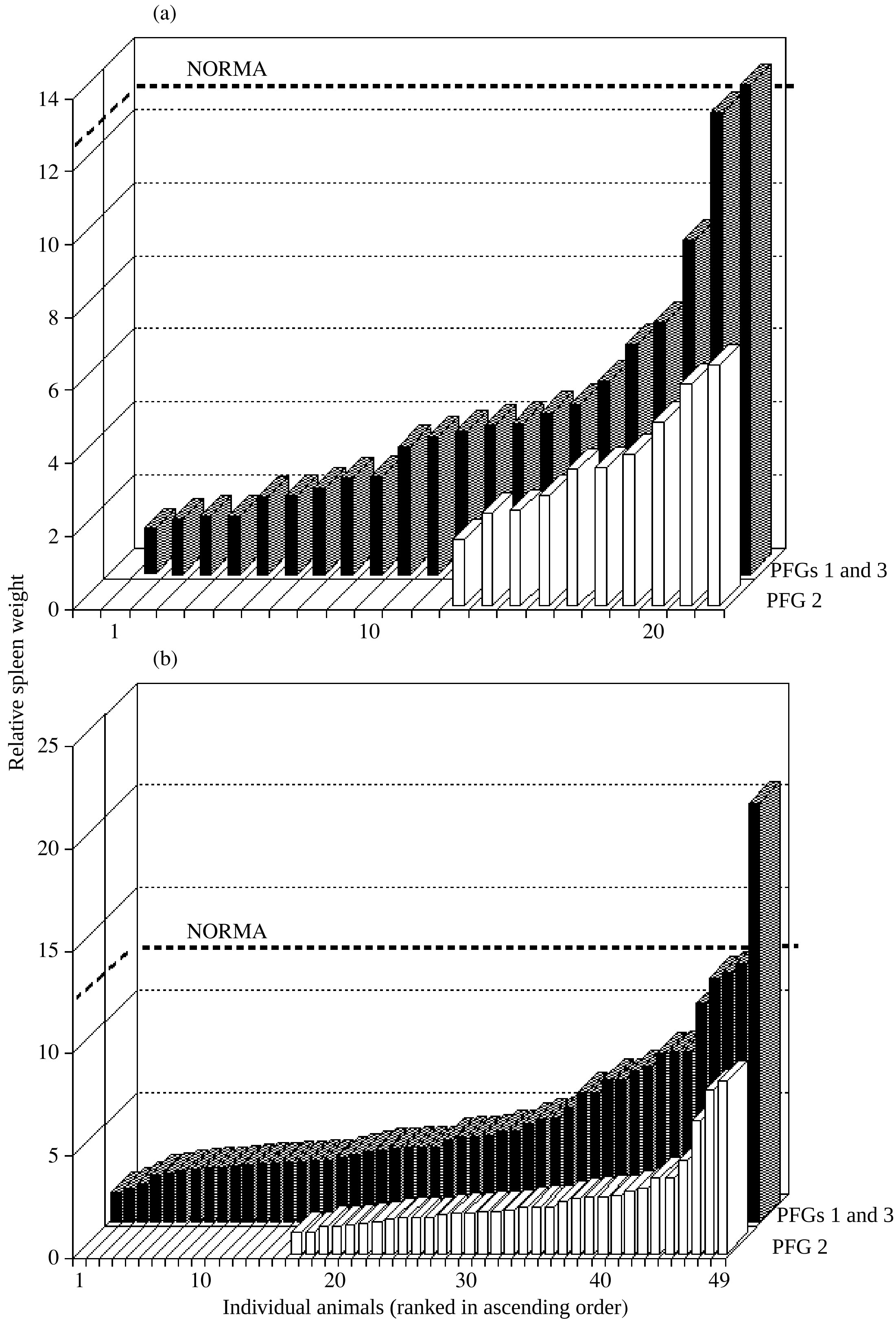


**Fig. 2.** Variation ranges of spleen index in (a) field vole and (b) common field mouse. PFGs 1 and 3 are reproductive groups; PFG 2 is a nonreproductive group. NORMA**,** the border between the norm and hypertrophy.

ulation (for more details on the types of ontogeny, see Olenev, 1989, 2002).

The possibility of dealing with pure intrapopulation groups is one of the main advantages of our functional approach. This substantially improves the accuracy of analysis and permits logical interpretation of its results. This is confirmed by the successful use of the functional approach in numerous studies (Olenev, 2002). The existence of alternative developmental pathways (two variants of ontogeny in small mammals) is regarded as the basis of the structural and functional adaptive rearrangements that allow the population to perform an adaptive maneuver in response to changes in environmental conditions.

We also used the *hepatosuprarenal coefficient* (Puzanskii, 1974; Korneev and Karpov, 1978), also called the index of well-being. Earlier (Olenev, 1987), a simplified method of the calculation of this coefficient for predicting the numbers of rodents was proposed.

## ECOLOGICAL ANALYSIS OF SPLEEN HYPERTROPHY

**The prevalence of spleen hypertrophy in rodents.** In the study region, spleen hypertrophy was found in three species of *Clethrionomys* voles but was virtually absent in *Microtus* voles and mice (Table 2). Thus, this phenomenon may be considered genus-specific, at least in the Ural region, although we do not exclude the possibility of spleen hypertrophy in any rodent species in other regions.

Note that the variation of the spleen weight within the normal range was characteristic of all species studied. This is confirmed by the distributions of the spleen indices of the field vole and common field mouse (Figs. 2a, 2b). Only in a few animals did the indices exceed the normal value. The entire subsequent analysis was based on the data on the European bank vole.

**The relationship between spleen hypertrophy and animal age (using PFG 3 as an example).** The method of determining the absolute age by the degree of age-related changes of teeth (Olenev, 1989) allowed us to estimate the relationship of the absolute age and spleen hypertrophy in voles. The earliest cases of spleen hypertrophy were found in 30-day-old voles from PFG 3 (maturing animals under one year of age). The spleen index in some of these voles exceeded 50‰, which suggested that the spleen could increase very rapidly. Note that the type of animal ontogeny could have already been determined at the age of 30 days, whereas the correlation between age and the presence of a hypertrophied spleen at this age was at the limit of significance ($r = 0.225$, $p = 0.101$). The situation was the same in the case of overwintered animals (PFG 1) ($r = 0.236$, $p = 0.066$).

The absence of hypertrophied spleens in juvenile animals might be accounted for either by passive immunity, which is preserved in voles until the age of one month, or by the absence of exposure to the deleterious factor. Apparently, the animals were not exposed to this factor until the beginning of active, independent life and sexual maturation. We may also assume that the longer the animal lives in the area where the deleterious factor is present, the higher the probability of exposure to it. This is especially true of the overwintered group (PFG 1), which had an old absolute age and matured

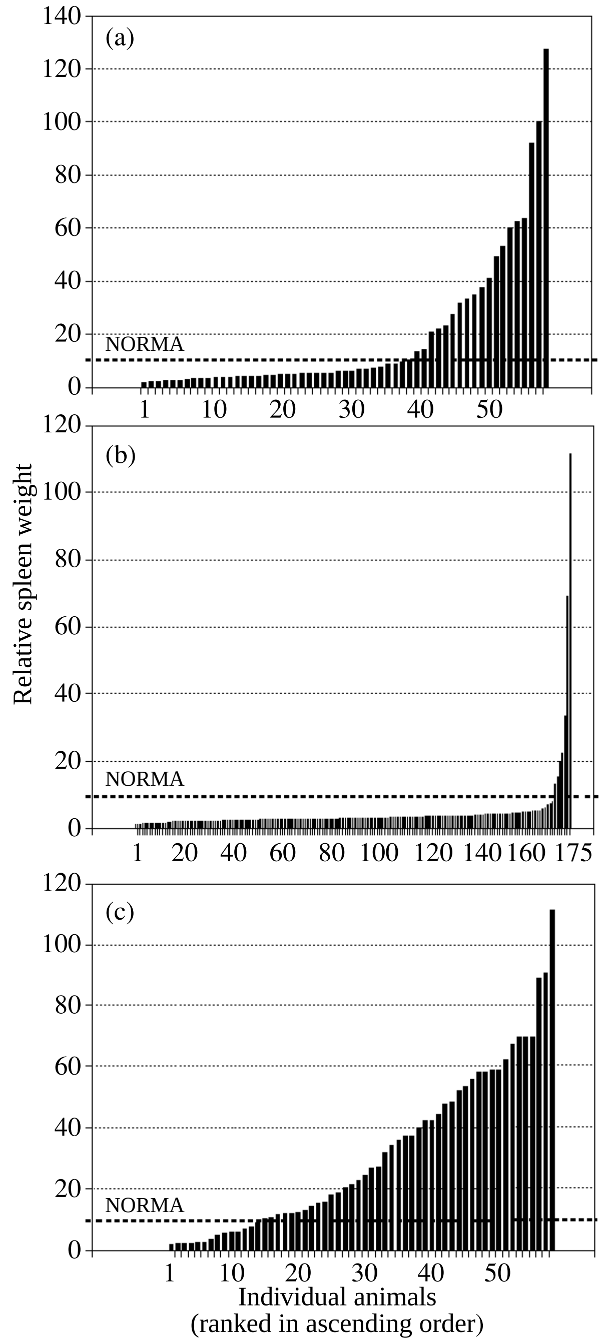


**Fig. 3.** Distribution of spleen indices in animals differing in the functional state: (a) reproductive young of the year (PFG 3), (b) nonreproductive young of the year (PFG 2), and (c) overwintered (PFG 1).

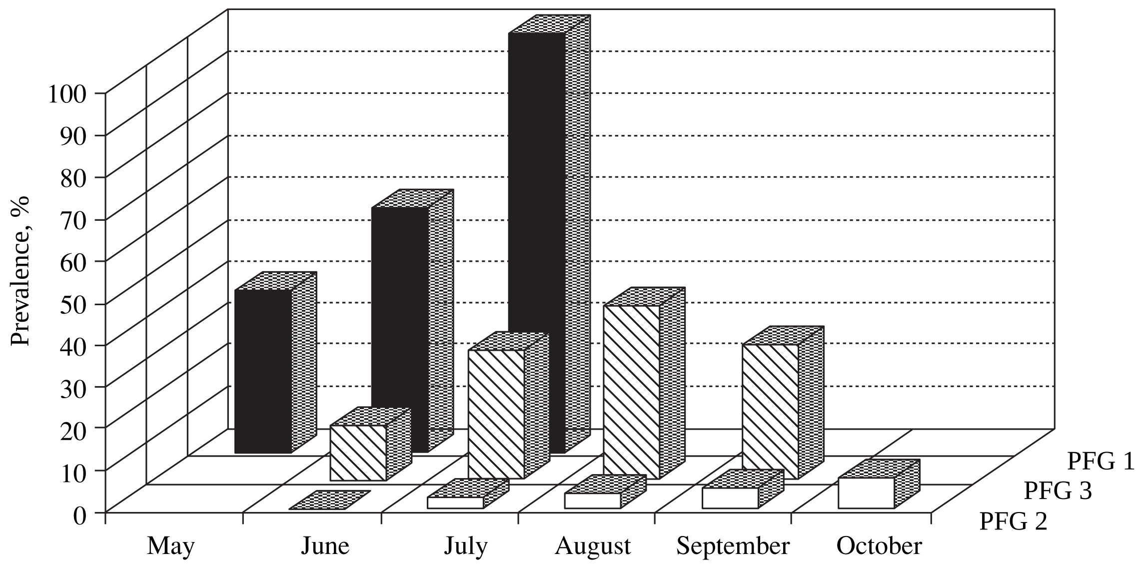


**Fig. 4.** Seasonal changes in the prevalence of spleen hypertrophy in animals differing in the functional state (mean values for a period of several years).

almost simultaneously in spring, i.e., synchronously developed a high metabolic rate. In this group, the spleen was hypertrophied, on average, in 35% of the animals in early May and in almost all animals by the end of the reproduction period.

Thus, spleen hypertrophy as a response of the animals to a hypothetical deleterious factor may develop very rapidly. Probably, the absolute age is important only as a correlative of the duration of the animal's stay in the area exposed to the deleterious factors; however, *the functional state of the animals is the main determinant.* The animals with a high metabolic rate (reproducing animals) accompanied by a high mobility and frequent social contacts are the most sensitive.

Apparently, the low prevalence of spleen hypertrophy in PFG 2 (nonreproductive animals under one year of age) is determined by their increased resistance to a wide range of factors, e.g., ionizing radiation (Olenev and Grigorkina, 1998), toxic metals (Bezel' and Olenev, 1989), high population density (Kolcheva and Olenev, 1991), and some other factors, which are related to the low metabolic rate in these animals.

**The relationship between spleen hypertrophy and the type of ontogeny.** We studied the variation range of the spleen in animals from different functional groups reflecting different phases of ontogeny types (Fig. 3). As seen from Fig. 3, the prevalence of spleen hypertrophy was the highest (77.6%) in overwintered animals (PFG 1); in reproductive young of the year (PFG 3), the prevalence was considerably lower (39.7%). In nonreproductive young of the year (PFG 2), spleen hypertrophy with spleen indices as high as 120‰ was also found; however, its prevalence was below 10%. Note that some animals had no definite functional status when they were caught; they were at the maturation stage and could not yet have been included in PFG 3 according to the criteria used (see above); therefore, the prevalence of spleen hypertrophy was actually lower.

A comparison between two groups of reproductive animals (PFGs 1 and 3) showed that the mean spleen indices in these groups (32.1 and 17.4‰, respectively) differed significantly ($t = 2.99$, $p = 0.003$). The standard deviations of the spleen indices in these groups (26.9 and 25.6, respectively) did not differ significantly ($f = 1.11$, $p = 0.693$). PFG 2 significantly differed from both PFG 1 and PFG 3 ($t = 6.18$ and $t = 2.37$, respectively).

Note that, although different PFGs considerably differed in the prevalence of spleen hypertrophy, the variation ranges of spleen index and, especially, its maximums, were almost the same. This confirms that the differences were essentially qualitative.

**Seasonal changes in the prevalence of spleen hypertrophy.** Among the overwintered voles caught in May, there already were animals with hypertrophied spleens, with the spleen indices reaching very high values (100‰ in some cases). Apparently, the hypertrophy began earlier, synchronously to maturation. The pattern of changes in the prevalence of spleen hypertrophy was almost the same in all cohorts (Fig. 4). All groups exhibited a distinct increase in the prevalence of spleen hypertrophy, with almost all PFG 1 animals having hypertrophied spleens by the end of the reproduction period. The prevalence of spleen hypertrophy in reproductive voles under one year of age (PFG 3) was lower than in overwintered voles (PFG 1); it was only 30% by the end of the reproduction season. Although the general trend toward an increased prevalence of spleen hypertrophy persisted during the reproduction period, this parameter varied in different years. This was accounted for by quantitative changes in the composition of the groups (in some years, the relative sizes of the first cohorts in populations were considerably reduced because of a high mortality, and the proportion of nonreproductive animals among young of the year constantly increased). Under varying environmental conditions, the proportion of reproductive young of the year (PFG 3) in populations rapidly changed with time. During 27 years of our studies, this proportion varied from 0 to 93% in PFG 3 (Olenev, 2002), whereas overwintered animals (PFG 1) reproduced under practically any conditions.

The time course of the season- and age-related changes in the absolute and relative weight of the spleen in bank voles born in different seasons confirmed that these oscillations had a regular pattern reflecting the population metabolic rhythms related to sexual maturation, growth, and reproduction.

**The spatial distribution of animals with spleen hypertrophy: the effect of specific conditions in different biotopes.** Two types of biotopes were most common in the study region. Earlier (Olenev, 1981), it was found that the populations of different biotopes may differ in several parameters, including age composition. In order to exclude the possible relationship of the specificity of habitats and populations in different biotopes with spleen hypertrophy, we performed comparative analysis using the data for the period 1997–1999. To compare dry and moist biotopes with respect to this parameter, we used Student's $t$ test with Fisher's φ-transformation. We did not find any significant differences between biotopic groups in the proportion of animals with hypertrophied spleens ($t$ = 0.45). This allows us to conclude that animals with spleen hypertrophy were uniformly distributed over the area.

**Population dynamics and prevalence of spleen hypertrophy.** We analyzed the relationship between the increase or decrease in the prevalence of spleen hypertrophy and the phases of rodent population dynamics over a 14-year period (Fig. 5). We found a significant negative correlation between the relative numbers of animals and the prevalence of spleen hypertrophy (Spearman's rank correlation coefficient was $R = -0.68$, $p = 0.01$). This relationship may be explained by the characteristic pattern of changes in the functional structure of the population (Olenev, 2002). In the years when the relative numbers are high, the proportion of reproductive animals under one year of age is usually lower than in the years when the numbers are low. Spleen hypertrophy is characteristic of reproductive animals, which may account for the observed relationship.

In the five years from 1997 to 2001, we, for the first time during the 27 years of studies, observed a permanent growth in animal numbers, which was untypical of this population. This was accompanied by a considerable increase in the prevalence of spleen hypertrophy, which exceeded the data observed during long-term studies.

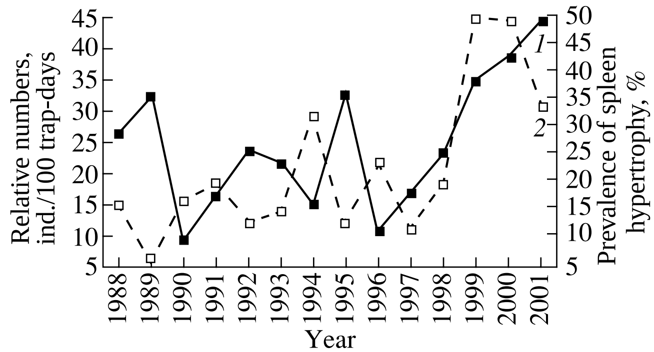


**Fig. 5.** The relationship between (*1*) relative numbers of animals and (*2*) prevalence of spleen hypertrophy, annual average values.

**Analysis of the groups of reproducing animals with respect to hepatosuprarenal coefficient.** This coefficient is generally regarded as an integrated index of the energy potential of animals or the index of well-being (Korneev and Karpov, 1978). The hepatosuprarenal coefficient may be used as an indicator of unspecific resistance of individual animals or populations. Its value is directly proportional to the energy required for regulatory and compensatory mechanisms.

When comparing animals with normal and hypertrophied spleens from the same PFGs, we did not find significant differences in this coefficient: for PFG 1, $t$ = 0.104 ($p$ = 0.917) and $f$ = 1.58 ($p$ = 0.241); for PFG 3, $t$ = 0.186 ($p$ = 0.85) and $f$ = 1.51 ($p$ = 0.391).

**Biological differences between animals with normal and hypertrophied spleens.** There were no noticeable external differences between the animals with normal and hypertrophied spleens. Both groups normally reproduced, matured within the same period, produced equal numbers of litters of similar size per reproduction season, had the same life span and survival rate, similar morphological parameters, etc. According to the values of their hepatosuprarenal coefficients, these animals had equally high resistances and energy potentials. In other words, spleen hypertrophy had no noticeable effect on the animal vital activity in natural populations. The phenomenon was asymptomatic. Nor was spleen hypertrophy sex-related, although the prevalence of spleen hypertrophy in males was nonsignificantly higher.

The results of our ecological analysis will promote future studies, especially medical ones, aimed at revealing concrete factors responsible for spleen hypertrophy.

## CONCLUSIONS

(1) We showed the scale of the phenomenon of spleen hypertrophy. The detailed analysis of the relative spleen weight in the rodents inhabiting the study region

demonstrated that spleen hypertrophy occurred in three species of *Clethrionomys* voles and was absent in *Microtus* voles and mice. Thus, the phenomenon, at least in some geographic regions, is genus-specific.

(2) For the dominant species (the bank vole), we determined the variation range of the spleen relative weight, which was very wide (from 1 to 125‰). The limits of the norm and hypertrophy were determined for the populations of six rodent species inhabiting the study region.

(3) We demonstrated a distinct direct relationship between the type of ontogeny and spleen hypertrophy. This phenomenon was especially characteristic of the reproductive portion of the population, including reproductive young of the year (PFG 3) and overwintered animals (PFG 1), i.e., the animals with high metabolic rates. Thus, mature and sexually active animals are the first to be included into the risk group.

(4) The progressive increase in the prevalence of spleen hypertrophy during the reproductive season occurred in all age groups (cohorts). The main determinant was the functional state of the animal, whereas the age was of little significance.

(5) The changes in animal numbers and the prevalence of spleen hypertrophy negatively correlated with each other. In the years when animal numbers decreased, the prevalence of spleen hypertrophy increased, and vice versa.

(6) Animals were distributed over the area nonuniformly because of the specificity of the populations of different biotopes with respect to several parameters, mainly demographic. However, the populations did not differ from one another in the prevalence of spleen hypertrophy.

(7) We believe that this parameter may be used as a new, specific ecological marker, which, on the one hand, indicates the presence of a deleterious factor in the population and, on the other hand, serves as an indicator of the state of environment. However, parameters of the spleen can hardly be used as morphological or physiological indices, mainly because of their considerable variability.

(8) Spleen hypertrophy is not accompanied by any deviations in vital activity, at least, with respect to the parameters estimated in this study; i.e., this phenomenon is asymptomatic. This suggests that the populations studied have adapted to an unknown deleterious factor(s) during a prolonged coevolution.

(9) Individual variation of characters is an important dynamic characteristic of a population, which reflects its responses to a wide range of external factors. Therefore, it is especially important to correctly estimate the degree of their variation. Taking into account the giant variation range of spleen index, it seems reasonable to analyze the norm and hypertrophy as two qualitatively different states when estimating the parameters of the spleen in various studies. In addition, the nature of external factors and the duration of exposure should be taken into account. For example, industrial pollution, to which animal populations have been exposed for several decades, usually causes changes within the normal range; whereas natural factors, to which they have been exposed for several hundreds or thousands of years, usually cause hypertrophy.

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