THE PARTICULARITIES OF THE ECOLOGICAL-GENETIC DIFFERENTIATION OF MICROTIN SPECIES IN THE ANTHROPIZED LANDSCAPE OF THE REPUBLIC OF MOLDOVA

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Abstract. Genus *Microtus* is presented in Republic of Moldova by two sibling species from "arvalis" group, *Microtus arvalis* (s. str.) and *Microtus rossiaemeridionalis*. Material were collected in 12 sites. A total of 516 genetic tests were performed. 188 individuals of *M. arvalis* and 92 – *M. rossiaemeridionalis* were identified through genetic methods. Both species has coexistence. Most findings of *M. arvalis* were in agrocoenosis, and *M.rossiaemeridionalis* in arboreal-shrubby coenoses.

Keywords: *Microtus*, voles, marking, genetic testing, density, agrocoenoses.

Rezumat. Particularitățile diferențierii ecologo-genetice a speciilor de microtine în lanșaftul antropizat din Republica Moldova. Genul *Microtus* este reprezentat în Republica Moldova de două specii surori din grupa „arvalis", *Microtus arvalis* (s. str.) și *Microtus rossiaemeridionalis*. Materialele au fost colectate în 12 locuri. Au fost efectuate în total 516 teste genetice. 188 de indivizi *M. arvalis* și 92 – *M. rossiaemeridionalis* au fost identificați prin metode genetice. Ambele specii populează simpatric biotopurile. Cele mai multe descoperiri ale speciei *M. arvalis* au fost în agrocenoză, iar ale speciei *M. rossiaemeridionalis* în cenozele arboricole-arbusticole.

Cuvinte cheie: *Microtus*, marcare, testare genetică, densitate, agrocenoze.

INTRODUCTION

The rodent fauna of the Republic of Moldova has been studied quite well (LOZAN, 1971), but as a result of the change in concepts regarding the taxonomy of microtines, the need arose to re-evaluate the data regarding their ecology (MALÂGHIN, 1983). The adaptive capacities of microtine species under the conditions of the agricultural landscape were determined in terms of the constantly changing trends of their adaptation strategies. The results obtained will be used to develop a forecast of the numerical strength of the object of study and its dynamics under the influence of anthropogenic impact and climate change in order to minimize the risks of affecting the food and medical-sanitary security of the country (MUNTEANU, 2003). Knowledge of the laws and ways of adaptation of mammals to different environmental conditions and, especially, to the anthropogenic landscape, dynamic by its nature, represents one of the central directions of theriology. The solution of this problem is possible only as a result of a systematic and detailed study of the population structure of the species in different ecological conditions and at different phases of the dynamics of the herd. Systematics is developing quite intensively as a result of the use by researchers of methods that allow a deeper understanding of the essence of the biological species.

The typological-morphological concept, according to which the species represents a totality of morphologically similar individuals, is replaced by the biological concept, which reveals the essence of the species as a dynamic biological system, consisting of a series of populations, reproductively isolated from the populations of other species (MALÂGHIN & DEULIN, 1979, SYTNIC, 1999; TKADLEK, 2001). There is a whole system of isolation mechanisms that prevent hybridization of some species with others. This is the basic condition for the segregation of species. From this point of view, the twin species *Microtus arvalis* (Pallas, 1778) and *Microtus rossiaemeridionalis* (Ognev, 1924) are of great importance, as well as those biological, ecological and genetic features that form the basis of their identification.

The information in the literature on the genetic differentiation of the studied species of microtines in correlation with spatial distribution is quite limited. In the Republic of Moldova, for the first time, a thorough analysis of the problem of elucidating and establishing the features mentioned above was undertaken. A higher probability of detecting twin species in some groups is determined by ecological conditions. Some ecological, genetic and cytological research was carried out in the 80s-90s of the 20th century (GAICENCO et al., 1975; ARAI et al., 1988; ZAGORODNIUC, 1991; ZAGORODNIUC et al., 1994; MIHAILENKO, 1993; SKIRIUTĖ, 2000). The research carried out fills the existing gaps.

MATERIALS AND METHODS

Samples for genetic research were collected from individuals captured in 12 locations in the districts of Ialoveni (Sociteni, Dănceni, Horăști), Ungheni (Rădenii-Vechi, the "Plaiul Fagului" scientific reserve) and Edineț (Ruseni) during the period 1985-2024. For this purpose, 1 ha marking nets installed on alfalfa fields, forest strips, orchards, pastures, wheat fields, where microtines have a higher population, were used. A total of 516 genetic tests were performed. The density was determined based on the results obtained over 4-5 days using snap traps or from marking nets over a week (NISTREANU et al., 2021). The populations were described using two categories of parameters: the general relative population and of different groups of adults and according to population structure indices - the share of each group in the

population. The absolute population was determined - the total number of individuals per hectare in the marking sectors, as well as the relative population - the number of individuals per 100 snap traps captured during one night (NAUMOV, 1956). In the captured animals the following parameters were recorded: species, sex, age, morpho-physiological indices, physiological and reproductive status. All captured individuals were subjected to detailed processing: weighing, determination of body length, tail, leaflet and ear, the external appearance and condition of the generative organs were described. Age was determined by external appearance, body mass and degree of wear of molars. The population size of the studied species was expressed as the number of individuals per unit area. This expression represents the unit of measurement, widely used in ecology and known as absolute density. The study of the spatial structure of populations in agrocenoses, determination of the numerical population, activity of individuals, areas of individual sectors were carried out on marking nets. Individuals were captured using traps, located on 4 ha nets at a distance of 20 m, and on 1 ha nets - at 10 m from each other and - directly - at colonies. Diagnosis of the species by the method of hemoglobin electrophoresis in polyacrylamide gel with a concentration of 8% was carried out in the laboratory by the Maurer method (MAURER, 1971; DOBROHOTOV et al., 1982). Tris-HCl (pH = 8.9) was used as a gel buffer solution, and tris-glycine (pH = 8.3) as a buffer solution for electrodes. The type of hemoglobin was determined in erythrocyte hemolysates, albumins and transferrins - in blood plasma and acetate hydrogenase - in kidney extracts, using standard histochemical staining methods (KUTNIUK et al., 1986). The variability of gene loci was assessed according to the heterozygosity degree $H=1 - \sum qi\ 2$, where qi - the frequency of allele i in the sample. The nomenclature and symbols are assessed according to Zakijan et. al. (ZAKIJAN et al., 1984).

RESULTS AND DISCUSSIONS

Being very sensitive to food conditions and heat exchange, as well as relatively sedentary, microtines inhabit different biotopes depending on the season and climatic peculiarities of the year, which is explained not only by dispersal into new habitats, but also by survival in different resorts. During the season with favorable conditions, a rapid increase in their numerical population and their dispersion occurs, with the formation of new populations. This process continues until optimal conditions are maintained in the respective resorts. Then, after the general deterioration of the living conditions, populations disappear from those habitats, where the factors become extremely unfavorable. On the contrary, they remain viable where vital needs correspond to a minimal level of ecological requirements (JACOB & TKADLEC, 2010).

The habitats of microtines in each region of the range, taking into account the predilection and spatial variability, can be divided into refuge stations, which ensure the existence of populations in critical periods of the year, and dispersal stations, where only certain generations can survive (JÁNOVÁ, 2003). In arid regions, shrub thickets, forest edges, glades, and forest curtains serve as refuge stations in the summer. In the northern regions of the range, where a rather critical situation is created during the cold period of the year, refuge resorts serve the non-floodable sectors during spring and autumn, and the snow cover is relatively moderate: forest edges, hill slopes, and meadows. In regions with intensive agriculture, agrotechnical measures are of decisive importance (BRYJA et al., 2005). There was a process of changing the resorts in terms of refuge habitats of the studied species of microtines. In the optimum sectors of the range, microtines survive in most of the territory, except for predominantly humid sectors, and in the southern regions, irrigated crops serve as refuge resorts.

According to other researchers, the most frequently encountered species of microtines in the republic is *M. arvalis*, being reported in about 66% of the registered locations (ZAGORODNIUC et al., 1994). The studied microtine species are widely distributed. The range of each of them covers the entire territory of the country (SYTNIC et al., 1995; SÎTNIC, 1999; SYTNIC, 1999; TIKHONOVA et al., 2006). However, some clear trends in spatial and biotopic differentiation have been recorded. *M. arvalis* is more frequently found in agrocenoses and, primarily, in perennial forage grasses, and *M. rossiaemeridionalis* – in arboreal-shrub cenoses (forest curtains, orchards). For the first species, the territory of the country represents an optimum area, and for the twin species this geographical space constitutes the western limits of the distribution. *M. rossiaemeridionalis* was not identified in Romania and in the regions bordering Ukraine, primarily in the Chernivtsi region (TESLENKO, 1986). Tab. 1. presents the ratio between the species of the genus *Microtus* in different biotopes.

The species *M.arvalis* and *M.rossiaemeridionalis* were differentiated by the type of hemoglobin (Hb). The basic fraction of hemoglobin is identical in both species, and the minor (fast) one is missing in *M. arvalis* and is a marker in *M. rossiaemeridionalis* (DOBROHOTOV & MALÎGHIN, 1982). A total of 280 individuals were diagnosed, of which about 2/3 belonged to the species *M. arvalis*. The population of both species was determined absolutely in all localities (Table 1).

Systematization of the data, taking into account the fluctuation of the numerical population, demonstrated the predominance of the species *M. arvalis* in all phases of oscillation, the share being higher in the phase of decrease (Table 2).

The blood plasma proteins, albumin and transferrin, were studied in 56 individuals, of which 35 belonged to the *M. arvalis* species and 21 – to the *M. rossiaemeridionalis* species.

Albumin (Alb) has an identical electrophoretic mobility in both species, being represented by a single type. Only two rare variants were determined in the *M. arvalis* population. Thus, this protein is monomorphic in both species.

Transferrin (Tf) was represented by some and the same types in the *M. arvalis* and *M. rossiaemeridionalis* populations.

Table 1. The numerical share of individuals of the *Microtus* genus species in the anthropized landscape.

N/o	Locality, biotope	*M. arvalis*		*M. rossiaemeridionalis*	
		n	%	n	%
1.	Sociteni, pasture with a group of trees	2	66,7	1	33,3
2.	Sociteni, alfalfa field – forest strip	5	41,7	7	58,3
3.	Horăşti, alfalfa field – forest strip	28	68,3	13	31,7
4.	Sociteni, alfalfa field – forest strip	30	90,9	3	9,1
5.	Sociteni, forest strip along the alfalfa field	5	41,7	7	58,3
6.	Dănceni, old orchard	17	58,6	12	41,4
7.	Horăşti, alfalfa field – forest strip	7	53,8	6	46,2
8.	RădeniiVechi, alfalfa field-forest	32	100,0	0	0,0
9.	Dănceni, alfalfa field-group of trees	1	7,7	12	92,3
10.	Ruseni, alfalfa field – forest strip	5	20,8	19	79,2
11.	Dănceni, old orchard	24	82,8	5	17,2
12.	Horăşti, alfalfa field-wheat field	32	82,1	7	17,9
	Total	188	67,1	92	32,9

Table 2. Fluctuation of the weight (%) of microtine species at different phases of the oscillation.

Species	Peak phase	Decline phase	Depression phase	Growth phase
M. arvalis	62,5 %	77,8%	57,1%	68,6%
M. rossiaemeridionalis	37,5%	22,2%	42,9%	31,4%
Total effective	56	45	42	137

According to the data obtained, in both species this protein is polymorphic, being controlled by three codominant alleles: TfA, Tf B , Tf C (they are listed in order of decreasing mobility of the corresponding protein variants). In both species the most frequent allele is Tf B , the Tf C allele is rare; The interspecific differentiation at the Tf locus is small. The level of variability of this locus in the *M. arvalis* population (H = 0.336) is slightly lower compared to the *M. rossiaemeridionalis* population (H = 0.453).

Lactate dehydrogenase (Ldh-1 and Ldh-2) was studied in 124 individuals, of which 91 belonged to the species *M. arvalis* and 33 – *M. rossiaemeridionalis*. The enzyme was represented by three types – ¬ in the population of the first species and by one type – the second. The Ldh-1A allele was identified in both species, which determines the "fast" variant, and the Ldh-1B allele only in *M. arvalis*, which determines the "slow" variant. The second locus was invariable. It is worth noting that only in two out of five localities in M.arvalis was the Ldh-1B allele identified, in the others – only the Ldh-1A allele (Table 3).

Table 3. Ldh-1 locus allele frequency in the *M. arvalis* species population.

Locality	$Ldh\text{-}1^A$	$Ldh\text{-}1^B$
Sociteni, pasture with a group of trees	1,000	0,000
Sociteni, alfalfa field – forest strip	0,700	0,300
Horăşti, alfalfa field – forest strip	0,857	0,143
Dănceni, old orchard	1,000	0,000
Horăşti, alfalfa field-wheat field	1,000	0,000
Mean value	0,911	0,089

In general, the Ldh-1B allele was less frequent in the *M.arvalis* population, and the level of variability of the Ldh-1 locus is low (H=0.162).

The level of polymorphism, in general, is high in perennial grass fields, where ecological conditions are more favorable. However, it is impossible to explain the variability only by the action of selection, without taking into account the effect of isolation and gene drift, especially during the phase of population depression, when the importance of the "founder effect" increases. We demonstrate this fact on the example of a simple single-locus model. Let the population consist of a series of isolated groups of individuals with a reduced population, with an identical frequency of alleles A and B, and qA=0.9 and q B =0.1. If by chance only two individuals from each group participate in the formation of the next generation, then by decomposing the binomial (qA + qB)2 the probability of groups with a different concentration of alleles A and B is calculated. It turns out that only in 44% of the groups both alleles are preserved, and in 66% of the groups the presence of allele A will be recorded and allele B will be eliminated, which is consistent with the experimental data regarding lactate dehydrogenase. In the population, however, the frequency of alleles A and B will remain invariable.

CONCLUSIONS

It was established that *M.arvalis* is more frequently found in agrocenoses, and *M. rossiaemeridionalis* – in arboreal-shrub cenoses. For the first species, the territory of the country represents an optimum zone, and for the twin species this geographical space constitutes the western limits of distribution. M.arvalis dominates by about 66%.

It was determined that transferrin (Tf) in both species is polymorphic, being controlled by three codominant alleles: TfA, Tf B , Tf C . In both species, the most frequent allele is Tf B, the Tf C allele is rare. The level of variability

of this locus in the *M. arvalis* population (H = 0.336) is slightly lower compared to the *M. rossiaemeridionalis* population (H = 0.453).

Lactate dehydrogenase was represented by three types -- in the population of the first species and by one type – in the second. The Ldh-1A allele was identified in both sible species, which determines the "fast" variant, and the Ldh-1B allele only in *M.arvalis*, which determines the "slow" variant. The second locus was invariable.

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