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BIOINDICATOR EPIGENETIC CHARACTERISTIC OF THE ZOOMONITOR WOOD MOUSE (APODEMUS SYLVATICUS) FOR ENVIRONMENTAL CONTAMINANTS IN AGROECOSYSTEMS

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***Abstract**: Frequency distribution of phenotype realization of discrete alternative craniological traits – markers of genotype structure of wood mouse (Apodemus sylvaticus) in populations inhabiting the agroecosystem in North Eastern Bulgaria was determined.*

The obtained characteristics of epigenetic polymorphism of studied craniological morphogenetic markers reveals the possibility to be applied as information parameter in biomonitoring of agroregion environment condition through the zoomonitor A. sylvaticus in the agroecosystem in Bulgaria.

Introduction

After decades of exploitation of soils in agroecosystems, newly dominant ecological factors have arisen. They change greatly under anthropogenic impact and from a new complex factor in the agricultural environment. The mass usage of present-day resources of plant protection (pesticides and fertilizers) and pronounced desire for intensification of agriculture through artificial improvement of soil fruitfulness by fertilization causes vast changes in the environment of agro-ecosystems. Using pesticides and fertilizers is not environmentally sustainable and can cause deadly changes in the soil since it becomes full of heavy metals, chemicals, nitrates, phosphates, and other harmful waste. Today we can see the harmful effects and changes in soil fertility because of our past intensive use of the land, we can see the damages such as (i) chemical contamination with heavy metals, pesticides, petroleum products exceeding health regulations including increased salinity and acidity; (ii) contamination with organic and mineral fertilizers and waste; (iii) physical degradation (wind and water erosion including anthropogenic aspects), oversaturated and swampification, the results of burning of field stubble and plant waste.

Anthropogenic impact on environmental processes in agroecosystems has become one of the most important ecological factors in defining existing biological systems. We conclude that it's insufficient to evaluate environmental habitats by studying merely the chemical-analytic control of abiotic components, and furthermore difficult to determine critical thresholds for the technical loading of the fields.

Independent application of chemical-analytical control in monitoring studies at the ecosystem level for determination of environmental thresholds is also restricted by the fact

that in reality, plants and animals are influenced by complex and interrelated physical, chemical, and biological factors. Depending on the nature, intensity and time of influence of particular agents their joint action can create essentially different effects – additive, synergistic, or opposing effects.

In nature, in most cases occur a joint action of the polluters of different types, low concentrations and doses of influence, which results in synergistic increase of the genetic effects. Because of this when we plan and carry out eco-monitoring, we have to use such test-criteria and test subjects, which determine correctly the degree of complex technical loading of the environment. This possibility can be realized through the application of bio-monitoring.

With bio-monitoring, the opposite of chemical-analytic control, it is possible to estimate correctly and prognosticate the deviations in the biological systems state from the norm of reaction, which has been caused by an actual complex of anthropogenic factors. Although in rather limited cases, bio-monitoring results could be bound with a distinct influential factor, it could provide an integral assessment of the consequences for the living creatures caused by a complex of contaminated substances, as is more typical.

With biological monitoring it's critical to select appropriate bioindicators. Small mammals (*Rodentia*) are excellent indictors for biomonitoring because they have a number of features which make them preferred zoomonitors: high population numbers, non-migratory, well-known genetics and anatomy, short gestation period, and food similar to that of the man.

The significance of morphological, physiological and behavioral features of small mammals, which have strict quantitative expression, is difficult to be estimated comparatively in forming a bioindicator value for environmental change. Recently, the results of a purely population method are applied as highly informative to the reaction of a population of wild animals to the appearance of new influential factors in the environment. This method is the phenetic analysis, based on the analysis of morphogenetic variation in natural populations carried out through evaluation of polymorphism of a large number of morpho-craniological features from sufficient number of individuals.

On the grounds of the revealed frequency distribution of realized phenotypes of discrete alternative features – genotypic markers, the present study aims: (i) to determine the specific values for morphogenetic variation in populations of the wood mouse (*A. sylvaticus*) inhabiting agroecosystems in Northeastern Bulgaria; (ii) to evaluate its uniqueness; (iii) to develop a basis for analysis of the character of the morphogenetic variation of this zoomonitor in agroecosystems throughout Bulgaria.

Materials and Methods

A sample of 82 individuals of the wood mouse (*A. sylvaticus*) inhabiting an agricultural ecosystem in the environs of the Zlatna Niva country near the city of Shumen (Northeastern Bulgaria) was studied. In order to carry out comparative analysis of the specific features and uniqueness of the studied population from Zlatna Niva (G_1), data from five other populations were included: Western Stara Mountains (G_2); Rila Mountains (G_3); Central Danube Plains (G_4); Southeastern Dobrudja (G_5); Southwestern Bulgaria (Rupite region) (G_6). These data were described by Chasovnikarova and Markov (1999) and represent the basis for evaluation of epigenetic characteristics and population divergence of the wood mouse in Bulgaria.

Species determination was done in a laboratory through craniometrical analysis. A cranial discriminant function expanded from a genetically determined group of individuals of *A. sylvaticus* from Bulgaria (Chasovnikarova, Markov, 2005) was used to classify wood mice individuals.

The pheno-genetic analysis of the studied wood mouse populations was based on 20 aberrations. Non-metric craniological variation was evaluated on the basis of the

manifestation of qualitative cranial features: presence or absence of orifices for nerves and blood vessels, presence of additional bone structures, etc. The reading of these features was done on the left side of the skull under stereo microscope with 12x magnification.

Craniological feature complex (Hedges, 1969) and statistical methods (Berry, 1968; Smith, 1973; Sjovold, 1973) were applied in estimation of the epigenetic polymorphism and divergence of studied populations. Epigenetic variation (Vi), epigenetic uniqueness (MU), and epigenetic distance (MMD) between each studied population and every other population were calculated.

Results and Discussion

The frequency distribution of studied non-metric features provided characteristic population epigenetic cranial polymorphism of the wood mouse in the studied ecosystem and was specific for each feature. Some of the features don't manifest polymorphism and some exhibit a significantly different frequency distribution from the mean value of the epigenetic polymorphism in the studied features (Fig. 1).

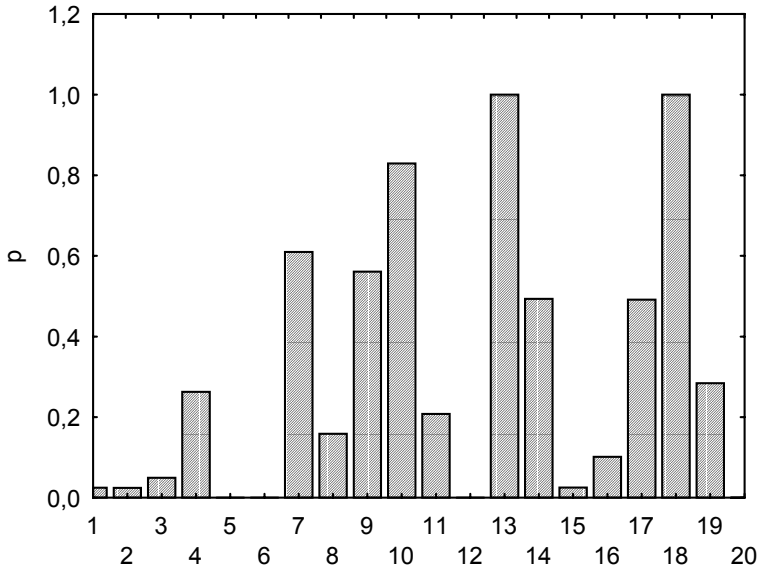

Fig. 1. Frequency distribution (p) of the studied non-metric cranial features for the wood mouse (*A. sylvaticus*) in agroecosystems in NE Bulgaria (area of Zlatna niva; G_1).

The analysis of distribution of polymorphism found in the studied craniological features in the wood mouse population in agricultural ecosystem of Northeastern Bulgaria (area of Zlatna Niva; G_1) showed that:

1. Features: "frontal fontanelle – *present*" (№ 5), "maxillary foramen I – *absent*" (No. 6), and "foramen sphenoidale laterale ventrale – *present*" (No.12), "third molar – *absence*" (No. 20) - null occurrence;
2. Features: "processus pterygoideus – *absent*" (No. 13) "accessory mental foramen – *present*" (No. 18), had maximal frequency of occurrence;
3. Features: "maxillary foramen I – *double*" (No. 7), "foramen palatinum minus anterius – *absent*" (No. 10), "foramen hypoglossi – *single*" (No. 17) had comparatively high frequency of occurrence;
4. Features: "frontals - *together*" (No. 4), "frontal foramen – *double*" (No. 8), "foramen palatinum majus – *double*" (No. 9), "foramen sphenoidale medium – *present*" (No. 11), "foramen ovale – *single*" (No. 14) and "mandibular foramen – *double*" (No. 19) had comparatively average frequency of occurrence;

5. Features: “preorbital foramen – *double*” (No. 1), “interfrontal – *present*” (No. 2), “frontals – *separate*” (No. 3), “foramen infraovale – *double*” (No. 15), “foramen pterygoideum – *double*” (No. 16) had comparatively low frequency of occurrence.

Comparative assessment of epigenetic variation (Vi = 0.101) of the of the wood mouse population in agricultural ecosystem of Northeastern Bulgaria and populations in other regions (Fig. 2) show that the studied population exhibits the lowest epigenetic variation. It is very similar to the absolute value of the epigenetic variation of the Rila Mountain population (G_3), differs greatly from the epigenetic variation in the Danube plains (G_4) and Southeastern Dobrydja (G_5) populations, and is also very different from the epigenetic variation in the Western Stara Mountains (G_2) and Southwest Bulgaria (Rupite region; G_6) populations.

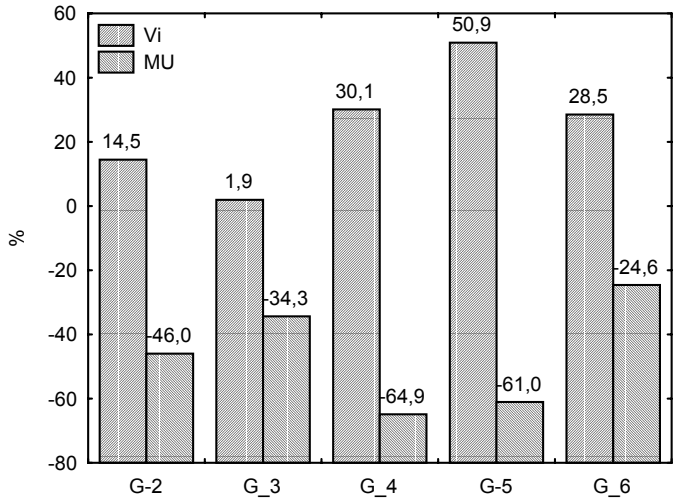

Fig. 2. Comparative values (%) of epigenetic variation (Vi) and uniqueness (MU) of the population in Northeastern Bulgaria (Zlatna Niva region), towards the populations in natural ecosystems (Chasovnikarova and Markov, 1999) in the Western Stara Mountains (G_2); Rila Mountains (G_3); Central Danube Plain (G_4); Southeastern Dobrudja (G_5); Southwestern Bulgaria (Rupite region; G_6).

All established population epigenetic distances between the wood mouse population from Northeastern Bulgaria and those from natural ecosystems in Bulgaria are statistically significant ($p<0.025$).

The epigenetic divergence of the population from Northeastern Bulgaria agricultural ecosystem reveals its epigenetic cranial uniqueness (MU = 3.55; Fig. 2), which has the highest value of the populations in this study, and is most different from the neighboring populations in Northern Bulgaria – the population from the Central Danube Plain (G_4) and Southeastern Dobrydja (G_5).

Comparative evaluation of inter-population epigenetic distances between wood mouse population in agricultural ecosystem in Northeastern Bulgaria and other natural populations of the species in Bulgaria (Chasovnikarova and Markov, 1999), showed that by its epigenetic characteristics this population is (Fig. 3): (i) very different from the other natural populations, all of which have 50-60% inter-population similarity; (ii) very isolated from the neighboring populations in Northern Bulgaria, all of which have a high level of similarity between them; (iii) reveals close intra-population epigenetic distance compared with the Rila Mountains population, which lives under specific mountainous ecological conditions.

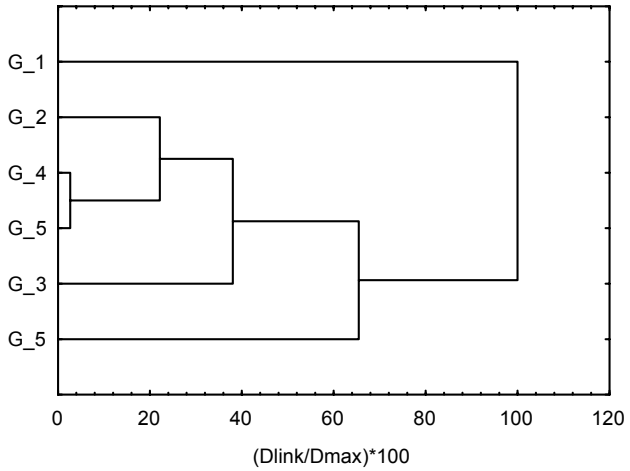

Fig. 3. Comparative evaluation between epigenetic distances of populations of the wood mouse (*Apodemus sylvaticus*; MMD) from agroecosystems in Northeastern Bulgaria (Zlatna Niva region; G_1) and from natural ecosystems in Western Stara Mountains (G_2); Rila Moutains (G_3); Central Danube Plain (G_4); Southeastern Dobrudja (G_5); Southwestern Bulgaria (Rupite region; G_6).

The comparative analysis of epigenetic variation revealed statistically significant inter-population distances between studied populations and indicative epigenetic cranial uniqueness of the wood mouse population from agricultural ecosystem in Northeastern Bulgaria. The results show that comparing the distribution of non-metric craniological markers of realized phenotypes and evaluating morphogenetic variation of the common wood mouse (*Apodemus sylvaticus*) is a viable option for including the epigenetic analysis as a part of biological monitoring. Therefore this species is an appropriate zoomonitor to track changes in the correlation of morphs and the appearance of new phenotype tendencies under amplified influence of anthropogenic stimulations, represented by new complex factors of the environment in the agro-regions.

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