ORIGINAL ARTICLE

Ecological aspects of multi-locus variability of DNA markers in animals

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Abstract

Aim: The work presents the algorithm for calculating the potential number of genetic combinations with an increase in the animal population to infinity. **Methods:** Calculations were carried out using the methods of bias corrected form for the Chao 1 and the 1st order jackknife, based on the multi-locus variability of DNA markers (RAPD and ISSR) in two specially protected species of *Lucanus cervus* (stag-beetle) and *Helicopsis striata* (steppe snail). **Results:** According to the obtained data, the groups, inhabiting relict biocenoses, are the most prominent among the populations of two species. There is the largest number of multi-locus genotypes, most of which are unique.The most monomorphic are the populations, inhabiting technogenic territories, because strongly isolated and small groups of animals are formed here very often. Their drift of genes leads to a decrease in allelic diversity. **Conclusion:** In addition, it has been established, that dapper animals (insects) are better adapted for existence in anthropogenic environment, than sedentary species (snails).

Key words: Anthropogenic impact, beetles, DNA, multi-locus genotypes, populations, potential genetic diversity, snails

INTRODUCTION

It is known, that Shmalkhausen[1] calls allelic diversity as “mobilization reserve” of the population, which provides it with a more stable existence in time. Based on this view, it is possible to assess the viability and, in part, the further evolutionary fate of natural populations, both vulnerable and common species. At the same time, the level of genetic heterogeneity of such groups is often estimated on the basis of samples with a limited number, containing only a small part of the population allele fund. The problem is aggravated by the fact, that often a limited number of loci (allozymes or DNA markers) are used for analysis, that does not allow to estimate the state of population genetic pools accurately. Usually, to solve this problem, it is necessary to either increase the number of selected individuals (that is undesirable for rare and vulnerable species) or expand the number of expensive biochemical markers. At present time, there is a mechanism for calculation the potential genetic diversity of populations, in case of an increase in the sample size to infinity. Such an analysis is carried out, for example, using nonparametric methods: The bias corrected form for the Chao 1 method[2] and the 1st order jackknife method.[3]

METHODOLOGY

As an example, we used data on the frequencies of DNA locus alleles (RAPD and ISSR) from our previous studies of the population structure of two specially protected species: a sedentary terrestrial steppe snail *Helicopsis striata* (9 populations)[4] and a highly-dapper stag-beetle *Lucanus cervus* (4 populations),[5,6] inhabiting the south of the Central Russian Upland.

To assess the potential genetic diversity of populations, we calculated multi-locus combinations for each of the selected

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Received: 09-09-2017
Revised: 17-09-2017
Accepted: 21-09-2017

individuals. Then, in each group, the total number of multi-locus genotypes (N_{MLG}) and the number of unique multi-locus genotypes (N_{MLG-1}), i.e., those combinations, which were noted in one single sample, were estimated. Further, based on the distribution of multi-locus genotypes frequencies, the potential genetic diversity, expected in case of increase in the sample size to infinity (N_{max}), was defined for each population. All calculations were carried out using SPADE software.[7]

EXPERIMENTAL

The distribution pattern of multi-locus genotypes in the populations under study is shown in Figures 1 and 2, and the calculated indicators of potential genetic diversity are presented in the Table 1.

According to the obtained data, the 7th and 8th groups stand out the most strongly among the populations of *H. striata*.

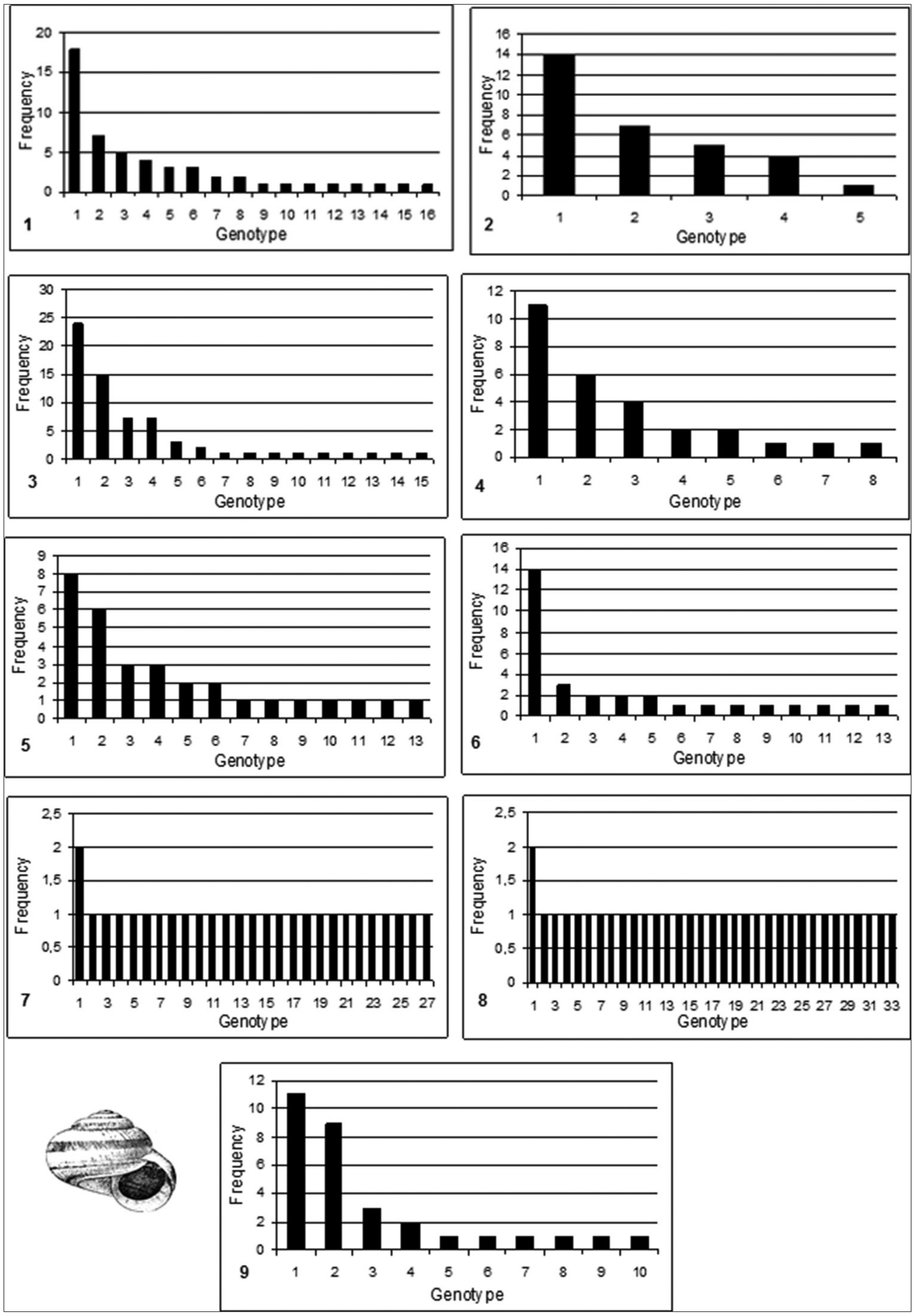

Figure 1: The distribution of multi-locus genotypes of DNA markers in populations of *Helicopsis striata* (the names of populations: 1 - Gubkin; 2 - Melavoe; 3 - Teleshovka; 4 - Khmelevoe; 5 - Nagol'noe; 6 - Kalyuzhnyi yar; 7 - Kreidyanaya mountain; 8 - Divnogor'e, 9 - Yamskaya steppe[4]

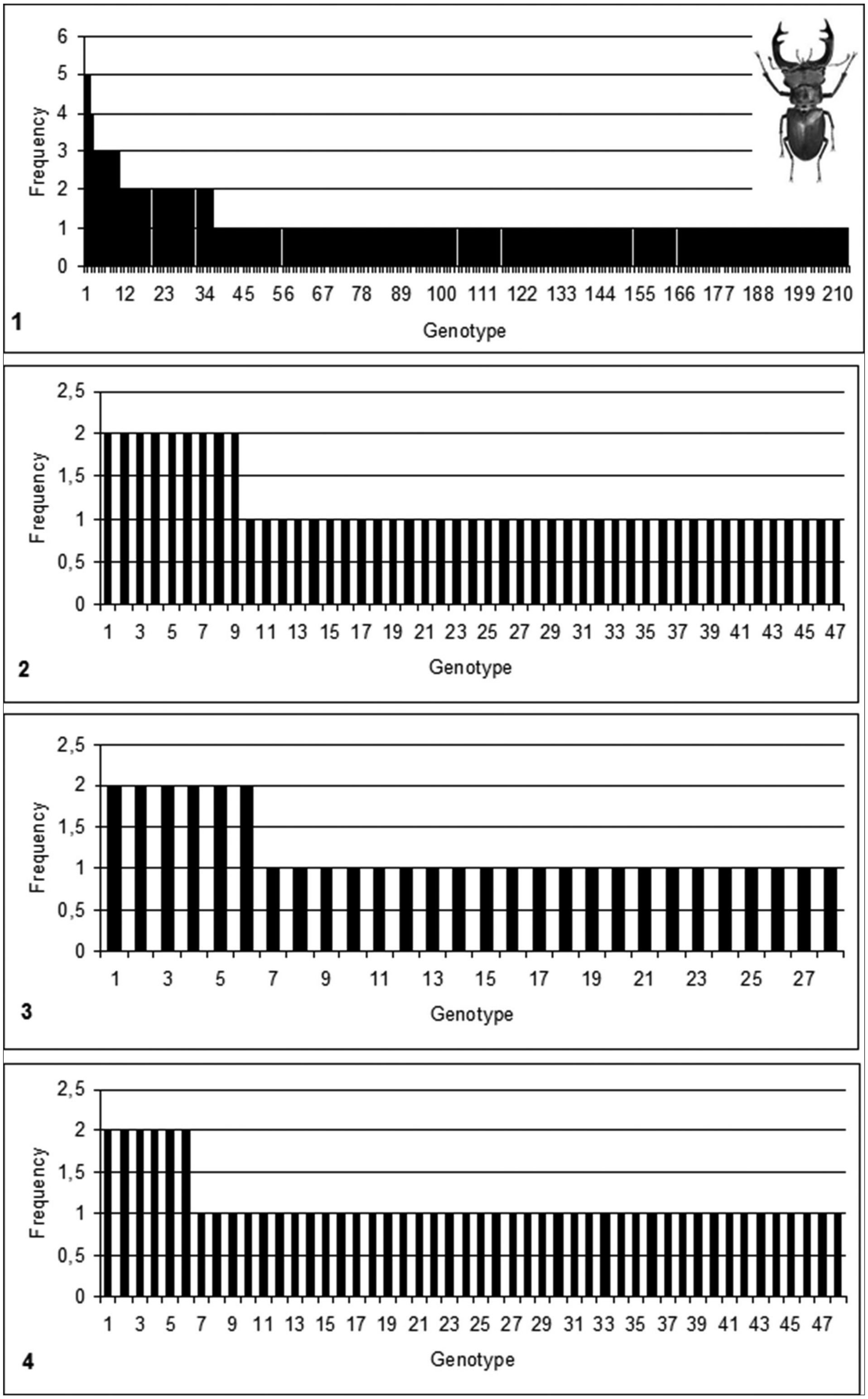

Figure 2: The distribution of multi-locus genotypes of DNA markers in populations of *Lucanus cervus* (the names of populations: 1 - Central, 2 - Western, 3 - Northern, 4 - Eastern)[5]

These populations inhabit the relict biocenoses. In these groups, the greatest number of multi-locus genotypes was noted, which turned out to be unique for them. The 2nd group was the most monomorphic. It inhabits the area of influence of the ore dressing and processing enterprise of Kursk magnetic anomaly.

It should be noted, that the actual genotypic diversity in individual samples often does not correlate with the calculated expected genotypic diversity, because the latter depends not only on the total number of detected combinations but also on their distribution in the analyzed group (i.e., on the number of individuals with one or another combination).

Table 1: The number of defined multi-locus genotypes and assessments of potential genetic diversity, obtained by different methods, for the populations of *H. striata* and *L. cervus*

Species	Population	N	N_{MLG}	N_{MLG-1}	Method			
					Chao 1-bc		The 1st order jackknife	
					N_{max}±SE	95% CI	N_{max}±SE	95% CI
H. striata	1	52	16	10	25.2±8.7	17.9-60.2	23.8±3.9	19.1-35.9
	2	31	5	4	5.0±0.4	5.0-5.0	6.0±1.4	5.1-12.6
	3	67	15	8	32.7±15.9	18.9-95.1	23.9±4.2	18.7-36.4
	4	28	8	4	9.0±1.8	8.1-18.4	10.9±2.4	8.7-19.9
	5	31	13	9	19.8±6.9	14.3-48.5	19.8±3.7	15.5-31.2
	6	31	13	8	19.8±6.5	14.4-45.8	20.7±3.9	16.0-32.7
	7	28	27	27	183.7±101.1	76.3-525.1	52.1±7.0	41.6-69.9
	8	34	33	33	273.7±149.0	111.8-768.1	64.1±7.8	52.1-83.5
	9	31	10	8	17.3±7.9	11.3-51.3	15.8±3.4	12.0-26.7
L. cervus	1	263	212	188	787.7±140.0	570.9-1135.9	389.3±18.8	356.2-430.1
	2	56	47	35	116.0±31.8	76.2-210.3	84.3±8.6	70.9-105.3
	3	34	28	21	60.0±18.8	39.0-121.0	49.4±6.5	39.9-66.2
	4	54	48	35	168.7±57.7	97.6-341.6	89.2±9.0	75.0-111.0

H. striata: Helicopsis striata, L. cervus: Lucanus cervus, SE: Standard deviation, CI: Confidence interval

Among the populations of *L. cervus*, the 1st group has the most abundant genetic pool, both real and potential. However, such a sharp difference in the number of combinations can be partly explained by the large number of analyzed individuals. The fewest combinations were found in the 3rd group, which inhabited the same anthropogenically altered area as the population of *H. striata* (N2). This fact indicates that under the influence of humans, there is a decrease in the genetic diversity of natural populations, due to the changes of biotopes and the death of some part of individuals. In addition, strongly isolated and small groups of animals are often formed in industrial zones, where the genetic drift leads to a decrease in genetic diversity. This was demonstrated by us on other groups of animals many times.[8-12]

Attention is also drawn to the fact that despite an equal number of analyzed loci, *L. cervus* populations are more polymorphic than *H. striata* populations. This is probably facilitated by the high mobility of beetles compared to snails. Due to the migration activity, the populations can exchange by different alleles and, thus, maintain a high level of genetic diversity.

DISCUSSION

Thus, using the described method, it is possible to determine the state of population genetic pools of the analyzed species more accurately. This will allow to assess their viability more objectively, and to make more realistic predictions about their further evolutionary fate.

CONCLUSIONS

The analysis showed, that the greatest genotypic diversity is observed in populations, inhabiting undisturbed relict biocenoses. Under conditions of anthropogenic influence, a significant decrease in allelic combinations is observed. In addition, it has been established, that dapper animals (insects) are better adapted for existence in anthropogenic environment than sedentary species (snails).

REFERENCES

1. Shmalkhausen II. Evolution Factors: Theory of Stabilizing Selection. Moscow: Nauka; 1968. p. 451.
2. Chao A. Species richness estimation. In: Balakrishnan N, Read CB, Vidakovic B, editors. Encyclopedia of Statistical Science. New York: Wiley; 2005. p. 7907-16.
3. Burnham KP, Overton WS. Estimation of the size of a closed population when capture probabilities vary among animals. Biometrika 1978;65:625-33.
4. Snegin EA. Estimating the state of population gene pools of the specially protected *Helicopsis striata* (*Mollusca*, *Gastropoda*, *Pulmonata*) species based on DNA markers. Russ J Genet Appl Res 2017;7:135-44.
5. Snegin EA. Assessment of viability of populations of a stag-beetle *Lucanus cervus* L, 1758 in forest-steppe landscape. Eurasian Entomol J 2011;10:137-42.
6. Snegin EA. Analysis of gene flow between generations of various years in population of stag beetle (*Lucanus cervus L.*) based on RAPD and ISSR DNA markers. Adv Environ Biol 2014;8:9-12.

7. Chao A, Shen TJ. SPADE; 2009. Available from: http://www.chao.stat.nthu.edu.tw. [Last accessed on 2015 Nov 28].
8. Snegin EA. Assessment of the state of population gene pools of terrestrial mollusks in conditions of influence of ore dressing combines from the example of *Bradybaena fruticum* Mull. (*Gastropoda*, *Pullmonata*). Russ J Genet Appl Res 2011;1:379-89.
9. Snegin EA. Analysis of genetic variability of terrestrial mollusk populations *Bradybaena fruticum* Müll. Using RAPD and ISSR markers. Belgorod state university scientific bulletin. Nat Sci 2011;15:37-43.
10. Snegin EA. Analysis of genetic variability in populations of a terrestrial snail *Chondrula tridens* Müll. (*Gastropoda*, *Pulmonata*), based on the RAPD and ISSR markers. Russ J Genet Appl Res 2014;4:444-54.
11. Snegin EA, Shapovalova EA, Polynov VA, Shapovalov AS. The state of genetic pools and the estimation of the effective number of populations of indicator species of the rodent mice under the influence of the mining enterprises. Belgorod state university scientific bulletin. Nat Sci 2014;17:95-103.
12. Snegin EA. Temporal dynamics of the genetic structure and effective size of *Bradybaena fruticum* Müll. (*Mollusca*, *Gastropoda*, *Pulmonata*) populations in the south of the central Russian upland. Russ J Ecol 2015;46:260-6.

Source of Support: Nil. **Conflict of Interest:** None declared.