

Kramarenko S.S., Ph.D. in Biology (Candidate of Biological Sciences), Ass.Professor,
E-mail: KSSNAIL@rambler.ru

*Mykolayiv State Agrarian University, Parizhskoi Kommuny str. 9, Mykolayiv 54021,
Ukraine*

GENETIC STRUCTURE AND EFFECTIVE SIZE POPULATION OF THE LAND SNAIL *CEPAEA VINDOBONENSIS*, INTERMEDIATE HOST OF TREMATODA IN THE SOUTHERN UKRAINE

*A high polymorphism at RAPD-marker for studied population of the land snail *C.vindobonensis* was observed ($h = 0,236$). However, genetic subdivided of this population ($Gst = 0,3142$) was generally determined by random factors, and but geographic distances between samples. The significant importance of the gene flow ($Nem = 1,288$) for supporting of the population genetic uniformity was shown. The Wright's "island model" with the discrete half-isolated patches for studied population may be assumed because low estimates of the population's land snail *C.vindobonensis* effective size was based on LD-method between seven RAPD loci ($Ne = 19,2$).*

Key words: *genetic structure, RAPD-marker, effective size of population, *C.vindobonensis*, Ukraine*

Introduction

In Southern Ukraine and the Crimea dicrocoeliosis is regarded as one of the most important parasitic diseases with both economical, showing high prevalence in cattle, and medical significance (Sverlova et al., 2006). This disease is caused by the trematode *Dicrocoelium dendriticum* (Rud., 1819), which needs a land snail as intermediate host to complete its life cycle. No integrated program aiming at stopping or at limiting the transmission of the parasite, associating the control of intermediate snail hosts to other control strategies is currently carried out at the national level. The development of an effective strategy of integrated control, with the precondition of a good knowledge on the epidemiology of this parasitosis requires including, among other things, the study of population dynamics of intermediate hosts.

Materials and methods

The species

Cepaea vindobonensis (Ferussac, 1821) is a simultaneously hermaphroditic helicid land snail that it common in different natural and artificial habitats of the Southern-eastern Europe, including the Ukraine and southern Russia (Shileyko, 1978). Snails were collected from series of 7 sampling sites on the city park "Dubki" in Mykolayiv, Southern Ukraine. In order to minimize genetic artefacts of population differentiation, snails were collected from an area as small as possible, i.e. no more than 5 x 5 m. Only sexually mature adult snails (characterized with a lip at the edge of the shell aperture)

were collected. Because of the low density of adults the number of individuals sampled per collection site was small, ranging from 10 to 20. All samples in the present survey were collected between 09h00 and 14h00 on several sampling occasions in October 2007.

RAPD analysis

Random amplified polymorphic DNA (RAPD) marker assays are quicker and more efficient in assessing genetic variation of populations in a moderately short time, while not requiring a prior knowledge of the genome structure of the organism studied. Random DNA segments are amplified via the polymerase chain reaction (PCR) by using primers consisting of small inverted repeats (10 bp) of arbitrary nucleotide sequences (Welsh, McClelland, 1990). The RAPD procedure produces a complex pattern of anonymous DNA fragments from the whole genome, some of which are polymorphic between individuals. RAPD studies of the population structure are increasingly becoming available for terrestrial snail (Armbruster et al., 2007; Hille et al., 2003; Wirth et al., 1997). In the study one primer (OPA-01) was employed on seven-population samples, examining 80 snail in total (Kramarenko, in press).

Genetic data analysis

RAPD fingerprints provide dominant genetic signals. I used POPGENE v. 1.32 to analyse the presence/absence matrix of RAPD bands. Using the fraction of double-recessive phenotypes (band absent), the bias frequency of the null allele is $q = \sqrt{x}$.

Molecular variation was calculated for Nei's gene diversity index h (Nei, 1972). Estimates of genetic differentiation between populations were calculated as estimator G_{st} (Nei, 1973) using POPGENE software. For two alleles at a locus, as applicable in RAPD analysis, G_{st} is identical to Wright's F_{ST} . Corresponding estimates of gene flow (Nem), i.e. the average effective number of migrants exchanged between populations of each generation, were based on the inverse relationship between G_{st} and Nem (Wright 1951):

$$Nem = \frac{1}{2} \left(\frac{1}{G_{st}} - 1 \right).$$

The linkage disequilibrium method

Linkage disequilibrium (D) between alleles at two gene loci is defined as the difference between the observed frequency of a two-locus gamete and its expected frequency based on random association and population allele frequencies. D can be estimated directly from gametic frequencies; however, for the vast majority of natural populations only genotypic data are available, which means that gamete frequencies cannot be reconstructed with certainty because of ambiguity regarding gametes that unite to form double heterozygotes. In the latter case, the most commonly used method for estimating linkage disequilibrium is Burrows' D , which is simple to calculate and, does not depend on the assumption of random mating.

We estimated N_e in the snails from via the linkage disequilibrium (LD) method (Hill, 1981):

$$N_e = \frac{1}{3 \cdot \left(r^2 - \frac{1}{S} \right)},$$

were, S – the number of individuals sampled to estimate population parameters.

We used the Burrows method to calculate the squared correlation of allele frequencies (r^2) at pairs of loci. The overall mean r^2 for a sample was computed as the weighted average r^2 over the $L \cdot (L-1)/2$ pairwise comparisons of different gene loci, with L being the number of polymorphic loci.

Results

For the seven sampled populations of *C.vindobonensis* and with the OPA-1 primer a total of seven different DNA fragments were detected, ranging in length from 185 bp to 540 bp. The calculated degree of polymorphism is similarly high in all the populations tested - 53,1%. However, the number (and proportion) of polymorphic loci varied between samples - from 0% (sample #2) to 85,7% (Sample # 4). The Sample #2 are characterized by being monomorphic for all seven loci examined. The average Nei's genetic diversity (h) was 0,236 and varied between samples – from 0 to 0,389.

The overall G_{st} value based on allele frequencies was $0,3142 \pm 0,0308$, corresponding to an N_{em} value of 1,288 (from 0,611 to 2,135 for different loci).

Thus, intrapopulational substructuring or pooling of subpopulations that differ in genetic composition may have been contributed to the unexpectedly high intrapopulation variation found in *C.vindobonensis*.

The linkage relationship coefficients (r^2) between any two of the seven RAPD loci *C.vindobonensis* and correspondent values of N_e is presented in Table 1.

Table 1. The linkage relationship coefficients (r^2 ; above diagonal) between any two of the seven RAPD loci *C.vindobonensis* and correspondent values of N_e (below diagonal) (∞ - infinite effective size population)

Loci	OPA01-1	OPA01-2	OPA01-3	OPA01-4	OPA01-5	OPA01-6	OPA01-7
OPA01-1	X	0,07941	0,08399	0,24146	0,24146	0,00568	0,02982
OPA01-2	5,0	X	0,04361	0,18988	0,00021	0,00040	0,00154
OPA01-3	4,1	10,7	X	0,04800	0,00014	0,00630	0,00861
OPA01-4	1,4	1,9	8,1	X	0,01807	0,00376	0,00123
OPA01-5	1,4	∞	∞	37,0	X	0,04897	0,13870
OPA01-6	∞	∞	∞	∞	7,8	X	0,11148
OPA01-7	14,6	∞	225,3	∞	2,6	3,1	X

The r^2 -values across all pairs of loci varied from 0,00014 to 0,24146, and median is 0,02982. Estimates of effective population size (N_e) are based on linkage disequilibrium were 19,2 (95% confidence interval: 1,5 – ∞) for “Dubki” population of the land snail *C.vindobonensis* at 2007.

Greenwood (1974) estimated the effective size *C.nemoralis* populations as between 190 and 7,000 individuals. He later corrected these values (Greenwood, 1976) such that N_e lay between 95 and 6,000. Selander and Kaufman (1975), working on *Helix aspersa*, considered the snails in their study area to be in small, discrete colonies, and estimate N_e as about 15, on the basis of heterogeneity between colonies in allozyme frequencies. This figure is very similar to those estimated here for *C.vindobonensis*, using genetic markers also. Thus, the Wright’s “island model” with the discrete half-isolated patches for studied population may be assumed because low estimates of the population’s land snail *C.vindobonensis* effective size was based on LD-method between seven RAPD loci ($N_e = 19,2$).

Conclusion

A high polymorphism at RAPD-marker for studied population of the land snail *C.vindobonensis* was observed ($h = 0,236$). However, genetic subdivided of this population ($G_{st} = 0,3142$), was generally determined by random factors, and but geographic distances between samples. The significant importance of the gene flow ($N_{em} = 1,288$) for supporting of the population genetic uniformity was shown.

The Wright’s “island model” with the discrete half-isolated patches for studied population may be assumed because low estimates of the population’s land snail *C.vindobonensis* effective size was based on LD-method between seven RAPD loci ($N_e = 19,2$).

Literature

- Armbruster G.F.J., Hofer M., Baur B.* Effect of cliff connectivity on the genetic population structure of a rock-dwelling land snail species with frequent self-fertilization // *Bioch. Syst. Ecol.* – 2007. – V. 35. – P. 325-333.
- Greenwood J.J.D.* Effective population numbers in the snail *Cepaea nemoralis* // *Evolution.* – 1974. – V. 28. – P. 513-526.
- Greenwood J.J.D.* Effective population number in *Cepaea*: a modification // *Evolution.* – 1976. – V. 30. – P. 186.
- Hill W.G. Estimation of effective population size from data on linkage disequilibrium // *Genet. Res.* – 1981. – V. 38. P. 209-216.
- Hille A., Liebal K., Mosch B., Pellmann H., Schlegel M.* An RAPD (Random Amplified Polymorphic DNA) analysis of genetic population structure of *Balea biplicata*

(Gastropoda: Clausiliidae) in fragmented floodplain forest of the Elster/Saale riparian system // Bioch. Genet. – 2003. – V. 41. – P. 175-199.

Kramarenko S.S. Analysis of the genetic structure of the land snail *Cepaea vindobonensis* (Gastropoda; Pulmonata; Helicidae) population based on RAPD-marker // Vestn.Zool. (in press).

Nei M. Genetic distance between populations // Amer.Nat. – 1972. – V. 106. – P. 283-292.

Nei M. Analysis of gene diversity in subdivided populations // PNAS. – 1973. – V. 70. – P. 3321-3323.

Selander R. K., Kaufman D.W. Genetic structure of populations of the brown snail (*Helix aspersa*). I. Microgeographic variation // Evolution. – 1975. – V. 29. – P. 385-401.

Sverlova N., Khlus L., Kramarenko S. et al. The fauna, ecology and intraspecific variation of the land snail in urban environment. – L'viv, 2006. – 226 p.

Shileyko A.A. The land snail of superfamily Helicoidea. – Leningrad, 1978. – 384 p.

Welsh J., McClelland M. Fingerprinting genomes using PCR with arbitrary primers // Nucleic Acids Res. – 1990. – V. 18. – P. 7213-7218.

Wirth T., Baur A., Baur B. Mating system and genetic variability in the simultaneously hermaphroditic terrestrial gastropod *Balea perversa* on the Baltic island of Öland, Sweden // Hereditas. – 1997. – V. 126. – P. 199-209.

Wright S. The genetical structure of populations // Ann.Eugen. – 1951. – V. 15. – P. 323-354.

Анотація

Для дослідженої популяції наземного молюска *C.vindobonensis* встановлений високий рівень поліморфізму за RAPD-маркером ($h = 0,236$). Однак, генетична структурованість популяції ($Gst = 0,3142$) у більшому ступені обумовлюється випадковими факторами, а не географічною відстанню між окремими вибірками. Продемонстрована значна роль потоку генів ($Net = 1,288$) для підтримання генетичної єдності популяції. “Острівна модель” С.Райта з дискретними, напівізольованими колоніями може бути запропонована для молюска *C.vindobonensis* через дуже низьку оцінку ефективної чисельності популяції, що отримана з використанням LD-методу ($Ne = 19,2$).

Ключові слова: генетична структура, RAPD-маркер, ефективна чисельність популяції, *C.vindobonensis*, Україна