UDC 594.382

Kramarenko S.S., Ph.D. in Biology (Candidate of Biological Sciences),
Ass.Professor,
Kramarenko A.S., student [©]
E-mail: KSSNAIL@rambler.ru
Mykolayiv State Agrarian University, Parizhskoi Kommuny str. 9, Mykolayiv 54021, Ukraine

A NEW INDIRECT METHOD FOR EFFECTIVE SIZE POPULATION ESTIMATING OF THE LAND SNAIL *CEPAEA VINDOBONENSIS*, INTERMEDIATE HOST OF TREMATODA

A new model for the effective population size estimating of the land snail C.vindobonensis based on RAPD-marker's genetic interpopulation variation has been presented. The median estimates of Ne for the land snail C.vindobonensis metapopulation is 5,5 individuals, with 95% CI - from 2,8 to 50,5 individuals.

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

Introduction

Any population, which is finite in number, is subject to the random genetic changes, known as genetic drift. The gradual loss of population genetic diversity is one of the most important consequences of these random changes. The inverse relationship between population size and rate of loss of genetic diversity has a long history in population genetics. However, this loss is not determined by the total population size (census size - *Nc*), and its effective size (effective size - *Ne*). The concept of effective population size was first introduced by S. Wright (Wright, 1938) and is aimed primarily at correcting the influence of various demographic factors on the genetic variability within populations.

In an ideal population of effective size is equal to the total population, however, in most real populations of effective size is always less, sometimes much less than real. Deviation in the sex ratio, variation in size of the family, significant fluctuations in population size among generations are important demographic factors, which are usually reduced Ne. The result of all these demographic factors is that individuals of one generation does not contribute equally to the gene pool of the next generation and, therefore, remains only a limited account of genetic material. Effective population size can be estimated, if the above population traits are known, but it is a very rare occurrence, especially for natural populations.

Difficulties in obtaining estimates *Ne* directly on the basis of demographic data led to the development of numerous methods for its indirect estimates using molecular genetic data (Wang, 2004). Thus, the main purpose of this study was to obtain estimates of effective size for a population of the land snail *Cepaea*

---

vindobonensis (Fer., 1821) using two indirect approaches, based on the consideration of the variability of molecular genetic markers (RAPD-marker).

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. For more details see S.Kramarenko (2009a; 2009b).

RAPD analysis

In the study one primer (OPA-01) was employed on seven-population samples, examining 80 snail in total. For more details see S.Kramarenko (2009a; 2009b).

Methods of *Ne* estimating

For the estimation of effective population size of the land snail *C.vindobonensis* we have used two methods. The first is R.Lande and G.Barrowclough (1987) method (L-B model), according to which the degree of genetic differentiation expected between separate subpopulations is equal:

$$Fst = \frac{1 - t_k}{1 + t_k}, \tag{1}$$

where

$$t_k = \exp - \left\{ \begin{array}{l} \left(\dfrac{1}{Ne}\right)\left[\ln(K - 0,5) + 0,5772\right] + \left(\dfrac{1}{2 \cdot Ne}\right)\left[1,6449 - \dfrac{2}{2 \cdot K - 1}\right] + \\[2mm] + \left(\dfrac{1}{3 \cdot Ne}\right)\left[1,202 - \dfrac{2}{(2 \cdot K - 1)^2}\right] \end{array} \right\}, \tag{2}$$

where K is the number of subpopulations (in our case, $K = 7$).

The second method of an estimation of effective population size (*Ne*) has been offered by us. Using the method of simulation, we found that in L-B model dependence between the estimates of *Ne* and *Fst* values is feedback. However, the shape of the curve obtained depends also on the magnitude of allele frequencies. (We used a model with two phenotypic groups, reflecting, as in the case of RAPD-markers, the process of complete dominance of one allele over another.) The plots of the effective size of model populations dependent on the level of between-population genetic differentiation for different values of recessive allele are presented on Figure 1.

Results

As a whole, for a plane which are passing through these curves, the following formulae can be used:

$$Ne = \frac{0,007214 + 0,002962 \cdot Fst^{-1,00372}}{0,006199 \cdot q^{0,68818}} \qquad (3)$$

This given model with enough high reliability describes empirical curves ($R^2 = 99,6$ %). In addition, we verified this model by comparing the obtained on the basis of its assessment of the effective population sizes with real values used in modelling (Table 1). As we can see (Table 1), in the range of allele frequencies from 0,3 to 0,9 mean absolute error of this model is only 5,2%. While for very low frequencies of allele, the model (3) gives a very biased estimates of effective population size (mostly, it overestimates them).
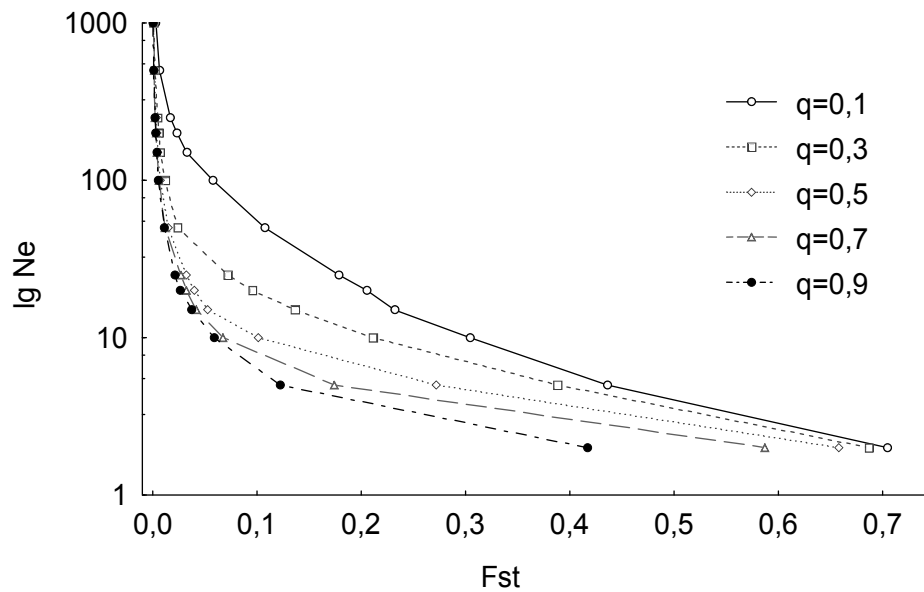

Figure 1. Plots of the effective size of model populations dependent on the level of between-population genetic differentiation for different values of recessive allele

Thus, the model (3) was used to calculate the effective population size of the land snail *C.vindobonensis* based on RAPD-marker.

As an indicator of genetic differentiation between local populations of the land snail *C.vindobonensis* we have been used *Фst* (Excoffier et al., 1992), *Gst* (Nei, 1987) and *θ* values (Weir, Cockerham, 1984).

333

Table 1

Estimates of effective size of model population, obtained on the basis of model (3) and actual *Ne* values

Actual *Ne* values	Frequency recessive allele (*q*)				
	0,1	0,3	0,5	0,7	0,9
5	11,0	5,5	4,7	5,0	5,5
10	13,4	7,9	9,6	10,6	10,1
50	27,6	49,5	55,1	53,7	48,7
100	46,5	96,1	106,4	101,9	101,1
500	371,0	511,5	494,7	483,1	528,4
1000	773,0	1022,9	990,1	897,9	1058,2

Estimates of effective size population of the land snail *C.vindobonensis*, obtained using two models based on three different values of between-population genetic differentiation are presented in Table 2.

Table 2

Estimates of effective size population size of the land snail *C.vindobonensis*, obtained using two models based on three different values of between-population genetic differentiation (*F*-statistics)

Locus	*Фst*		*Gst*		*θ*	
	L-B model	model (3)	L-B model	model (3)	L-B model	model (3)
OPA01-1	-	-	6,5	4,4	-	-
OPA01-2	40,0	21,3	4,0	3,4	85,0	43,2
OPA01-3	8,5	5,7	3,9	3,4	17,0	9,8
OPA01-4	8,8	5,6	3,7	3,2	16,7	9,5
OPA01-5	-	-	6,9	4,9	-	-
OPA01-6	7,4	5,3	3,0	3,1	14,5	9,0
OPA01-7	3,2	3,2	2,6	2,9	4,8	4,0

For two loci (OPA01-1 and OPA01-5) values of genetic differentiation among local populations of the land snail *C.vindobonensis* were equal to zero, therefore they could not be used to estimation *Ne*.

The median estimates of *Ne*, obtained on the basis of different models, is *Me* = 5,5 individuals, with 95% *CI* - from 2,8 to 50,5 individuals (Table 2). In general, for *Fst* and *θ* values has been obtained fairly consistent estimates of *Ne*, although the latter figures were approximately twice as high. *Gst* values were the most conservative estimates and *Ne*, obtained using these coefficients, were the lowest. As for L-B model and model (3), last from them has appeared to be stable in the field of low and very low *Fst* values. Thus the model offered by us for the dominant genetic markers does not inflate the estimates *Ne*.

Discussion

From the theoretical statements and estimations, a *Ne* between 50 and 200 seems generally to indicate the threatened status, and at *Ne* below 50, the driftless reproduction and even the survival of the population is uncertain (Bodo, 1999; Maijala, 1999).

Several researchers have addressed the question of appropriate minimum effective size of livestock populations. From a consideration of the net genetic response in economic merit in dairy cattle breeding, Goddard and Smith (1990) suggested 40 as a minimum effective size. Another approach toward defining minimum effective size was considered by Meuwissen and Woolliams (1994), which balanced inbreeding depression and gain in fitness through natural selection. This resulted in recommendations of the order of 30 to 250. An effective population size of at least 500 is needed if genetic variation in the long term should not decrease (Franklin, Frankham, 1998).

Estimates of *Ne* for populations of the land snail *C.vindobonensis*, obtained in this study are higher than those that has been obtained earlier for the same population with using LD-method (Kramarenko, 2009a). However, these estimates Ne are an order of magnitude lower than those that has been previously obtained based on demographic data (Kramarenko, 2009b). As a whole it is possible to notice, that the estimates of *Ne* for *C.vindobonensis* are significantly lower than 500 individuals. This suggests that an important mechanism for the formation of genetic metapopulation structure this species are random processes (e.g., founder effect and random genetic drift), especially in urbanised habitats.

Literature

Bodo I. The minimum number of preserved populations // FAO Animal Production and Health Paper. – 1999. – V. 104. – P. 91-105.

Excoffier L.*,* Smouse P.E., Quattro J.M. *Analysis of molecular variance inferred metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites // Genetics. – 1992. – V. 131. – P. 479-491.*

Franklin I.R., Frankham R. How large must populations be to retain evolutionary potential? // Anim. Conserv. – 1998. – V. 1. – P. 69–70.

Goddard M.G., Smith C. *Optimum number of bull sires in dairy cattle breeding // J. Dairy Sci. – 1990. – V. 73. – P. 1113–1122.*

Lande R., Barrowclough G.F. Effective population size, genetic variation, and their use in population management / In Soule M.E. (ed.): Viable Populations for Conservation. – Cambridge: Cambridge University Press, 1987. – P. 87-123.

Maijala K. Monitoring animal genetics resources and criteria for priorization of breeds // FAO Animal Production and Health Paper. – 1999. – V. 104. – P. 73-85.

Kramarenko S.S. Genetic structure and effective size population of the land snail *Cepaea vindobonensis*, intermediate host of trematoda in the Southern Ukraine // Science Herald of S.Gzhyc'ky Lviv National University of Veterinary Medicine and Biotechnology. – 2009a. – V. 11, No. 2(41), part 4. – P. 346-350.

Kramarenko S.S. Analysis of the genetic structure of the land snail *Cepaea vindobonensis* (Gastropoda, Pulmonata, Helicidae) population based on RAPD-marker // Vest. Zool. – 2009b. – V. 43, No. 5. – P. 449-455.

Meuwissen T.H.E., Woolliams J.A.. Effective sizes of livestock populations to prevent a decline in fitness // Theor. Appl.Genet. – 1994. – V. 89. – P. 1019–1026.

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

Wang J. Estimation of effective population sizes from data on genetic markers // Phil. Trans. R. Soc. B. – 2005. – V. 360. – P. 1395-1409.

Weir B.S., Cockerham C.C. Estimating F-statistics for the analysis of population structure // Evolution. – 1984. – V. 38. – P. 1358-1370.

Wright S. Size of population and breeding structure in relation to evolution // Science. – 1938. – V. 87. – P. 430–431.

Анотація

Запропоновано новий метод для оцінювання ефективної чисельності популяції *(Ne)* наземного молюска *C.vindobonensis* на підставі внутрішньопопуляційної генетичної мінливості за RAPD-маркером. Медіана оцінок *Ne* метапопуляції наземного молюска *C.vindobonensis* складає 5,5 особин (з 95% довірчим інтервалом: 2,8-50,5 особин).

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