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CAPACITIES FOR POPULATION-GENETIC VARIATION AND ECOLOGICAL ADAPTATIONS

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In contemporary science of population genetics it is equally complex and important to visualize how adaptive limits of individual variation are determined, as well as to describe the amount and sort of this variation. Almost all century the scientists devoted their efforts to explain the principles and structure of biological variation (genetic, developmental, environmental, interactive, etc.), basing its maintenance within existing limits mostly on equilibria proclaimed by *Hardy-Weinberg rules.* Among numerous model-organisms that have been used to prove these rules and demonstrate new variants within mentioned concepts, *Drosophila melanogaster* is a kind of *queen* that is used in thousands of experiments for almost exactly 100 years (CARPENTER 1905), with which numerous discoveries and principles were determined that later turned out

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to be applicable to all other organisms. It is both, in nature and in laboratory, that *Drosophilids* were used to demonstrate the basic principles of population-genetic variation that was later applied to other species of animals. In ecological-genetic variation their richness in different environments could be used as an exact indicator of the status of a determined habitat, and its population-genetic structure may definitely point out to a possibility that specific resources of the environment start to be in danger to deteriorate, or to disappear in the near future. This paper shows clear-cut differences among environmental habitats, when populations of *Drosophilidae* are quantitatively observed in different wild, semi-domestic and domestic environments, demonstrating a highly expressed mutual dependence of these two parameters.

A crucial approach is how to estimate the causes that determine *the limits* of biological, i.e. of individual and population-genetic variation. The realized, i.e. adaptive variation, is much lesser than a total possible variation of a polygenic trait, and in this study, using a moderately complex gene-enzyme system, is estimated to be smaller than 0.2%. For an allozymic system based on 9 loci at three *D. melanogaster* chromosomes, the estimate is that chromosomal types are reduced, on the average, to ca. 3% during meiotic divisions, and that available gene-enzyme combinations are reduced further 15 times in gamete selection. So finalized metabolic or adaptive developmental programs are emphasized to be the basic targets of Darwinian selection, rather than chromosomes or individual genes, that are involved in these programs.

Key words: population genetics, *Drosophila*, variability

A .ECOLOGICAL ADAPTATIONS IN WILD AND IN ANTROPOGENIC ENVIRONMENTS

In our Laboratory, the Chair of genetics and evolution, we are mainly working in the field of *population genetics,* using a few model-organisms. One of the most frequently used experimental organism is the fruit fly *Drosophila melanogaster,* but we also had to study the distribution, variability and population abundance of all the members of family *Drosophilidae.* So far 59 species have been described (KEKIĆ 2002), according to which the region of former Yugoslavia was one of 5-6 richest in Europe.

We are applying quantitative studies to estimate the ecological status of specific populations and variability of the species, trying to estimate how synantropic is a habitat according to a proportion of domestic species of *Drosophila* (PARSONS and STANLEY 1981). One of such studies was based on ca. 80.000 collected and determined individuals of *Drosophilidae* in different localities and habitats of former Yugoslavia. The ratio of domestic species (*D. melanogaster & D. simulans)* to typically wild species (*D. obscura & D. subobscura),* may give us a rough impression about the ecological status of a specific environment.

Table 1 show that, e.g., in forest at Jastrebac Mountain (Serbia) the two species from *obscura* group are almost 235 times more abundant, whereas in forest at Fruška Gora Mt. (near the city of Novi Sad) subobscura/obscura flies are 125 times more numerous than melanogaster/simulans flies. On the other hand, in green parks of Belgrade *melanogaster* group individuals are about twice more frequent than those two from *obscura* group species. In Old Slankamen, a settlement at the bank of Danube river, ca. 50km north of Belgrade, melanogaster/simulans flies are even 10 times more numerous than subobscura/obscura flies.

In extremely domesticated environments, at balconies of apartments and inside of apartments, it is very rare to find the exemplars of wild obscura group of *Drosophila* species (KEKIĆ 2002). In specific microhabitats, in barrels with fruits prepared for destilation of home made brandy, wild species of Drosophila are also not observed (KEKIĆ, 1997).

Table 1. The ratios of quantitative presence (in no. of individuals) of two wild (obscura group) and two domestic species (melanogaster group) of Drosophila in collections from former Yugoslavia.
"W" = wild, "SD" = semidomestic and "D" = domestic habitats.

Locality Habitat	D. subobscura + D. obscura	D. melanogaster + D. simulans	Ratio
Mt. Jastrebac[/1]	2.777	13	234 : 1
forest "W"	+ 259	+ 0	
Mt. Goč[/1]	2.818	22	162 : 1
forest "W"	+ 749	+ 0	
Mt. Durmitor[/2]	6.897	66	142 : 1
forest "W"	+ 2.612	+ 1	
Mt. Fruška gora[/3]	5.410	45	125 : 1
forest "W"	+ 194	+ 0	
Vinci[/4]	1.840	2.826	0.7 : 1
around messuage "SD"	+ 1	+ 3	
Mt. Fruška gora[/5]	2.052	3.288	0.6 : 1
orchard "SD"	+ 22	+ 6	
Belgrade[/5+6]	7.795	944	0.5 : 1
Park "SD"	+ 66	+ 16.247	
Old Slankamen[/7]	653	7.163	0.1 : 1
around messuage"SD"	+ 30	+ 0	
Belgrade[/6]	3	2.178	0.001 : 1
balkonies "D"	+ 0	+ 57	
Barels with[/8,9]	0	13.288	0 : 1
fermenting fruit "D"	+ 0	+ 61	

[/1] Bächli & Kekić 1983; [/2] Kekić & Bächli 1991; [/3] Kekić & Bächli 1983; [/4] Kekić et all 1995-1998; [/5] Kekić et all 1999a; [/6] Kekić et all 1992; [/7] Kekić et all 1996; [/8] Kekić 2003; [/9] Kekić et all 1983.

B. The limits for individual-genetic variation

There are two facts which contemporary ecological and developmental genetics have to accept:
(1) The new progenies do not develop on the basis of random combinations of parental g e n e s, but rather on different combinations of specific developmental p r o g r a m m e s;
(2) Enormous variability of prospective genotypes has to be drastically reduced to such adaptive combinations of allelogenes which may provide appropriate metabolic pathways. Consequently, it is of crucial importance to estimate how small is the proportion of such adaptive variation in a population, as a basic unit of evolution.

To answer this question, we undertook the following experiment, where, using *Drosophila melanogaster* as a model-organism, a multi-locus electrophoretic variation at nine polymorphic gene-enzyme systems has been carefully surveyed in everyone of 4 x 100 inspected individuals, F2 progenies from the nature (see, e.g., MARINKOVIC 1999). These nine loci (6Pgdh; Gpdh, Adh, Hk-2; Sod, Pgm-1, Est-C, Odh & Acph-1) are prevalently involved in a metabolic process of sugar and phosphorus cycle, with specific relationships to four most common cosmopolitan inversions in *D.melanogaster.* The capacity of their polymorphism, with three of loci (Gpdh, Pgm-1, Acph-1) having 3 alleles (i.e. six genotypes), and the rest of them at least two alleles (i.e. min. three genotypes each), amounts more than 78000 combinations of present alleles, i.e. possible genotypes. A random chance to find two individuals with identical combination of available alleles at studied nine loci is 1/78000 squared, i.e. about one in six billions!

Among 400 individually analysed *D. melanogaster* flies we did not determine that many genotypes, as could be expected, but a total of only 160 specific genotypes. Only 82 genotypes were unique, 54 were repeated in 2 or 3 individuals, and 24 appeared even in 4-22 individuals. It means that a sharp selection exists for such adaptive combinations at nine studied loci, which is clearly manifested especially at five third-chromosomal loci (Sod, 3-34.6; Pgm-1, 3-43.4; Est-C, 3-47.7; Odh, 3-49.2; Acph-l, 3-101, 1; DOAN and TREAT-CLEMONS, 1982). In the group of 24 highly repeated genotypes, present in 189/400 inspected individuals, only 4 allelogenic combinations at mentioned five loci exist, out of 972 possible. The second group of 54 genotypes found in 2-3 individuals each, are based on 19 combinations of third-chromosomal loci. And, finally, the group of 82 unique (non-repeatable) genotypes is based on 29 present combinations.

Since we succeeded to survey all repeatable and unique variation of genotypes in 400 *D. melanogaster* flies, we could also demonstrate that an increase of rare alleles at nine studied loci is evident toward such groups of genotypes which are less repeatable, in contrast to those with higher fitness which have the highest degree of homozygosity for most frequent alleles. This suggests that in our definition of fitness, or adaptive value, we have to include not only the frequency of some genotypes, but also their capacity for variation in a population. Here, we

may distinguish a restricted number of genotypes which are providing the proliferation of individuals in this population under optimal conditions, whereas a majority of less frequent genotypes may provide a survival of this population in changed and unpredictable conditions (see, also, MARINKOVIĆ 1997).

Table 2 gives a possibility to estimate how large could be the reduction of specific genotypes during meiotic divisions, and how much as a consequence of gamete selection. Total reduction of available 1^{st}, 2^{nd} & 3^{rd} chromosomes, carrying studied combinations of allelogenes at nine loci, amounts 1/833 in A-group of the most frequent genotypes, and drops down to 1/42 in C-group of realized unique genotypes. Further reduction during gamete selection is 4 - 23-fold, which gives a rough estimation of total reduction of available combinations which amounts 1000-3300 times for here studied nine marker-loci, in three observed fitness-samples. Total reduction is almost 500-fold (from 78000 to 160 realized genotypes, i.e. 0.2%), in our sample of 400 analysed *D. melanogaster* male adults.

Table 2. The numbers of observed versus potential genotypes at nine loci in 400 individuals of Drosophila melanogaster: A - repeated in 9 - 22 individuals; B - repeated in 2 - 3 individuals; C - non-repeated (unique) genotypes.

Genotypes (Individuals)	Selected combinations of 1^{st}, 2^{nd} & 3^{rd} chromosomes	Realized adult genotypes	Total reduction
A (189)	1/833	24/96 (1/4)	1/3332
B (129)	1/77	54/1026 (1/19)	1/1463
C (82)	1/42	82/1914 (1/13)	1/966
(400)	1/36	160/2244 (1/14)	1/504

The four samples of here studied flies (4 x 100 inspected individuals) did not show significant difference in allozymic frequencies at nine loci, so that we were able to summarize cumulative results from 100 to 400 individuals. In described samples of 100, 200, 300 and 400 D. *melanogaster* individuals, the number of genotypes found was 65, 111, 140, and 160, respectively. Such a trend of gradual increase could be theoretically extrapolated for larger numbers of individuals (e.g., by a computer simulation), and results obtained are presented in Table 3, for 100 - 1500 individuals.

It can be seen that the increase of the number of genotypes has an asymptotic character, reaching a limit of no more than 210-220 genotypes (for our model with nine loci) for population size of more than 1500 individuals.

Table 3. Determined and extrapolated changes in the numbers of genotypes (repeated and unique) at nine studied loci, depending on the size of a Drosophila melanogaster population.

Determined changes				
No. Individuals	100	200	300	400
No. Genotypes	65	106	140	160
Repeated Genotypes	44	64	76	78
Unique Genotypes	21	42	64	82

Extrapolated changes						
No. Individuals	500	700	900	1100	1300	1500
No. Genotypes	173	187	195	200	203	205
Repeated Genotypes	85	91	93	95	96	97
Unique Genotypes	88	96	102	105	107	108

It means that number of co-adaptive genotypes for such a complex system could be more than ten times smaller than number of individuals, amounting less than 0.5% of all possible genotypes for such a system. These are quite real numbers for *a Drosophila* population, and a complex of 8-10 gene-enzymes could be an average size *metabolic system* for a specific part of *developmental program*, which can be a real *target of selection*.

Special strategy could be based on a relationship between highly repeatable versus rare or unique genotypes (that increase more steeply in numerous populations), and this should be one of basic sorts of balancing polymorphism based on a relationship between optimal homogeneity and individual variability (MARINKOVIC and AYALA, 1975). Owing to the fact that here analysed flies are 400 males (with the absence of recombination) from F2 generation of wild ancestors, the electrophoretic screening of one such individual may yield the allele associations for wild chromosomes, carrying also the markers for other blocks of genes which may influence the fitness properties of studied genotypes.

Since different *combinations* of present alleles are providing above-mentioned multi-locus genotypes, their gene polymorphism seems not to be very much different in groups from a few hundreds to many thousands individuals of a population. This may regulate the constitution of a population in extremely different ecological conditions and in succeeding generations, i.e. after bottle-neck situations. Since we have specific combinations *of* allelogenes at groups of loci, the large variability of newly established combinations can be produced again in just a few of the following generations, e.g., after a winter depression. This wise strategy at population-genetic level, contributes clearly to the maintenance of biological diversity in the nature, keeping it in limits that are adaptive and profitable for every species of living organisms.

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KAPACITETI ZA POPULACIONO-GENETIČKU VARIJACIJU I EKOLOŠKU ADAPTACIJU

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I z v o d

U savremenim istraživanjima u oblasti populacione genetike podjednako je kompleksno i značajno utvrditi kako su determinisani adaptivni limiti individualnog variranja, kao i opisivanje obima i vrste variranja. Gotovi celo stoleće istraživači su usmerili velike napore za objašnjenje principa i structure biološkog variranja (genetičko, razvojno, ekološko, interaktivno itd.) bazirajući održavanje obima varijabilnosti unutar postojećih limita u ravnoteži proklamovanoj *Hardy – Weinber* pravilima.

Među brojnim model -organizmima, korišćenim u istraživanjima za potvrđivanje tih pravila i demonstracijom novih varijanti unutar pomenutih koncepta, *Drosophila melanogaster* je kao kraljica – model organizam, korišćena u hiljadama eksperimenata u toku skoro 100 godina istraživanja (Carpenter, 1905). Brojna otkrića i principi utvrđeni u tim istraživanjima su se pokazala primenjivim i za druge organizme.

Rezultati istraživanja u prirodnim i laboratorijskim uslovima u kojima je *Drosophyle* korišćena u demonstraciji osnovnih principa populaciono – genetičkog variranja, kasnije su primenjeni u istraživanjima drugih animalnih vrsta. U ekološko – genetičkom variranju njihova striktnost u različitim uslovima spoljne sredine može da se koristi kao egzaktan indikator statusa određene sredine a utvrđena populacionao – genetička struktura može definitivno da ukaže na mogućnost da stanje specifičnih resursa okoline može doći u opasnost da bude pogoršano ili da ti resursi nestanu u bliskoj budućnosti. Ovaj rad pokazuje da postoje jasne razlike između uslova sredine, kada su populacije *Drosophyla* kvantifikovane u izučavanim različitim divljim, semi – domaćim i domaćim ekološkim sredinama, demonstrirajući visoko izraženu obostranu zavisnost ova dva parametra.

Ključni pristup je kako da se utvrdi uzrok koji determiniše *limite* biološkog individualnog i populaciono – genetičkog variranja. Ostvareno variranje kao što je adaptivno je mnogo niže od ukupnog potencijalnog variranja poligenih osobina i u ovim istraživanjima, korišćenjem umereno kompleksnog gen - enzim sistema je utvrđeno da je niže od 0,2%. U korišćenom sistemu alozima u 9 lokusa kod tri *D. melanogaster* hromozoma, pretpostavka je da su tipovi hromozoma redukovani u proseku za oko 3% u toku mejoze a da su moguće gen – enzim kombinacije redukovane za sledećih 15% u toku selekcije gameta. Ovi rezultati su

osnova da se naglasi da su programi metabolizma ili programi razvića bazni ciljevi Darvinove selekcije pre od hromozoma ili individualnih gena koji su uključeni u te programe.

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