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DEVELOPMENTAL PROGRAMS IN SIMULATED AND OBSERVED MULTI-LOCUS GENOTYPES OF *DROSOPHILA MELANOGASTER*

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Abstract — Allozymic variation at nine polymorphic loci that control the phosphorus-sugar metabolic cycle was compared in 400 observed *Drosophila melanogaster* flies and in randomized samples of their 160 genotypes. Five samples combined independently in computer replicas exhibited an increased but mutually quite similar shape of variation, significantly different, however, from experimentally observed frequencies of genotypes in 400 F2 progenies of wild flies from a natural population. Such comparison of simulated and observed genotype frequencies supports our previous findings that within any species a restricted number of realized programs for different traits exists, and that such developmental programs are basic targets of natural selection, as well as the global units of inheritance.

This is one in a series of experiments on the adaptive limits of population-genetic variation conducted over the last decade using as a model the most studied species during the past century, *Drosophila melanogaster.*

Key words: Multi-locus genotypes, developmental programs, *Drosophila melanogaster*

INTRODUCTION

Biological progress in living systems is based on a permanent increase of their variability (i.e., complexity), together with a simultaneous increase of their harmony (i.e., homogeneity). A balance between these two properties tells us how successful a system is in its evolutionary progress.

Individual genes with their allelic forms are rarely the units of the functional hereditary variation in living organisms. A majority of traits are polygenically controlled, so that an orchestrated activity of groups of genes is the reality, with developmental programs expressed in succeeding generations of the progenies. This is why we have, within a species, a restricted number of realized programs for different traits, answering the question why individuals of any particular species are so similar to each other. This approach differs from the numerous efforts of population geneticists in the last century emphasizing the *sources of biological variation* among individuals of a species.

The basic problem is to explain how this huge potential variation could be limited and reduced to adaptive combinations of allelogenes. As indicated by our experience with *Drosophila*, in a population with more than a few thousand individuals, the number of existing genotypes for a metabolic system controlled by 8-10 polymorphic loci does not exceed more than 0.5% of theoretically possible combinations of allelogenes (Marinković, 1999, 2002). In an analysis of nine polymorphic and 16 monomorphic genes which may control the phosphoryc-sugar metabolic system in fruit flies, we concluded that out of ca. 80 thousand possible combinations of allelogenes, no more than 200-220 genotypes can exist in a large population of *Drosophila melanogaster*, suggesting an extremely restrictive number (<0.3%) of adaptive combinations of genes.

Such adaptive combinations, and not individual genes or chromosomes, are the basic targets of natural selection. Since different *combinations* of the alleles present provide such multi-locus genotypes, their gene polymorphism seems to be not very much different in groups ranging from a few hundred to a few thousand individuals of a population. This may regulate the constitution and destiny of a population in succeeding generations under extremely different ecological conditions.

Our experiments continuing these studies employ *D. melanogaster* as a model species, a *queen among entomological representatives,* one which has been used for more than 100 years (Carpenter, 1905) for numerous discoveries that later turned out to be applicable to all other organisms.

MODEL EXPERIMENT

In a series of papers published since 1997 (Marinković, 1997, 1999, 2002, 2005; Kovač and Marinković, 1999; Marinković and Kekić, 2007), the adaptive limits of population-genetic variation were estimated in an observation model of 400 individual genotypes of *D. melanogaster,* whose detailed electrophoretic analysis permitted a synchronous survey at nine polymorphic loci controlling a complex metabolic system. It was found that 6Pgdh is located in the first chromosome (1-0.64); Gpdh (2-20.5), Adh (2-50.1), and Hk (2-73.5) in the second; and Sod (3-24.6), Pgm-1 (3-43.4), Est-C (3-47.7), Odh (3-49.2), and Acph-1 (3-101.1) in the third (Doan, 1982).

Due to an enormous number of possible combinations of allelogenes present (ca. 78,700), it was expected that every individual, out of 400 observed, should have a different and unique genotype, with a theoretical chance (based on the second Mendelian principle) that in 1/200 cases, i.e., in just two individuals by chance, could an identical genotype be eventually found. However, it was not so, and a total of only 160 genotypes were determined at nine loci among 400 individuals, 82 of them being unique and even 78 being repeatable from two to 22 times. This already tells us how restrictive adaptive variation is in a metabolic system, this time controlling, primarily, the sugar-phosphorus gene-enzyme circle.

Aside from previously published data that try to explain the meaning and the origin of such a restricted adaptive variation in our model with nine polymorphic

gene-enzyme loci (Marinković, 1999, 2002, 2005), we shall here attempt to give an additional contribution to our analysis. For this purpose, we compared data obtained so far in 400 studied flies with randomized combinations of available genotypes fed into five computer systems**.** The available genotypes are those that appeared in 400 adult flies, the F2 progenies of wild ancestors caught from a natural population. Combining them in a randomized way in five computer systems should give us an idea as to how much they could differ from the observed distribution of adaptive population-genetical variation within our model system (Table 1).

The total number of simulated genotypes in five computer systems (as the average of 400 trials) varied between 185 and 202, compared with only 160 among experimentally observed 400 *D. melanogaster* individuals. The proportion of unique genotypes varied from 123 to 144 (i.e., 68 + 1%), which is notably higher than among directly observed individuals (82, i.e., 51%). Genotypes that are repeated five to 28 times appeared in "computer samples" only 13.2 out of 193 times (on the average), which gives a value of 6.8%; on the other hand, this value in the experimentally observed sample was 11.3%, obtained from 18 out of 160 highly adaptive genotypes.

Table 1. Computer-simulated and experimentally observed genotype frequencies at nine polymorphic loci in 400 individuals of *D. melanogaster.*

Appearance	Expected (simulated) genotype frequencies in 5 x 400 trials					Genotype frequencies observed in 400 individuals
	I	II	III	IV	V repl.	
1x	123	144	134	124	129	82
2	22	23	24	32	26	33
3	16	7	10	14	16	21
4	9	13	9	6	5	6
5	4	6	2	4	1	4
6	2	1	5	3	1	1
7	1	1	0	2	3	3
8	1	1	1	0	2	2
9	1	0	2	2	0	3
10	0	0	2	1	0	1
11x	0	1	0	1	1	1
12	1	1	1	0	0	0
13	1	2	1	1	2	0
14	1	0	0	1	1	0
15	2	0	0	0	1	1
16	0	0	0	0	0	0
17	0	0	0	0	0	1
18	0	1	0	0	1	0
19	0	1	0	0	1	0
20	0		1	0		0
21x	0		1	0		0
22	0			0		1
23	0			0		
24	1			0		
28x				1		
No. genotypes	185	202	193	192	190	160
Unique genotypes			Exp. 68 + 1%			Obs. 51%
X2 obs/exp=41.0 (p<0.001)						

The variation coefficients in five randomized samples were 9.1, 9.6, 9.7, 9.7, and 10.0%, whereas in the experimentally observed sample this value was 9.2%. The average value of genotype repeatability was only 2.08+.20, compared with 2.50+.23 in the observed sample.

The data presented in Table 1 clearly show that the frequencies observed in our sample of 400 genotypes polymorphic at nine loci in the three largest chromosomes of *D. melanogaster* significantly differ from randomly simulated frequencies presumed by classical population-genetic rules of random combination of available allelogenes. This leads to maintenance of a restricted proportion of adaptive genotypes in following generations as a factor acting in addition to Hardy-Weinberg rules, which alone would not be capable of ensuring repeatability of such relationships.

Results published recently (Marinković and Kekić, 2007) and modified in our Table 2 show how great could be the reduction of observed versus potential genotypes during meiotic and gamete selection, depending on their repeatability, from

Table 2. Ratios of observed versus potential genotypes at nine polymorphic loci after chromosomal and gamete selection in 400 *Drosophila melanogaster* individuals: A - repeated in 4-22 individuals ; B - repeated in 2-3 individuals ; C - non-repeated (unique) genotypes (see also Marinković and Kekić, 2007).

Genotypes (Individuals)	Selected combinations of chromosomes	Realized adult genotypes	Total reduction
A (189)	1/833	24 (1/4)	1/3332
B (129)	1/77	54 (1/19)	1/1463
C (82)	1/42	82 (1/23)	1/966
(400)	1/36	160 (1/14)	1/504

unique to ones that appear in a few or in many (4-22) individuals of *D. melanogaster*. Reduction in unique genotypes amounts to 1 : 966, i.e., 42 times in selected combinations of chromosomes in meiotic divisions and 23 times during gamete selection. For highly repeated genotypes, this reduction is even greater (up to 0.3 promille). The total reduction from a potential of 78.700 to 160 realized genotypes is almost 500-fold among analyzed male adults, with estimation that the number of such realized genotypes will not be much greater (up to 210-220 genotypes at most), even in samples and populations that amount to a few thousand individuals.

Table 3 presents the average numbers of genotypes per each of nine allozymic loci and per each individual in samples with high, low, and absent repeatability of genotypes among 400 analyzed individuals. There is an obvious increase from the first to third category in almost all loci, as well as per each individual, where the mean value increases progressively from 1.33 in the A category to 1.68 in the C category. Among unique genotypes, all loci are variable, whereas among those that are highly repeatable about half of the loci are stable and virtually invariable, with another half (predominantly from the second chromosome) having observable variability.

Table 3. Average number of genotypes per each of nine allozymic loci and per each individual (X) in samples with the high (A), low (B), and absent (C) repeatability of genotypes among 400 analyzed individuals.

	Genotypes/ Individuals	First chromosome			Third chromosome			Second chromosme			
	N	6Pgd	Sod	Odh	Acph	Pgm	Est	Hk	Adh	aGpd	X+SE
A	24/189	1.04	1.00	1.00	1.00	1.14	1.43	1.19	2.10	2.03	1.326+.147
B	54/129	1.48	1.08	1.21	1.06	1.53	2.35	1.44	2.00	2.13	1.587+.161
C	82/82	1.44	1.21	1.22	1.24	1.39	2.56	1.71	1.95	2.41	1.682+.173

We also succeeded in analyzing 800 third-chromosome *genomes* from our sample of 400 *D. melanogaster* flies. It turned out that 464 (58%) were monomorphic for the most common allele at five observed loci, 288 (36%) had a mutation at one of the loci, and 48 (6%) were recombinants carrying two or three such mutations. Phylogenetically, we estimate that the last group should be the youngest and the first one the most ancestral, although exact evolutionary routes are not so easily predictable.

CONCLUSIONS

The basic question to which scientists now need to answer is how can individuals within a species be so much similar to each other, and not why and how they are so different, which was analyzed in detail during the past century. Based on classical rules of genetics and evolution, individual variability should be much greater than what we find in reality in the nature.

Contemporary geneticists have to accept that new progenies of living organisms do not develop on the basis of random combinations of parental chromosomes and allelogenes, but rather on the basis of different combinations of a limited number of existing *developmental programs*. Polygenic complexes determining these programs are the real 'targets' of Darwinian selection, as well as the basic units of inheritance (Marinković, 1997, 1999, etc.).

Multidimensional relationships among genes involved in the control of a complex metabolic cycle can be observed on at least two interdependent levels – structural and functional. The structural level is related to the determination of gene arrangements that are selected during meiotic divisions in individuals, giving rise to specific variation of chromosomal genomes, i.e. among produced gametes (Krimbas and Powell, 1992; Mestres et al., 1998; Živanović et al., 2000). The functional approach provides information about the properties and genotypes of those genomes that yield viable zygotes which succeed in developing into adult individuals (Gavrilets and DeJong, 1993; Saura et al., 1998; Živanović and Marinković, 2004). We evaluated the complex relationships between these two basic levels by observing a group of polymorphic genes whose locations in a specific chromosome are known, and which are involved in the control of a specific metabolic process.

Apart from selectional (i.e., external) criteria, we emphasize that a basic role in evolutionary development of such complex systems is played by systemic (intrinsic) factors, i.e., by the rules of 'auto-synthesis' of a well-established system, directing restrictive variation of available possibilities, on which Darwinian selection can operate (Milojević, 1956; Crkvenjakov and Drmanac, 2007).

In structural and physiological systems, *evolutionary auto-synthesis* determines the direction and limits within which *Darwinian selection* can operate in an organism. These two forces should be considered together, and not as a part of one of them (natural selection), an approach that gives a limited choice of allelic combinations in determining which ones are inappropriate and need to be eventually eliminated.

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