Arch. Biol. Sci., Belgrade, 61 (4), 573-579, 2009 DOI:10.2298/ABS0904573M

EVOLUTIONARY PROGRAMMED DEVELOPMENT AS THE BASIS OF DARWINIAN SELECTION: A REVIEW

D. MARINKOVIĆ

Serbian Academy of Sciences and Arts, 11000 Belgrade, Serbia

Abstract — The sources of biological variation are numerous and versatile. The basic problem is to explain how this huge potential variation could be limited and reduced to adaptive combinations of allelogenes and characters. It has been estimated that, in a population of *Drosophila melanogaster* with a few thousands of individuals, the number of existing genotypes for a metabolic system controlled by 8-10 polymorphic loci, would not exceed more than 0.5% of possible combinations of genes. Based on individual allozyme analysis of such a system in 400 flies, less than 1 pro-mile of possible combinations of three largest chromosomes of this species could be present in spermatozoa of an adult male, before they enter a competition to produce viable zygotes. Such adaptive combinations are targets of natural selection, realized through a restricted number of developmental (metabolic) programmes, being also the units of inheritance. The basic role in evolutionary development of such systems have intrinsic factors, i.e., the rules of auto-synthesis of well established programmes, directing a restrictive variation of adaptive variants with which Darwinian selection can operate.

Key words: Allozymes, programmed developments, Darwinian selection

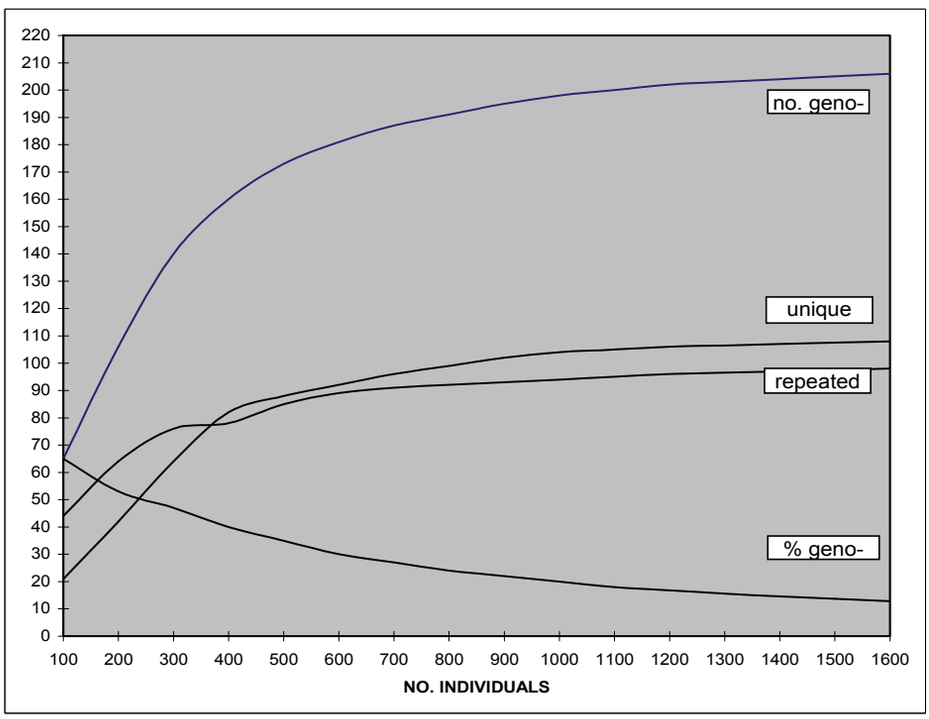
UDC 5

INTRODUCTION

Biological progress in a great majority of living organisms 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. Extensive variation may lead to an anarchy with a disbalance of the system, and too big homogeneity to a monomorphism, with non-adaptive perspectives in variable environments.

The accumulation of data from numerous genetic studies suggests that individual genes (i.e., 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 a reality, with developmental programmes expressed in succeeding generations of the progenies. This is why we have, within a species, a restricted number of realized programmes for different morpho-physiological traits, answering the question why individuals of any particular species are so much *similar* to each other (Marinković, 1997, 1999, 2008; Kovač and Marinković, 1999).

The basic problem is how this huge potential variation could be limited and reduced to adaptive combinations of allelogenes. According to our experience with *Drosophila* (a century-model for genetical research; Carpenter, 1905), in a population with more than a few thousands of 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 available gene alleles. In an analysis of nine polymorphic and 16 monomorphic genes which may control phosphor-sugar metabolic system in fruit flies, we concluded that out of ca. 80 thousand possible combinations of allelogenes, no more than 200-220 genotypes exist in a large population of *Drosophila melanogaster*, suggesting an extremely restrictive number (<0.3%) of adaptive combinations of genes (Marinković, 2002, 2005; Marinković and Kekić, 2007).



No. individuals	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600
No. genotypes	65	106	140	160	173	181	187	191	195	198	200	202	203	204	205	206
% genotypes	65	53	47	40	35	30	27	24	22	20	18	16.8	15.6	14.6	13.7	12.9
Repeated genotypes	44	64	76	78	85	89	91	92	93	94	95	96	96.5	97	97.5	98
Unique genotypes	21	42	64	82	88	92	96	99	102	104	105	106	106.5	107	108	108

Fig. 1. The changes in the number and proportions of genotypes in a system of nine polymorphic loci, depending on the size of a *D. melanogaster* population.

Such *adaptive combinations*, and not individual genes or chromosomes, are basic targets of natural selection. They are realized through a restricted number of developmental (metabolic) programmes, which could be also the basic units of inheritance. Since different *combinations* of present alleles are providing such multi-locus genotypes, their gene polymorphism seems not to be very much different in groups from a few hundreds to a few thousands individuals of a population (Fig. 1). This may regulate the constitution of a population in extremely different ecological conditions in succeeding generations, i.e., after bottle-neck situations (Marinković, 2001, 2004, 2005).

A MODEL EXPERIMENT

In the mid 1980s we started, at the University of California in Davis, the experiment with the *individual analysis* of allozyme polymorphisms in *Drosophila melanogaster,* being capable, using all the experience of this Laboratory (Head Prof. F. J. Ayala) and some of ours from Belgrade, to determine such variation in every individual of 300 wild fly progenies for eleven polymorphic loci. Previously we determined that an additional 16 loci, involved mainly in *sugar-phosphorus gene-enzyme system*, are allozymically monomorphic, so that we did not involve them in our further analysis. Somewhat later we succeeded to analyse individually another 100 flies for the same polymorphic loci, and since these

two samples turned out to be not significantly different in allelic frequencies, further analyses could be proceeded in these 400 individuals together, as F2 progenies of wild flies from the nature. Obtained results have been published in relatively few papers (e.g., Marinković, 1999, 2002), succeeding to present a part of numerous rules expressed in such a multigenic variation of a relatively large sample of studied individuals.

Individual variabilities were allozymically analysed at nine loci (6Pgdh-Gpdh-Adh-Hk-Sod-Pgm-Est-Odh-Acph), since at two loci (Ao and Xdh) this variation was not expressed clearly. The loci considered in our report are prevalently involved in a metabolic process of sugar and phosphorus circle, and they have specific relationships to most common cosmopolitan inversions in *D. melanogaster*. The capacity for their polymorphism, with two of loci having 3 alleles (i.e., six genotypes each) and the rest of them having 2 alleles (i.e., three genotypes each) amounts more than 78.000 combinations, i.e., possible genotypes.

Individual variation in the observed sample of 400 *Drosophila* males was so much limited that out of ca. 78.700 possible combinations of determined alleles at nine polymorphic loci, only 160 variants (0.2%) have been detected (Marinković, 1999, 2001, 2004). In spite of a minor theoretical chance that two individuals may have by chance the same genotype at nine loci, we found such genotypes repeated 2-22 times in 78 variants, whereas 82 variants (i.e., only 20% individuals) had unique genotypes, theoretically expected in everyone of 400 inspected flies.

Observing the variation in 400 second and third chromosomal pairs separately (Marinković, 2002, 2005), we found 22 2^{nd}-chromosomal genotypes in three marker loci out of 27 (Gpdh, Adh, Hk-2), whereas in five marker loci of the third chromosome (Sod, Pgm-1, Est-C, Odh, Acph-1) only 37 genotypes were discovered out of 972 possible combinations of alleles (3x6x3x3x6). Out of so expected 814 combinations of 2^{nd} and 3^{rd} chromosomal types (22x37), only 127 were present among 400 individuals, which is 0.5% of all possible (26.244) allozyme combinations. From discovered genotypes it could be extrapolated that all eight types of 2^{nd} chromosomes could be present in spermatozoa of studied flies, as well as 24 out of 72 types of 3^{rd} chromosomes. Out of expected 192 and theoretically possible 576 combinations of 2^{nd} & 3^{rd} chromosomal types in zygotes, only 127 have been realized at eight observed loci.

EVOLUTIONARY ORIGIN OF RESTRICTED BIOLOGICAL VARIATION

Table 1 presents 22 observed genotypes discovered at second, and 37 at third chromosomes, for eight studied loci in 400 flies. It could be seen that the individuals with most of homozygous loci are the most frequent and that those which have more of mutant alleles in homo- or heterozygous states, are less frequent. No. 1. geno-, homozygous for the most frequent alleles in Gpdh, Adh and Hk 2^{nd}-chromosomal loci, combines with 21 3^{rd}-chromosomal genotype in 81 individuals, whereas no. 20, heterozygous at Gpdh and Adh and homozygous for a mutant allele at Hk locus, combines with only three 3^{rd}-chromosomal genotypes in three individuals.

The genotypes with a series of homozygous loci for most frequent alleles could be evolutionary initial and more ancient, whereas more polymorphic ones could be of a more recent origin. This is more obvious in Table 2, with a kind of prospective evolution of 13 most frequent (out of 37) genotypes of five 3^{rd}-chromosomal loci, present in 360 out of 400 inspected *D. melanogaster* flies. The probably "original" and most ancient genotype with most frequent 100 (=1) allele homozygous at all five loci (Sod/Pgm/Est-C/Odh/Acph), has been found in 130 out of 400 observed individuals, combined with 17 2^{nd}-chromosomal genotypes. Genotypes with mutants at four or five loci have not been found at all, but in six genotypes mutations were present at three out of five loci, combined with only 1-3 2^{nd}-chromosomal variants (see, also, Table 1).

The "original genotype" may designate a structure which appeared:

- further in the past when a metabolic system had been established; when a species separated from

Table 1. Genotypes found at 2nd and 3rd chromosomes for eight allozyme markers among 400 *D. melanogaster* individuals (1=100/100; 90=90/90; F=F/F, etc.). Numbers in brackets are combinations with the genotypes of the other chromosome.

II N (geno)	Chr. Gpd	Adh	Hk	**Geno-no.**	Sod	III Pgm	Chr. Est	Odh	Acph	N(geno)
81(21)	1	F	1	**1**.	1	1	1	1	1	130(17)
50(11)	93/1	F	1	**2.**	1	1	1/103	1	1	88(20)
53(14)	93/1	F/S	1	**3.**	1	96/1	1	1	1	43(11)
47(10)	1	F/S	1	**4.**	1	96/1	1/103	1	1	21(8)
19(5)	1	F/S	1/103	**5.**	1	1	103	1	1	20(9)
19(7)	1	F	1/103	**6.**	1	1	1	98/1	1	14(5)
20(6)	93/1	F	1/103	**7.**	1	1	1/103	98/1	1	13(7)
17(7)	93/1	F/S	1/103	**8.**	90/1	1	1	1	1	6(3)
17(7)	1	S	1	**9.**	90/1	1	1/103	1	1	8(4)
12(3)	93/1	S	1	**10.**	90/1	1	103	1	1	3(2)
15(5)	93	F/S	1	**11.**	1	103/1	1	1	1	4(4)
12(5)	93	F	1	**12.**	1	103/1	1/103	1	1	5(3)
6(4)	93	F	1/103	**13.**	1	1	1/103	1	94/1	4(3)
5(2)	93	F/S	1/103	**14.**	1	1	1	1	94/97	4(2)
3(2)	93	S	1/103	**15.**	1	96/1	103	1	97	4(3)
6(4)	93/1	S	1/103	**16.**	90	1	1/103	1	1	2(1)
5(3)	93/1	F	103	**17.**	1	96	1	1	1	4(3)
4(2)	1	F	103	**18.**	1	96/1	1	98/1	1	2(1)
2(2)	93	F	103	**19.**	1	1	1	98	1	2(1)
3(3)	93/1	F/S	103	**20.**	1	1103	98/1	1	2(2)	
3(3)	93	S	1	**21.**	90/1	96/1	1/103	1	1	2(2)
1(1)	1	S	1/103	**22.**	1	1	1/103	1	94	1(1)
	400 ind's (127 geno-)			**23.**	1	96	103	1	97	2(2)
				24.	1	1	1/103	1	97/1	2(2)
				25.	1	1	1	1	94/1	2(2)
				26.	90	1	1	1	1	1(1)
				27.	1	1	103	1	94/1	1(1)
				28.	90/1	1	1	1	94/1	1(1)
				29.	1	96/1	103	1	1	1(1)
				30.	1	1	1	1	97/1	1(1)
				31.	1	96/1	1	98	1	2(1)
				32.	90/1	1	1	98/1	1	1(1)
				33.	90/1	96/1	1	1	1	1(1)
				34.	1	1	1	1	97	1(1)
				35.	1	1	103	98/1	94/1	1(1)
				36.	90/1	1	1/103	1	97/1	1(1)
				37.	1	96/1	1	1	97/1	1(1)
							400 ind's (127 geno-)			

other members of its genus; in a more recent time when a specific chromosomal type appeared and later spread out. We should not exclude a possibility that one of chromosomal types with a moderate frequency could be the ancient or "original type", giving rise (through a multidirectional development) to a contemporary variation of observed population structures.

As new mutations first appear in heterozygous states, this can explain the importance of Dobzhansky's claims (e.g., Dobzhansky, 1970), that heterozygotes are of special importance for the maintenance of genetic balances in natural populations.

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 gametes (see, also, Krimbas and Powell, 1992; Mestres et al., 1998; Živanović et al., 2000, 2004). The functional approach gives the information about the properties of these genomes that yield viable zygotes and their genotypes, which succeed to develop into adult individuals (e.g., Marinković et al., 1987, 2007;

Table 2. Allozymic genotypes at five 3rd-chromosomal loci in 360 out of 400 *D. melanogaster* flies, with the numbers of corresponding genotypes at three 2nd-chromosomal loci (95/127), presenting a prospective order of evolutionary origin of gene-enzyme polymorphism in this metabolic system.

In(3L)		*C*		*In(3R)*			
Sod	**Pgm**	**Est-C**	**Odh**	**Acph**	**N**	2nd geno-
1/1	1/1	1/1	1/1	1/1	**130**	**17**
		1/103			**88**	**20**
	96/1				**43**	**11**
	96/1	1/103			**21**	**8**
		103/103			**20**	**9**
			98/1		**14**	**5**
		1/103	98/1		**13**	**7**
90/1					**6**	**3**
90/1		1/103			**8**	**4**
	103/1	1/103			**5**	**3**
		1/103		94/1	**4**	**3**
				94/97	**4**	**2**
	96/1	103/103		97/97	**4**	**3**
					360/400 ind's	***95/127 geno-***

Cluster et al., 1988; Gavrilets and DeJong, 1993). We evaluated the complex relationships between these two basic levels 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. Despite of selection (i.e., external) criteria, we emphasize that basic role in evolutionary development of such complex systems have systemic (intrinsic) factors, i.e., rules of a gradually 'programmed auto-synthesis' of a well established system, directing a restrictive variation of available variants with which Darwinian selection can operate.

STATEMENTS

1. The new progenies of living organisms do not develop on the basis of random combinations of parental chromosomes and allelogenes, but rather on different combinations of a limited number of existing *developmental programmes*. Polygenic complexes determining these programmes are the real 'targets' of Darwinian selection, as well as the basic units of inheritance.

2. Fitness of population genotypes is inevitably based on a balanced relationship between the frequency of their representatives and the amount of their polymorphisms. The higher their frequency, the lower could be their allelic polymorphism (and vice versa), and this seems to be the main adaptive strategy in natural populations for the maintenance of the limited numbers of polygenically controlled developmental programmes and characters.

3. The basic ancestral polygenic structure, being most conservative and monomorphic at many loci, could often be more frequently present than the later variants that provide individual variation. It protects original structure of this initial (e.g., metabolic) system and changes (mutates, evolves) gradually: (1) to provide a step-by-step increase in its adaptive complexity, and (2) to increase its own harmony, i.e., functional efficiency and homeostatic stability. The acceptance of new mutational changes (i.e., alleles) must be quite a risky and rare event, tested by extremely sharp systemic (intrinsic) and selection (external) criteria.

4. If we consider only third chromosomes of *D. melanogaster* (containing ca. 40% of all genes in this species), it turns out that (based on the observed five loci markers) no more than 5% of their allozymic types exist in reproductive cells that enter gametogenesis. Using 9-loci markers for 3rd, 2nd, and 1st chromosomes, the proportion of realized combinations drops down to less than 0.5% (Marinković, 1999, 2002, 2005). If we include some additional markers, less than one percent of all available 3rd-

chromosomal types, and less than one pro-mile of possible combinations of the three largest chromosomes of this species could be present in spermatozoa of an adult *D. melanogaster* male, before they enter a competition to produce viable zygotes.

5. Despite of selection (i.e., external) criteria, the basic role in evolutionary development of such complex systems have systemic (intrinsic) factors, directing a restrictive variation of available variants with which Darwinian selection can operate. The way how adaptive variation in a multigenic system can be limited is based on the fact that it gradually evolves from ancestral, less polymorphic structures, during a systemic process of a very conservative programmed auto-synthesis that gives rise to newly formed variants that, step by step, are more polymorphic, but also more stable and better adapted structurally and functionally. This harmony in improvements of polymorphism and stability is what we call *evolutionary progress*.

6. The basic question to which scientists should presently give the answer is how individuals within a species are so much *similar* to each other, rather than how they are so *variable* – that has been analysed in details during passed century. Based on classical rules of genetics and evolution, individual variability must be by far much greater, than one that we find in reality in the nature (see, also, Milojević, 1956; Crkvenjakov and Drmanac, 2007). Yet, the sources, qualities, and amounts of multigenic variation may well become the main topic of interest and investigation in future population-genetic studies.

REFERENCES

Carpenter, F. W. (1905). The reaction of the pomace fly (*Drosophila ampelofila* Loew) to light, gravity, and mechanical stimulation. *Am. Naturalist* **39,** 157-171.

Cluster, P. D., Marinković, D., Allard, R. W., and *F. J. Ayala* (1988). Correlations between developmental rates, enzyme activities, rDNA spacer-lengths, and adaptation in *Drosophila melanogaster. PNAS USA* **84,** 610-614.

Crkvenjakov, R., and *R. Drmanac* (2007). Biological evolution as natural programming. *Bull. Serb. Acad. Sci. Arts* **136 (45),** 1-108.

Dobzhansky, T. (1970). *Genetics of the Evolutionary Process.* Columbia University Press, New York.

Gavrilets, S., and *G. DeJong* (1993). Pleiotropic models of polygenic variation, stabilizing selection, and epistasis. *Genetics* **134,** 609-625.

Kovač, I., and *D. Marinković* (1999). Analysis of background-dependent genetic interactions without inbred strains. *Biochem. Genetics* **37,** 23-40.

Krimbas, C. B., and *J. R. Powell* (1992). Drosophila *Inversion Polymorphism*. CRC Press, Washington D.C.

Marinković, D. (1997). Multigenic polymorphism as a basis of population variability. *First Serbian Symposium in Population and Evolutionary Genetics*, *Tara 3-7.VI 1997. Serbian Genetic Society*, *Belgrade*, *Abstracts*, 1-2.

Marinković, D. (1999). Multigenic polymorphism as a source of adaptively restricted population variability. *J. Zool. Syst. Evol. Res*. **37,** 113-120.

Marinković, D. (2001). Genetic bases of the evolution of organisms. *Genetika* **34,** 53-58.

Marinković, D. (2002). Evolution of a multigenic system, In: *Genetics-Ecology-Evolution* (Eds. B. Ćurčić and M. Andjelković), 9-20. Monographs 6. Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade.

Marinković, D. (2004). The sources and limits of developmental polymorphisms. *Glas SANU* **59,** 19-27.

Marinković, D. (2005). The scopes and limits of adaptive variation. *Bull. Serb. Acad. Sci. Arts* **130 (43),** 139-150.

Marinković, D. (2008). Developmental programs in simulated and observed multi-locus genotypes of *Drosophila melanogaster.* In: *Advances in Arachnology and Developmental Biology. Papers Dedicated to Professor Božidar P. M. Ćurčić* (Eds. S. E. Makarov and R. N. Dimitrijević), 81-86. Monographs 12. Institute of Zoology, Faculty of Biology, University of Belgrade, Vienna-Belgrade-Sofia.

Marinković, D., and *V. Kekić* (2007). Capacities for population-genetic variation and ecological adaptations. *Genetika* **39 (2),** 93-102.

Marinković, D., Tucić, N., Moya, A., and *F. J. Ayala* (1987). Genetic diversity and linkage disequilibrium in *Drosophila melanogaster* with different rates of development. *Genetics* **117,** 513-520.

Mestres, F., Sanchez, J. and *L. Serra* (1998). Chromosomal structure and recombination between inversions in *Drosophila subobscura. Hereditas* **128,** 105-113.

Milojević, B. D. (1956). Thesen einer Evolutionstheorie. *Rec. Trav. Inst. Physiol. Develop. Genet. Select.* (*Belgrade*) **5,** 29-38.

Živanović, G., Andjelković, M., and *D. Marinković* (2000). Genetic load and coadaptations of chromosomal inversions. II. O-chromosomes in *Drosophila subobscura* populations. *Hereditas* **133,** 105-113.

Živanović, G., and *D. Marinković* (2004). Viabilities of originally natural O-chromosomal inversion of homo- and heterokaryotypes in *Drosophila subobscura. Hereditas* **139,** 128-142.

ЕВОЛУЦИЈОМ ПРОГРАМИРАНО РАЗВИЋЕ КАО ОСНОВА ДАРВИНИСТИЧКЕ СЕЛЕКЦИЈЕ

Д. МАРИНКОВИЋ

Српска академија наука и уметности, 11000 Београд, Србија

Потомство организама не настаје на основу случајних комбинација родитељских хромозома и алелогена, већ као резултат комбиновања ограниченог броја постојећих развојних *програма.* Комплекси полигена који одређују оствари-вање развојних програма су стварни предмет (циљ/таргет) дарвинистичке селекције (Маринковић 1997, 1999, 2002).

Анцестралне полигенске структуре, конзервативне и мономорфне на више локуса, могу бити најчешће присутне у оквиру индивидуалне генетске варијабилности, обезбеђујући међусобну сличност индивидуа у оквиру дате врсте. Оне гарантују одржање основне структуре иницијалних метаболичких система и мењају се (мутирају) да би постепено повећале адаптивну варијабилност, али и своју хармонију и хомеостатску стабилност.

Највећи део адаптивне полиморфности заснива се на комбинативној варијабилности полигенских комплекса, тако да опсег алелогенске различитости у популацији од пар стотина јединки може бити безмало исти као и у популацији од више хиљада индивидуа, што објашњава брзу обнову ових структура нпр. код инсекатских врста у пролећњим месецима, после оштре селекције током хладних зима. То је и основни механизам одржавања генетичке структуре у узастопним генерацијама и поред огромних осцилација у величини популација, уз Харду-Weинберг-ове равнотеже које се односе углавном на квалитативне особине.

Битну улогу у еволутивном развитку комплексних (структурних и метаболичких) система имају унутрашње (системске) промене, од којих зависи ограничена варијабилност варијанти на које дарвинистичка селекција може да делује. Оваква конзервативно програмирана *аутосинтеза* успешно напредује само ако су новонастали системи истовремено адаптивно полиморфнији, али и хомогенији од претходних, што се означава као *еволуциони прогрес.*

Основно питање на које савремена наука треба да одговори је како су индивидуе у оквиру сваке врсте у толиком степену међусобно *с л и ч н е,* за разлику од напора који су чињени током протеклог столећа да се објасне узроци и последице биолошке *различитости.* Величина, квалитет и еволуционо порекло индивидуалне мултигенске варијабилности треба да пруже одговор на постављено питање, у чему овај рад даје свој иницијални допринос.