UDK 575.113.39 : 17

MODIFIER VARIABILITY IN A NATURAL POPULATION OF DROSOPHILA SUBOBSCURA

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Alvarez, G., Zapata, C. and Fontdevila, A. (1980): *Modifier variability in a natural population of Drosophila subobscura.* – Genetika, Vol. 12, No. 1, 81–89.

Experimental evidence of the existence of modifier genes of the dominant morphological mutant *Bare (Ba)* in a natural population of *Drosophila subobscura*, in presented. The effect of these genes is to modify the expression of the *Ba* gene, in *Ba*/+ heterozygotes, towards the wild–type phenotype. It is also demonstrated that these modifiers are located on chromosome 0 of this species. Moreover, a large variability in modifier effect among chromosomes 0 of the natural population studied, is detected. The evolutionary meaning of the modifiers is discussed in connection with dominance evolution, canalization and structural genetic variability in natural populations.

INTRODUCTION

Population geneticists have made a large effort to study the genetic variability existing in natural populations. Chromosome, allozyme and viability variations are aspects of genetic variability which have been broadly studied. On the other hand, comparatively little attention has been devoted to the study of other types of genetic variability. Modifier genes are one of these types. Defined in a general way, modifiers are genes that change the phenotypic effects of other genes in a quantitative fashion. Modifier genes are important because they are often involved in penetrance, expressivity and modification of dominance phenomena (Belt and Burnet, 1972; Thompson and Thoday, 1972), which, in turn, are connected with the processes of evolution of dominance and canalization (Sved and Mayo, 1970).

In this work, experimental evidence for the existence of modifier genes of the dominant morphological mutants *Bare* of *Drosophila subobscura*, is presented. An attempt to determine distribution of these modifiers in a natural population was also carried out.

MATERIALS AND METHODS

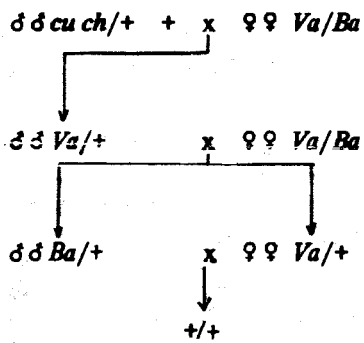
Marker strains. – The *ch–cu* and *Va/Ba* strains were used. These strains were kindly supplied by Dr. Sperlich from the University of Tübingen.

The *ch–cu* strain carries the recessive mutants *cherry (ch,* bright red eye color) and *curled (cu,* curled wings), both located on chromosome 0 of *Drosophila subobscura* (Koske and Maynard–Smith, 1954). In addition, this strain is homozygous for several allozymes and 3+4 arrangement, on chromosome 0.

The balanced lethal *Va/Ba* strain for chromosome 0 of *Drosophila subobscura* carries the dominant morphological mutants *Varicose (Va)* and *Bare (Ba)* (Koske and Maynard–Smith, 1954; Sperlich *et al.*, 1977). Both markers are lethal in the homozygous condition. *Va* produces irregular thickenings at the junctions of the wing veins, and *Ba* reduces variably the number of bristles. The *Va* chromosome carries an inversion complex to suppress recombination. The *ch* and *cu* mutants are also situated on this chromosome, and so the *Va/Ba* strain is heterozygous for these genes.

Isogenic lines. – Chromosomes 0 of a natural population of El Pedroso (Santiago, Spain) were isolated by crossing males of this population, individually, with females of the *ch–cu* strain. A single male (*+ +/cu ch)* of the offspring of each of the previous crosses was mated with females of the *ch–cu* strain. These crosses were performed to investigate the genetic composition of each chromosome 0 sampled, which was necessary for a linkage disequilibrium study to be reported elsewhere. During four generations ♂♂ *+ + cu ch* x ♀♀ *cu ch/cu ch* crosses were repeated in order to obtain individals with a similar genetic background to that of the *ch–cu* strain.

Then, the isogenization process of 0 chromosomes was performed by making the following crosses with the *Va/Ba* strain:



Finally, with the +/+ offspring of the last cross the corresponding isogenic line was established.

All the experiments presented in this paper were carried out on the medium described by David (1959) at 19°C and 60–70% humidity.

RESULTS AND DISCUSSION

In connection with a linkage disequilibrium study between chromosome arrangements and allozymes of chromosome 0 of *Drosophila subobscura*, several isogenic lines for this chromosome were obtained by crosses with the balanced lethal *Va/Ba* strain (see Materials and Methods). During the isogenization process a certain number of individuals which were wild–type for *Va* and *Ba* markers, appeared among the offspring of a substantial number of ♂♂ +/*cu ch* x ♀♀ *Va/Ba* and ♂♂ *Va*/+ x ♀♀ *Va/Ba* crosses (first and second crosses in the isogenization procedure, respectively). When the wild–type phenotype of these individuals was observed more carefully one was able to verify that, while some of them showed a perfect wild–type phenotype for *Va* and *Ba* markers, other individuals showed an absence of a few bristles. This suggested taht the wild–type phenotypic class could correspond to some individuals of the *Ba*/+ genotype in which the usual variable expressivity of *Ba* would be more displaced towards the wild–type phenotype than is costomary.

It is possible to explain the appearance of these wild-type individuals by postulating the presence of modifier genes which may attenuate or conceal the *Ba* gene effect in the *Ba*/+ individuals. These modifiers could originate either from the *ch–cu* strain of form the wild males of the natural population. This latter possibility implies that the modifiers would be most probably contained in the 0 chromosomes, since the remaining chromosomes of both ++/*cu ch* and *Va*/+ males belong mainly to the *ch–cu* strain in the former, and to the *ch–cu* and *Va/Ba* strains in the latter.

In order to check the first possibility, 10 ♀♀ *Va/Ba* = 10 ♂♂ *cu ch/cu ch* crosses were performed. In each cross, the number of bristles of the parental *Va/Ba* females and of 40 *Ba/cu ch* offspring were counted. In each individual dorsocentral (4) and scutellar (4) bristles were considered. The experiment was carried out using a factorial design, in which 4 *Va/Ba* lines (named 2, 3, 4 and 11) and 9 *ch–cu* lines were used. The results of this experiment are shown in Figure 1. In this Figure the results corresponding to different *ch–cu* lines are pooled, since the two way analysis of variance of the mean numbers of bristles of *Ba/cu ch* offspring (Table 1) did not detect statistically significant differences among *ch–cu* lines. In this same analysis, significant differences among *Va/Ba* lines are not detected. On the other hand, the analysis of variance of the mean numbers of bristles of parental *Va/Ba* females (Table 2) show significant differences among *Va/Ba* lines, as can be observed in Figure 1.

In order to detect differences between the *Ba* phenotype in each *Va/Ba* line and the phenotype of *Ba/cu ch* offspring heterozygotes, the averages of mean numbers of bristles of parental *Va/Ba* females and their *Ba/cu ch* offspring for each *Va/Ba* line were compared by means of t–dependent tests. This kind of test was used since a statistically significant correlation ($r = 0.50$, $P < 0.01$) was detected between the mean numbers of bristles of the parent females and their *Ba/cu ch* offspring. These t-dependent tests do not show statistically significant differences for the 3 and 4 *Va/Ba* lines ($t = 0.15$, $P > 0.05$ and $t = -1.03$, $P > 0.05$,

respectively). On the other hand, differences are statistically significant for the 2 and 11 *Va/Ba* lines ($t = 4.43$, $P < 0.01$ and $t = 4.54$, $P < 0.01$, respectively). In these cases, the values corresponding to the *Ba/cu ch* offspring are lower than that of their *Va/Ba* mothers (Figure 1). These results show that the phenotypic effect of the *Ba* gene is not reduced in heterozygotes with *ch–cu* chromosomes, and, thus, it is not probable that the *ch–cu* strain is carrying modifiers of the *Ba* gene.

Table 1. Analysis of variance of the mean numbers of bristles of Ba/cu ch offspring (data from Figure 1).

Source of variation	S.S.	d.f.	M.S.	F
Va/Ba lines	0.54	3	0.18	1.38^{NS}
ch–cu lines	1.78	8	0.22	1.69^{NS}
Error	3.03	24	0.13	
Total	5.35	35		

NS = not significant, $P > 0.05$

Table 2. Analysis of variance of the mean numbers of bristles of parental Va/Ba females (data from Figure 1).

Source of variation	S.S.	d.f.	M.S.	F
Va/Ba lines	11.73	3	3.91	8.06^{++}
Error	15.53	32	0.48	
Total	27.26	35		

++ = $P < 0.01$

The above conclusions fored us to consider the possibility that the modifier genes were included in the 0 chromosomes from the natural population. In order to test this hypothesis the following set of crosses was performed for each isogenic line (see Materials and Methods). First of all, 3 males of each isogenic line were crossed with 3 *Va/Ba* famales. Then 3 *Va/+* males of the offspring of this cross were mated with 3 *Va/Ba* females again, and the bristles of 30 *Ba/+* and 30 *Va/Ba* progeny individuals were counted. The *Va/Ba* individuals were used as a control, since the *Ba* chromosome is the same in *Va/Ba* and *Ba/+* individuals. Thus, it is possible to study the effect of wild 0 chromosomes on *Ba* expressivity relative to that of the same *Ba* chromosome in a *Va/Ba* individual. In this experiment 12 bristles for each individual (4 dorso–centrals and 4 scutellars, as in the previous experiment, plus 2 supra–alars and 2 post–alars) were considered. This experiment was carried out individually with two *Va/Ba* lines, one with a high (line 2) and the other with a low (line 3) number of bristles. When it was possible 4 replicates were performed for each *Va/Ba* line.

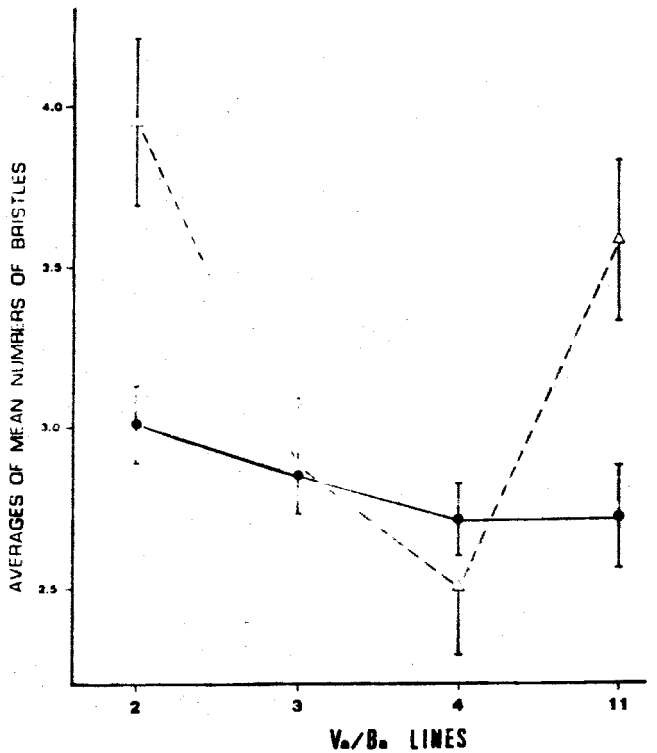


Fig. 1. Averages of mean numbers (with standard errors) of bristles of parental *Va/Ba* females (Δ) and *Ba/cu ch* offspring (•) from *Va/Ba* x *cu ch/cu ch* crosses.

Table 3 shows the results obtained in this experiment. In each replicate the difference (D) in mean nuber of bristles between *Ba/+* and *Va/Ba* (control) individuals was calculated. The mean ($\bar{D}$) of these differences was also computed for each 0 chromosome (isogenic line). Values of the two *Va/Ba* lines used were pooled, since only in two isogenic lines out of 20, were statistically significant differences detected between these two *Va/Ba* lines (by means of t tests). As can be observed in Table 3, $\bar{D}$ values were positive for all chromosomes 0 studied (with the exception of chromosome 57). However, in order to obtain the statistical significance of $\bar{D}$ values, t–dependent tests were performed to compare the averages of the mean numbers of bristles of *Ba/+* and *Va/Ba* individuals for each chromosome. These t–dependent tests were used, since a statistically significant correlation ($r = 0.49$. $P < 0.01$) between the mean numbers of bristles of *Ba/+* and *Va/Ba* individuals was detected. The t–dependent tests (not indicated in Table 3) showed the existence of statistically significant differences ($P < 0.01$) in all the studied chromosomes (chromosome 57 included). This demonstrates that all wild 0 chromosomes sampled in the natural population of El Pedroso (with the exception of chromosoma 57) carry a significant proportion of modifiers of the *Ba* gene relative to the *Va/Ba* controls. These modifiers are responsible for an increase

of the number of bristles in *Ba*/+ heterozygotes, which consequently show a phenotype very similar to that of the wild–type homozygote. Thus, dominance relationships in the *Ba* locus are also changed by these modifiers.

Table 3. Modifier effect of wild O chromosomes on the expressivity of Ba gene measured as D value (difference in mean number of bristles between +/Ba and Va/Ba individuals)

Chromosome number (isogenic line)	*Va/Ba* lines								D ± error
	line 3				line 2				
211	3.93	2,04	2,97	2.60	2.60	2.48	2.23		2.69 ± 0.23
34	2.56	4.56	3.56	3.90	2.72				3.46 ± 0.37
292	3.80	3.80	4.06		3.54	3.30	4.06	1.77	3.48 ± 0.30
51	2.90	2.73	2,89	3.22	3.86	3.7	2.56		3.05 ± 0.16
193	3.54	3.82	4.80	3.87					4.01 ± 0.27
35	3.30	1.77	1.97		2.00	1.74	2.08		2.14 ± 0.24
108	2.63	3.60	2.46	3.73	3.90	2.10	3.27	2.70	3.05 ± 0.23
132	4.80	2.86	2.46		4.26	3.60	2.57	3.34	3.41 ± 0.33
136	3.97	3.90	3.67	5.45	4.07	3.47	3.79	4.33	4.08 ± 0.22
220	6.67	5.33	6.74	6.86	5.05	4.46	3.96	4.04	5.39 ± 0.43
153	3.90	3.17	2.66	4.12	2.13	3.14	2.18		3.04 ± 0.29
57	−0.80	−0.68	−2.00	−1.00	−1.34	−2.16	−2.03	−3.47	−1.69 ± 0.33
294	1.40	1.80			1.40	0.60	1.87	0.83	1.32 ± 0.21
141	3.83				2.96	1.37	2.03	1.83	2.40 ± 0.44
47	3.34	3.66	3.90	2.60	2.23	2.98	2.81	2.80	3.04 ± 0.20
935	5.33	2.47	4.37	3.36	3.80	3.04	2.63		3.57 ± 0.38
713	1.96	1.93	3.53	1.59	0.67	2.24	2.64	3.66	2.28 ± 0.35
785	4.60	4.90	3.36	4.66	4.13	3.47	3.27	2.41	3.85 ± 0.30
793	3.43	3.73	1.83	3.20	2.37	2.74	2.55		2.84 ± 0.25
927	1.84	−0.11	2.13	3.87	2.12	3.67	4.39		2.56 ± 0.58
755	3.60	3.73	2.70		1.59	0.46	1.44		2.25 ± 0.53
258	4.40	4.25	4.46		3.77	2.33	3.76	4.51	3.93 ± 0.29
781	3.44	4.16	2.86	3.96	2.94	2.49	5.53	4.10	3.69 ± 0.34

The distribution of the mean differences ($\bar{D}$) of the studied wild 0 chromosomes is graphically depicted in Figure 2. Although the number of studied chromosomes is not high, one can observe, in outline, a large variability of modifier effects in this sample of chromosomes. This is fully confirmed by means of an analysis of variance of the D values of chromosomes (Table 4, chromosome 57 was removed from this analysis), in which statistically significant differences among chromosomes are detected.

It is not easy to understand the meaning of this large variability of modifier effects found in the 0 chromosomes of the studied natural population. However, several experiments by Rendel (1959 and 1962) have shown that scutellar bristles of *Drosophila melanogaster* are submitted to a canalization system which contributes to the stability of the wild–type phenotype. From this point of view, the modifiers of the *Ba* gene may be implicated in the canalization of the genetic system of the bristles of *Drosophila subobscura*. This view is also supported by the fact that for the majority of replicates the variance of number of bristles (not

indicated in Table 3) of *Ba*/+ is lower than the of *Va*/*Ba*. In spite of that *Ba*/+ genotypes show a mean number of bristles higher than that of *Va*/*Ba* genotypes in the majority of replicates. On this basis, the function of the modifiers would not be only restricted to the *Ba* locus, but could include a wider class of genetic and environmental factors disturbing the wild–type phenotype. If this were true, the *Ba* locus would have acted simply as a detector of a modifier genetic system of very extensive action. In this sense, Thompson (1973; 1975) has shown that many modifiers affecting the expression of several venation mutants in *Drosophila melanogaster* act on the character rather than on a specific mutant.

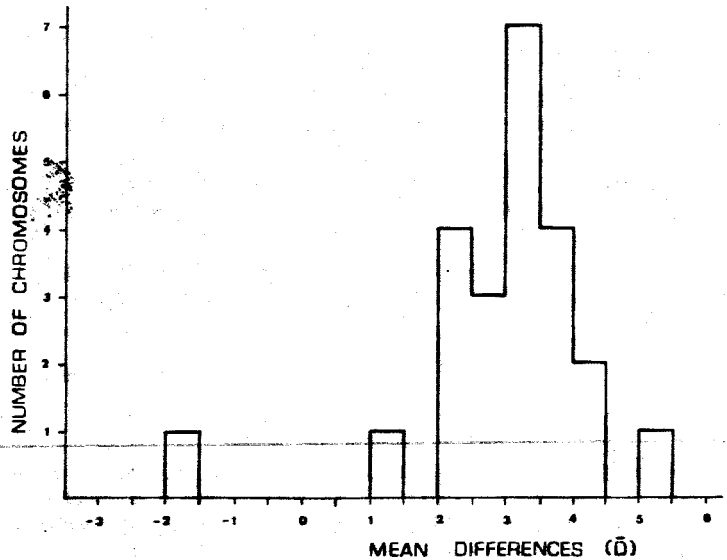


Fig. 2. Distribution of modifier effects ($\bar{D}$, see text for explanation) of wild O chromosome from an El Pedroso population of *Drosophila subobscura*.

Table 4. Analysis of variance of D values of different chromosomes.

Source of variation	S.S.	d.f.	M.S.	F
Chromosomes	107.90	21	5.13	6.74++
Error	98.31	129	0.76	
Total	206.21	150		

++ = $P < 0.01$

We are still far from knowing how large the effects of these modifier genes on evolutionary changes are. Evolution of dominance has been related traditionally

with this type of variability, although in different ways. This, Plunkett (1933) and Muller (1932) independently suggested that dominance could arise as a by–product of selection to stabilize a particular character. This argument circumvents the criticisms (Charlesworth, 1979; Wirght, 1977) of Fisher's theory of evolution of dominance (Fisher, 1928), based on the fact that selective pressure on modifiers, which are mostly represneted in a heterozygote state, is too low to account for the production of dominance (see Sved and Mayo, 1970, for a review). Recnetly, much variability inside allozyme loci has been attributed to modifier genes (Mc Donald and Ayala, 1978; Rawls and Lucchesi, 1974; Schwartz, 1976; Ward and Hebert, 1972; Wills and Nichols, 1972). Some of the rapid initial changes in allozyme fitness may be also attributed to the effect of this modifier variability (Fontdevila and Mendez, 1979). However, it is too soon to know whether both types of modifiers (morphological and allozymic) belong to the same or a different class. Further studies on modifier genes, such as the one we present here, will be necessary to unveil the importance of the class of variability in natural populations and its degree of incidence in the genome, as a whole. This knowledge is of extreme interest for an understanding of the maintenance of structural genetic variability which has been found in natural populations so far.

Acknowledgement. – The authors are grateful to Alejo Reigosa for his technical assistance in part of this work.

Received January 25th, 1980
Accepted May 5th, 1980

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MODIFIKATOR VARIJABILNOSTI U PRIRODNIM POPULACIJAMA DROSOPHILA SUBOBSCURA

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Izvod

U radu su izneseni eksperimentalni dokazi o postojanju u prirodnim populacijama *D. subobscura* gena koji modifikuju dominantni morfološki mutant *Bare (Ba)*. Efekat toga gena je modifikacija ekspresije *Ba* gena, u *Ba*/+ heterozigota u pravcu „divljeg" fenotipa. Pokazano je, takođe, da je ovaj modifikator lociran na 0 hromozomu. Takođe, uočena je velika varijabilnost u modifikacionom efektu među 0 hromozomima jedinki prirodne populacije koja je proučavana. U radu je diskutovana evoluciona uloga modifikatora u vezi sa evolucijom dominantnosti, kanalizacijom i varijabilnošću strukturnih gena u prirodnim populacijama.

Primljeno 25.I 1980.
Odobreno 5.V 1980.