THE GENETICS OF HOMEOSTASIS IN DROSOPHILA*

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The contribution of an individual of one generation to the gene pool of the next depends upon the ability of the individual to survive and to reproduce. This contribution, relative to that of other individuals of the same population, is a measure of the biological adaptive value of the individual in the succession of environments which it has encountered up to the close of its reproductive age. Similarly, the adaptive value of a population is determined by the average survival and reproductive capacities of the many gene combinations which are produced in this population. Since the environment is everchanging, an organism is exposed to a greater or lesser variety of environments. An organism is usually capable of carrying on its vital functions in all environments in which the species or population to which it belongs normally exists. Adaptation to a variety of environments is accomplished in two ways. First, most species and populations are polymorphic and consist of a variety of genotypes optimally adapted to different aspects and sequences of environments. Secondly, individuals respond to environmental changes by physiological and structural modifications. Modifications evoked by environmental variations recurrent in the environment of the species almost always tend to increase the probability of survival and reproduction of the organism. The organism adjusts itself to recurrent environmental changes in such a way that its functioning continues unimpaired; it is said to be homeostatic.[1]

Homeostatic mechanisms have been carefully studied by physiologists, especially in the higher vertebrates. It is nevertheless far from universally realized that homeostasis is conditioned by the genotype, and the different genotypes permit different degrees of homeostasis. The "wisdom of the body" is an outcome of the molding of the genetic structure of the species by natural selection in the process of evolution, and it cannot be understood outside this evolutionary context. As pointed out by several authors, especially by Schmalhausen,[2] genotypes which are favored most strongly by natural selection are those which condition homeostatic responses to recurrent environmental stimuli. Stimuli which were seldom or never encountered in the history of the species very often evoke adaptivity indifferent or even positively harmful responses, which Schmalhausen has called morphoses. Mutants and gene combinations which have not been historically established as normal constituents of natural populations of the species may be deficient in homeostatic responses even under usual

environments. These new or rare genotypes have not yet become fitted by natural selection to any particular environment. Homeostasis, like organic adaptedness in general, is not an inherent attribute of living matter as is often assumed by vitalists and by Lamarkians of various kinds.

The genetic mechanisms which underlie homeostasis have been explored very little. For obvious technical reasons, classical genetics preferred to deal with clean-cut genetic differentials regardless of their adaptive significance. We have been led to this problem by studies on the genetics of natural and experimental populations. Here the homeostatic properties of some genotypes contrast with a relative lack of such properties in others.

Experimental Procedures.—Four species of Drosophila are involved in

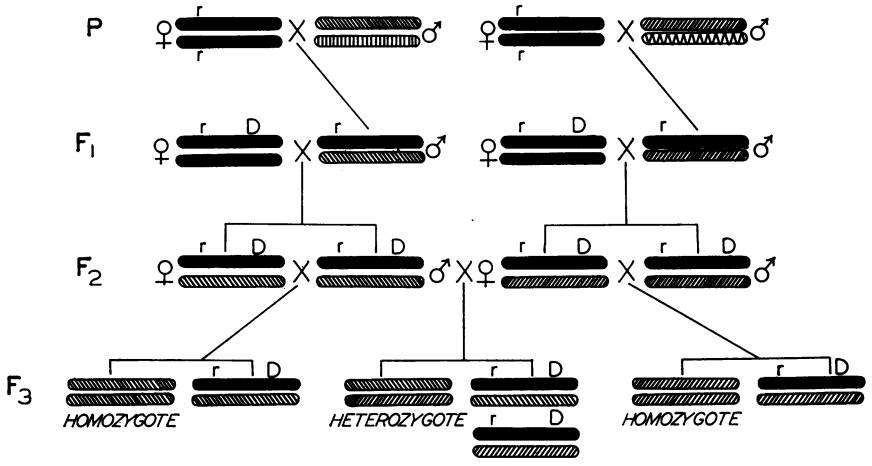

FIGURE 1

A generalized scheme of crosses used to obtain homozygotes and heterozygotes for various autosomes from wild and experimental populations. Black: chromosomes with mutant genes used as markers; *r*: recessive mutant; D: dominant mutant. Hachured: chromosomes, the effects of which are analyzed.

the present investigation, namely *D. pseudoobscura*, *D. persimilis*, *D. prosaltans*, and *D. melanogaster*. Samples of the natural populations of the first and second species were collected in the summer of 1951 in the Yosemite National Park region of California. Samples of *D. prosaltans* came from several localities in different parts of Brazil (Pirassununga, state of São Paulo, collected by Drs. C. Pavan and A. B. da Cunha; Ferreira Gomes, territory of Amapá, northeastern portion of the Isle of Marajó, state of Pará; Fordlandia, Pará; Içana, upper Rio Negro, Amazonas; and upper Rio Doce, Minas Geraes, collections of Dr. A. B. da Cunha and Th. Dobzhansky, made in 1952). All these population samples were brought or sent to the laboratory in New York and used to study the con-

cealed genetic variability due to autosmal recessive genes and gene combinations carried in the populations in question. *D. pseudoobscura* and *D. persimilis* have three pairs of large autosomes (the second, third, and and fourth chromosomes), and a pair of microchromosomes (the fifth chromosome). *D. prosaltans* has two pairs of large autosomes (the second and the third). The genetic variability in all of these autosomes, except the microsomes, has been studied. The results obtained will be described in detail elsewhere. Here it will be sufficient to state that in all cases a certain proportion of the chromosomes proved to be lethal in double dose, i.e., in homozygotes. Other chromosomes were semilethal, or subvital, or caused sterility, or modifications of the developmental rates, or morphological aberrations in the homozygotes. More important at present is that the techniques used in these studies were alike in principle in all cases, the essential features of these techniques being as follows (Fig. 1):

Males collected in natural habitats, or single sons of the females so collected, are crossed to females of a laboratory strain homozygous for suitable recessive mutant genes (*r*, in Fig. 1). A single male from each progeny (F_1) is outcrossed to females carrying in one of their chromosomes the same recessive marker, a dominant marker which is lethal to homozygotes (*D*, in Fig. 1) and an inversion which suppresses the recombination in the proper chromosome. In the next generation (F_2) flies of both sexes which show the dominant but not the recessive marker are selected in each strain. Such flies carry the same "wild" chromosome, as well as the chromosome with the marking genes. When they are inbred, one-third of their progeny (F_3) is expected to be homozygous for a "wild" chromosome (i. e., to carry two replicas of a certain chromosome descended from the wild ancestor). Two thirds of the progeny should carry this "wild" chromosome and the chromosome with the marker genes (Fig. 1). In reality, the homozygotes are often less numerous than expected because some of the "wild" chromosomes are deleterious to the homozygotes. If no homozygotes appear, the chromosome is lethal in double dose; if less than half of the expected proportion of the homozygotes survive, the chromosome is semilethal. (For more details about the method see previous publications.[3–6] For our present purposes the lethal and semilethal chromosomes may be disregarded. The remaining chromosomes, very roughly three-quarters of the total, fall within an approximately bell-shaped curve, somewhat truncated on the left owing to the exclusion of semilethals. It is with these remaining chromosomes, which may be conditionally referred to as "quasi-normal" that we are at present concerned.

The different quasi-normal chromosomes give different proportions of the homozygotes in the test cultures. Apart from the variability due to sampling errors, these differences are the result of two causes. First, the homozygotes for some of these chromosomes are more viable than others;

with sufficient data one can distinguish chromosomes which are subvital, normal, and supervital when homozygous. Secondly, the environment, the culture conditions, in which the tests are made are not uniform. Thus, some cultures are more crowded than others, or have unequal amounts of food, etc. The importance of this environmental component of the variance can be estimated from our data. Indeed, the tests were so arranged that the cultures of the final generation (the lowermost line in Fig. 1) were replicated for most of the chromosomes tested. A group of parents (F_2) were permitted to oviposit in a culture for 2–3 days, whereupon the parents were transferred for oviposition in a new culture bottle. For the different species and chromosomes, from one to five such transfers were made, giving from two to six replicate cultures. Now, if the viability of the homozygotes for a given chromosome is not sensitive to the environmental variations encountered in the replicate cultures, these cultures will give the same proportions of the homozygotes, within limits of the sampling errors. Conversely, an environmental sensitivity may result in statistically significant heterogeneities between the replicate cultures. This can be detected by the Brandt-Snedecor chi-square test for homogeneity among the replications.

Homozygosis for any chromosome is rare in any population of most species of Drosophila. In all but the very closely inbred populations the two chromosomes of each pair carried by most individuals differ in origin and thus have somewhat different gene complexes. Wild flies are usually heterozygotes rather than homozygotes. Corresponding heterozygotes can be obtained also in laboratory experiments. In the F_2 generation of the crosses shown in figure 1 one must now take females and males from the offspring of *different* wild progenitors. Such experiments have actually been made, using chromosomes which were quasi-normal as well as those which were lethal or semilethal when homozygous (see the middle of the F_2 and F_3 of Fig. 1). In these experiments the proportions of the flies heterozygous for the two "wild" chromosomes formed a nearly symmetrical bell-shaped distribution. As with homozygotes, the variance observed in this distribution may be the result of three causes. Apart from the errors of sampling, different chromosome combinations may give flies of different viability under one environment, or similar combinations may give different viabilities under different environmental conditions. The experiments with chromosomal heterozygotes were arranged like those that involved homozygotes. Most chromosome combinations were obtained in replicate cultures. The sensitivity of the heterozygotes to different environmental conditions may be tested by computing chi-squares for homogeneity of the replications.

The experiments involving *D. melanogaster* were somewhat differently arranged.[6, 7] During the course of regular sample analyses—similar in

technique and purpose to those described above for natural populations—an additional effort was made to find combinations of second chromosomes that exhibited "negative heterosis." The original matings that yielded homozygous and heterozygous wild type individuals were set up in such a way that the viability of individuals heterozygous for a given pair of second chromosomes could be compared with the viability of individuals homozygous for each of the two members of the pair. These matings were not replicated.

TABLE 1

SENSITIVITY TO ENVIRONMENTAL VARIATIONS SHOWN BY HOMOZYGOTES AND HETEROZYGOTES FOR CHROMOSOMES DERIVED FROM CERTAIN NATURAL POPULATIONS

		HOMOZYGOTES			HETEROZYGOTES		
SPECIES	CHROMOSOME	CHI-SQUARE	DEGREES OF FREEDOM	P	CHI-SQUARE	DEGREES OF FREEDOM	P
Pseudoobscura	II	144.34	81	<0.001	31.91	33	0.5
Pseudoobscura	III	212.24	86	<0.001	33.29	31	0.3
Pseudoobscura	IV	173.86	77	<0.001	44.09	30	0.05
Persimilis	II	225.77	184	0.018	74.92	99	0.96
Persimilis	III	519.28	329	<0.001	67.27	63	0.3
Persimilis	IV	327.43	269	0.008	99.05	66	0.004
Prosaltans	II	253.14	152	<0.001	48.07	47	0.4
Prosaltans	III	290.40	216	<0.001	72.36	54	0.046

TABLE 2

SENSITIVITY TO ENVIRONMENTAL VARIATIONS SHOWN BY HOMOZYGOTES AND HETEROZYGOTES FOR SECOND CHROMOSOMES FROM EXPERIMENTAL POPULATIONS OF *Drosophila melanogaster*. N IS THE NUMBER OF THE CHROMOSOMES OR CHROMOSOME COMBINATIONS STUDIED

	HOMOZYGOTES				HETEROZYGOTES			
POPULATION	N	CHI-SQUARE	DEGREES OF FREEDOM	P	N	CHI-SQUARE	DEGREES OF FREEDOM	P
1	20	88.35	75	0.50	11	32.16	40	>0.50
3	26	141.38	102	0.01	13	53.81	51	0.50
5	7	32.47	23	0.09	4	17.61	13	0.18
6	4	18.51	16	0.30	2	14.80	8	0.07
7	40	168.39	139	0.09	23	71.20	82	>0.50
TOTAL	97	449.10	355	<0.001	53	189.58	194	>0.50

Whenever a combination of cultures was found, however, in which the viability of the heterozygous individuals appeared to be less than that of the two homozygotes, replicate F_4 cultures were made using $D/+F_3$ (*Cy* $L/+$ in the case of *D. melanogaster*) flies as parents. Usually four or five, rarely three, replicate cultures of each type of homozygous and of the heterozygous crosses were made. The final decision as to the presence or absence of negative heterosis was based on these greatly increased numbers of flies. This slightly unorthodox reason for deciding which chromosomes to

test in replicate cultures would have the effect of selecting those chromosomes which when homozygous resulted in higher than average viabilities and chromosomal combinations that produced correspondingly lower viabilities. The Brandt-Snedecor chi-square test for homogeneity was used among these replications as it was in those described above.

Environmental Sensitivity of Homozygotes and Heterozygotes.—A summary of the results of the homogeneity tests for the replicate cultures is presented in tables 1 and 2. In both tables the cultures producing flies homozygous for certain chromosomes derived from natural or experimental irradiated populations are contrasted with cultures giving rise to flies with the two chromosomes of a pair derived from different progenitors.

It can be seen at a glance that the homozygotes generally show very significant heterogeneous viabilities in replicate cultures, while the heterozygotes usually do not. This situation has been emphasized in table 2 by summing the chi-squares and degrees of freedom for each type of replication. The individual entries in table 1, with few exceptions, agree in demonstrating the heterogeneity among homozygous replications and the homogeneity among the sets of heterozygotes. The data in table 2 are not so consistent; it is quite probable that replicated cultures of chromosomes from population 1, for instance, are not heterogeneous. The difference between tables 1 and 2 may result from any of three factors: (1) The number of *D. melanogaster* chromosomes analyzed was quite small. (2) The choice of chromosomes tested in replicate cultures from the experimental populations favored homozygotes with high viabilities. (3) The experimental irradiated populations of *D. melanogaster* were kept under relatively constant conditions where selection for homeostasis would be minimized; indeed, the Oregon-R strain of flies from which these populations were started has been kept under laboratory conditions for at least 25 years.

Environmental Sensitivity of Different Chromosomes.—Dobzhansky and Spassky[4] measured the viability of homozygotes for 26 second and 22 fourth chromosomes of *D. pseudoobscura* at three different temperatures: $16^1/_2°$, 21°, and $25^1/_2°$. They found that about half of these chromosomes showed significant differences in performance at the three temperatures, while the other half were not temperature sensitive. Their experiments, like those described in the present article, involved raising replicate cultures for every chromosome. Some of the chromosomes showed quite significant heterogeneities between the replicate cultures at all temperatures; others were heterogeneous at only one temperature; still others gave no heterogeneities. The environmental variable that caused the heterogeneity was in some instances quite obvious: different degrees of crowding in replicate cultures. The homozygotes for some chromosomes are sensitive to crowding and survive rather poorly in crowded cultures; other chromo-

somes seem to be insensitive to crowding, at least within the limits studied. When the heterogeneity is not correlated with crowding, it must be due to more subtle differences in culture conditions. Nutritional variables, such as different microflorae in different cultures, may be involved.

Examination of our data discloses a situation similar to that observed by the authors just cited. The contributions of some of the chromosomes to the chi-squares are much greater than those of other chromosomes. The over-all evidence shows that, on the average, the homozygotes possess less perfect homeostatic properties than do the heterozygotes; the evidence is not critical, however, in determining whether all homozygotes are deficient in homeostasis or whether some homozygotes are as adaptable as most heterozygotes (see, for instance, population 1, table 2). We have by no means explored the norms of reaction of the genotypes in question. It should be noted that in these experiments the environment was deliberately made as uniform as practicable in fairly large-scale work. Nevertheless, many homozygous genotypes proved to be sensitive to residual environmental diversities that failed to alter the viabilities of heterozygous individuals.

Discussion.—Cannon,[1] the greatest student of homeostasis, wrote as follows: "In an open system, such as our bodies represent, compounded of unstable materials and subjected continually to disturbing conditions, constancy is in itself evidence that agencies are acting, or ready to act, to maintain this constancy." And: "It is not supposed that the full display of homeostatic adjustments will be found in all forms of animals." Homeostasis is a matter of degree. In general, homeostasis with its consequent autonomy of life processes must confer a high selective advantage in most species. We do not know which physiological processes in Drosophila must be maintained constant to enable the development to proceed unimpaired, nor do we know the nature of the gene action that produces the buffered system for the constancy of such processes. The evidence of homeostasis is necessarily indirect. Nevertheless, heterozygotes are more uniformly successful in a variety of environments than are homozygotes; this suggests that the heterozygotes are better able than homozygotes to cope with these different environments and to maintain their internal milieu in functional order. Wigan[8] and, more recently, Robertson and Reeve[9] have reported similar phenomena within *D. melanogaster* and Mather[18] in *Primula sinensis*. In Robertson and Reeve's report it has been shown that environmentally caused variability for certain morphological characteristics is inversely proportional to the degree of heterozygosity. The generality of this correlation between homeostasis and heterozygosity is, of course, a matter of speculation at present. It should be emphasized, however, that our data are based on the relative frequencies of two classes of flies ($D/+$ and $+/+$) and, consequently, statistically homogeneous data

within sets of replications of heterozygous combinations indicate the existence of homeostasis within *both* classes of flies. The fact that a pronounced homeostasis exists among heterozygous individuals helps in our understanding of the genetic structure of populations. Within recent years it has been recognized that the genotype of a Mendelian population is a coadapted system. The evidence of this coadaptation has been obtained from studies on heterosis in inversion heterozygotes,[10, 11] on the adaptive values of experimental populations,[6, 7, 12] and on the breakdown of heterosis in the F_2 generation hybrids between natural populations of different localities.[13] Additional evidence[10, 12] has indicated that this coadaptation is based upon a genetic heterogeneity—not upon genetic unifomity and homozygosis within a population.

If heterozygosity—at least within some as yet unknown limits—results in an increase of homeostasis, then the extreme genetic heterogeneity within a population can be understood: selection for multiple alleles at many loci would act to minimize the frequency of homozygosity at any one locus. Coadaptation, then, leads to the formation of a gene pool containing those members of each series of alleles that are most likely to produce harmonious combinations with other alleles at the same locus and in combination with the alleles at all other loci. Selection must operate to choose a variety of alleles at every locus, thus avoiding the extreme sensitivity to environmental differences which characterizes many homozygotes; but it must also weed out those extreme variant alleles which often react with others at the same or other loci to produce morphoses. This results in the accumulation of a store of mutually compatible alleles—in other words, in a coadapted genetic system.

Note should be taken of the fact that not all of our data on heterozygotes show evidence of complete homeostasis. A significant chi-square (table 1) was found for fourth-chromosome heterozygotes in *D. persimilis*, and chi-squares that lie at the boundary of the conventional significance range for the fourth-chromosome heterozygotes in *D. pseudoobscura* and the third chromosome in *D. prosaltans*. This is not unexpected. First of all as mentioned above, in our experiments the viability of the homo- and heterozygotes for the wild chromosomes is measured against that of carriers of certain dominant mutant genes introduced as markers (*D*, in Fig. 1) and some of the statistical heterogeneity may arise from a lack of homeostasis in the mutant heterozygotes. Second, all heterozygotes need not possess equal homeostatic capacities; the conditions within standard laboratory cultures may well represent an environment that falls outside the range of those normally encountered in natural habitats. Furthermore, the coadaptation of the gene complexes in a population need not be so perfect that all individuals that carry two chromosomes from the same population will be equally versatile. A heterogeneity of viabilities of

heterozygotes has, indeed, been observed by Cordeiro[14] and by Wallace and King.[7] Lastly, occasionally inbreeding or the operation of chance must produce some individuals in nature—and in our heterozygous combinations as well—that carry a chromosome section in duplicate and are homozygous for that section.

Natural selection is to a great extent opportunistic, although interpopulation competition serves to limit this opportunism somewhat. Nevertheless, it is impossible to postulate abstract schemes of evolution without regard to the biology of the group in which selection is acting. It may be expected that coadapted gene pools and coadaptation of random combinations of chromosomes arising from these pools would be characteristic of crossfertilizing species with rather large effective population sizes. If inbreeding or selfing is the rule, it may be expected that homozygotes would show consistently high adaptive values in a variety of environments (see, for instance, Gustafsson[15]). Higher animals with their low fecundity and individual longevity may be expected to develop a different integration of their gene pools than would perennial plants with their great numbers of seeds and alternative asexual methods of perpetuation. Finally, microorganisms with their enormous reproductive potentials and extremely short generation time may rely upon a still different scheme; here gene mutation may serve as the main adaptive mechanism.

Summary.—We have studied the rates of survival in crowded cultures of individuals "homozygous" and "heterozygous" for chromosomes derived from natural and experimental populations of *Drosophila pseudoobscura*, *D. persimilis*, *D. prosaltans*, and *D. melanogaster*. The "homozygotes" carried certain chromosomes in duplicate, while the "heterozygotes" had the two chromosomes of a pair derived from different wild progenitors, or from different members of an experimental population. The experiments were so arranged that each chromosome combination was tested in replicate cultures. The environments in the replicate cultures varied because of different degrees of crowding and probably also because of variations in the quantity and quality of the food. The homozygotes often showed significantly different survival rates in the varying environments of the replicate cultures. Conversely, the heterozygotes gave usually uniform survival despite the environmental variations. It is inferred that the homeostatic adjustments are superior in heterozygotes than in homozygotes. The gene complexes carried in the homologous chromosomes in sexual and crossfertilizing populations are coadapted by natural selection to give high fitness in heterozygotes with most other chromosomes of the same population. The genotype of a Mendelian population is an integrated system, the parts of which are fitted together in the process of evolution.

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[1] Cannon, W. B., *The Wisdom of the Body*, W. W. Norton, New York, 1952.
[2] Schmalhausen, I. I., *Factors of Evolution*, Blakiston, Philadelphia, 1949.
[3] Dobzhansky, Th., Holz, A. M., and Spassky, B., *Genetics*, **27**, 463–490 (1942).
[4] Dobzhansky, Th., and Spassky, B., *Ibid.*, **29**, 270–290 (1944).
[5] Pavan, C., *et al.*, *Ibid.*, **36**, 13–30 (1951).
[6] Wallace, B., and King, J. C., *Am. Nat.*, **85**, 209–222 (1951).
[7] Wallace, B., and King, J. C., PROC. NATL. ACAD. SCI., **38**, 706–715 (1952).
[8] Wigan, L. G., *J. Genetics*, **46**, 150–160 (1944).
[9] Robertson, F. W., and Reeve, E., *Nature*, **170**, 286 (1952).
[10] Dobzhansky, Th., *Genetics*, **35**, 288–302 (1950).
[11] Dobzhansky, Th., and Levene, H., *Am. Nat.*, **85**, 247–264 (1951).
[12] Wallace, B., et al., *Genetics* (in press).
[13] Vetukhov, M. A., PROC. NATL. ACAD. SCI., **39**, 30–34(1953).
[14] Cordeiro, A. R., *Ibid.*, **38**, 471–478 (1952).
[15] Gustafsson, A., *Hereditas*, **32**, 263–286 (1946).
[16] Lerner, I. M., *Population Genetics and Animal Improvement*, Cambridge University Press, London, 1950.
[17] Lerner, I. M., and Demster, E. R., *Heredity*, **5**, 75–94 (1951).
[18] Mather, K., *Evolution*, **4**, 340–352 (1950).

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*HETEROGENEITY OF CLONES OF SACCHAROMYCES DERIVED FROM HAPLOID ASCOSPORES**

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It has been demonstrated[1, 2] in heterothallic strains of Saccharomyces, that crosses between clones derived from haploid ascospores sometimes produce asci which exhibit tetraploid segregation. One explanation for the occurrence of such asci is that they arise from tetraploid ascogenous cells which are in turn the result of fusion of diploid cells present in the parental clones.[1] This possibility points up the need for information regarding the composition of parental clones of haploid origin, in order to achieve