*AN EXTREME CASE OF HETEROSIS IN A CENTRAL AMERICAN POPULATION OF DROSOPHILA TROPICALIS**

BY THEODOSIUS DOBZHANSKY AND OLGA PAVLOVSKY

DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY, NEW YORK

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Natural populations of many species of *Drosophila* flies are chromosomally polymorphic. Two or more gene arrangements in the same chromosome, differing in inversions of blocks of genes, occur in the chromosome pool of many populations. Since the carriers of the different gene arrangements interbreed freely, some of the wild flies are structural homozygotes (which have the two chromosomes of a pair with the same gene arrangement), while other flies are structural heterozygotes (the two chromosomes with different gene arrangements). In at least some natural populations the structural heterozygotes exhibit heterosis, and the chromosomal polymorphism is, therefore, balanced.[1] The mean adaptive values of the heterotic heterozygotes are superior to those of the homozygotes in the environments which the population inhabits. In many instances the homozygotes are less viable than the heterozygotes. If a differential mortality favoring the heterozygotes takes place between the egg and the adult stage, the adult fly population contains relatively more heterozygotes and fewer homozygotes than are demanded by the Hardy-Weinberg equilibrium equation.[1] However, it is naïve to think that heterosis must always lead to differential mortality in preadult life. Adaptive superiority of the heterozygotes may as well be due to a greater fecundity, to greater longevity, or to more intense sexual activity of the heterozygotes compared with the homozygotes. Furthermore, the heterosis may be more pronounced in one sex than in the other, at one season than at another, or in some habitats which the population occupies than in others[2]. Balanced polymorphism is maintained by natural selection so long as the heterozygotes retain an over-all adaptive advantage over the homozygotes.

The purpose of the present article is to describe an instance of balanced polymorphism in a natural population in which the fitness of the homozygotes is extremely low, in fact approaching zero. Nevertheless, the population not only does not appear to be on the verge of extinction but is remarkably prosperous. One may infer that the heterozygotes in this population are quite well adapted to their environments.

Natural Populations Sampled.—Mr. William B. Heed, of the University of Texas, very generously sent us living *Drosophila* flies of the *willistoni* species group which he collected in Honduras in April and in El Salvador in May and August, 1954, while he was working at the Instituto Tropical de Investigaciones Cientificas at San Salvador. Among these flies there were individuals of the four sibling species of the *willistoni* group, namely, *D. willistoni*, *D. equinoxialis*, *D. tropicalis*, and *D. paulistorum.*[3] The females collected in nature were placed singly in regular laboratory culture bottles with banana-agar food and allowed to produce offspring. Most of them were inseminated and produced good crops of larvae. When the latter were fully grown, the salivary glands of one larva from the progeny of each female were stained in acetic orcein, and the chromosomes were examined for inversion heterozygosis. In the present article we are concerned only with the findings in *D. tropicalis.*

Four different configurations were observed in the left limb of the second chromosome (II L). Some larvae had no heterozygous inversions; others had a single subbasal inversion (represented schematically in the upper part of Fig. 1); others had a single submedian inversion (middle part of Fig. 1); still others had both inversions (lower part of Fig. 1). The subbasal inversion in our material is identical with that found by Townsend[4] in population samples from Haiti, the Dominican Republic, and Puerto Rico and pictured in his Figure 3. The submedian inversion lies apparently somewhat distally from the inversion shown in Townsend's Figures 1, 2, and 3. The subbasal and submedian inversions in our material are clearly independent, and an uninverted section can be seen between them in double heterozygotes (Fig. 1).

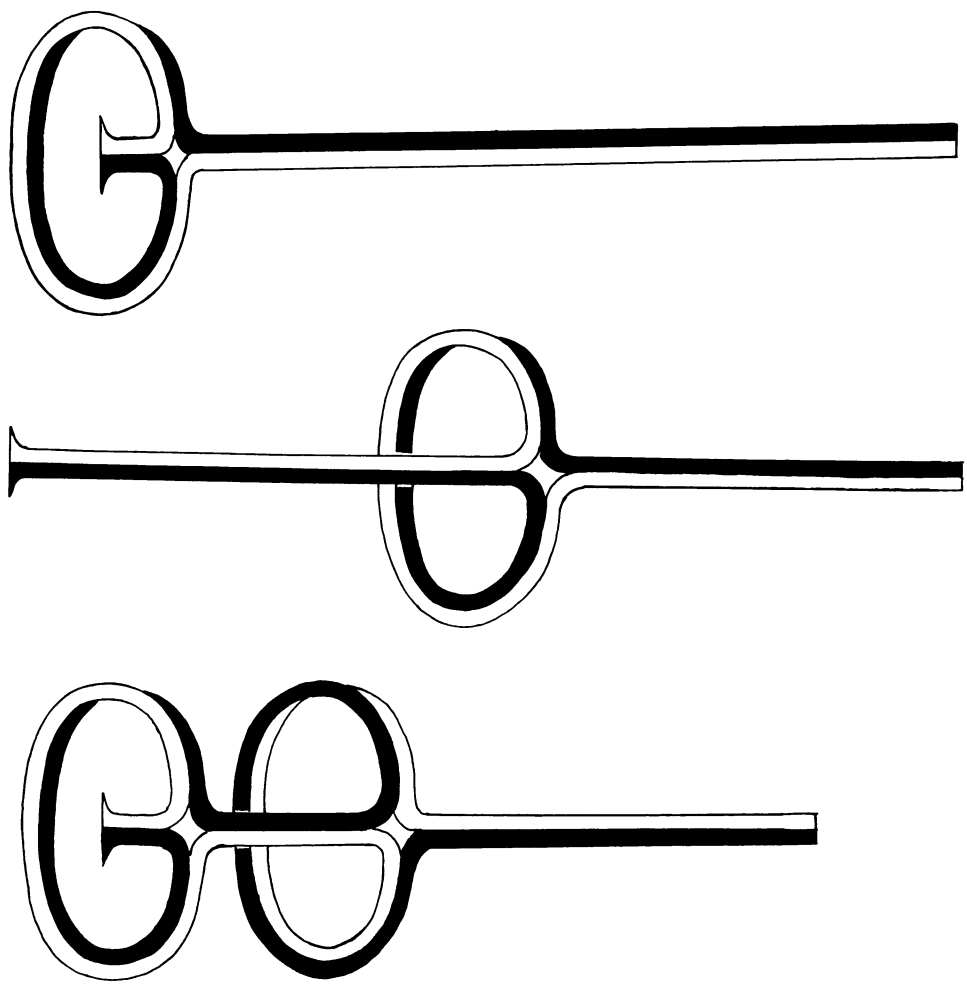

FIG. 1.—Schematic representation of the configurations in the second chromosome in heterozygotes for the subbasal inversion (*above*) for the submedian inversion (*middle*) and for both inversions (*below*).

Now, 74 of the females whose progenies we examined were collected by Mr. Heed in the vicinity of Lancetilla, Honduras; 6 near La Lima, Honduras; and 23 from the neighborhood of San Salvador. The chromosome configurations found by us were as shown in the accompanying tabulation. Among the 74 larvae from the

Locality	Free from Inversions	Subbasal Inversion	Submedian Inversion	Subbasal and Submedian
Lancetilla	18	45	4	7
La Lima	1	3	2	0
El Salvador	9	2	6	6

Lancetilla population, 52 or 70.3 per cent, were heterozygous for the subbasal inversion. This is significantly more than the 50.0 ± 5.8 per cent, which is the highest possible incidence of heterozygotes for a single inversion in a random-breeding Mendelian population in which the inversion homo- and heterozygotes are equally viable. It is most unlikely that the Lancetilla population of *D. tropicalis* has a breeding system which makes the two homozygotes mate preferentially to each other to produce heterozygous progenies. A more probable working hypothesis is that one or both inversion homozygotes are less viable than the heterozygotes and, consequently, that more heterozygotes than homozygotes survive to the late larval stage at which the chromosomes are studied.

This working hypothesis may apply only to the Lancetilla population. The El Salvador sample had only 8 subbasal inversions among 23 larvae examined, and the small La Lima sample had 3 heterozygotes among 6 larvae. According to Townsend,[4] the subbasal inversion is rare or absent in Cuba; and Dobzhansky, Burla, and Da Cunha[5] have not found it in Brazilian populations. Townsend (personal communication) found the subbasal inversion in progenies of 4 out of 10 females from the Dominican Republic. The observed frequency of the submedian inversion is in no population significantly greater than 50 per cent.

Experimental Population.—To test the above working hypothesis, an experimental population of *D. tropicalis* was set up in late May, 1954, in a wooden population cage of a type used in our laboratory for experiments with *D. pseudoobscura* and other species. The foundation stock of the experimental population consisted of the progenies of the Lancetilla females which hatched in the cultures from which the larvae were taken for the cytological examination the results of which are described above. Not all the cultures contributed equal numbers of parents to the foundation stock, but it is certain that this stock contained flies from most of the 74 Lancetilla cultures.

The population was allowed to breed freely for about 4 months in an incubator at 25° C. Samples of the eggs deposited in the cups of the population cage were then taken, and the larvae coming from them were raised under optimal conditions (with a refeeding with yeast) in culture bottles with banana food. Among the 150 larvae examined cytologically, the following situation was found:

Free from Inversions	Subbasal Inversion	Submedian Inversion	Subbasal and Submedian
14	111	0	25

As many as 90.7 per cent (136 out of 150) of the larvae were heterozygous for the subbasal inversion. It is evident that a differential mortality of the homozygotes occurs even under conditions of larval growth approaching optimum. Of course, there must exist two kinds of inversion homozygotes among these larvae, having in duplicate each of the two gene arrangements which give rise to the subbasal inversion configuration. *Drosophila tropicalis* is, unfortunately, not good enough as cytological material to enable us to distinguish between these homozygotes. However, if only one of the homozygotes were lethal and the other were fully viable, we would observe at most 66.7 ± 3.9 per cent of heterozygotes. The actually observed figure is significantly higher.

The question naturally arises whether any homozygotes at all survive to the adult stage, particularly under the conditions of crowding and competition for food obtaining in population cages and, presumably, also in some natural habitats. Instead of taking samples of the eggs deposited in the population cage, we have next withdrawn from the cage some of the cups with the nutrient medium in which masses of pupae about ready to hatch were found. When the adults emerged from these pupae, virgin females and males were taken and outcrossed, in individual cultures, to flies of the Brazilian race of the strain descended from a wild female collected at Palma in the state of Goiaz, and at Içana in the state of Amazonas, by Professor C. Pavan and the senior author. These strains are structurally homozygous. A complication arises because the Honduran flies are subspecifically distinct from the Brazilian flies. As shown by Townsend,[4] the West Indies are inhabited by the subspecies *D. tropicalis cubana,* while in Brazil there occurs *D. tropicalis tropicalis.* The subspecies do not cross as easily as do strains belonging to the same race; the hybrids show a short submedian inversion (not identical with the submedian inversion observed in the Honduran and Salvadorean populations), and the F_1 hybrid males are sterile. The population of Lancetilla belongs to the subspecies *cubana,* and more than half the single-pair matings between the Lancetilla and Palma flies fail to produce offspring. The Lancetilla flies cross freely and produce fertile hybrids with flies from Cuba.

When the Lancetilla × Palma hybrid larvae are obtained, they show in the II L chromosome the submedian interracial inversion described by Townsend[4] and also one of the four following configurations: (A) a subbasal inversion, like Lancetilla flies; (B) a submedian inversion, like Lancetilla flies; (C) both the subbasal and the submedian; and (D) neither the subbasal nor the submedian. At least two larvae were examined cytologically from each of the Lancetilla × Palma cultures. If these two larvae showed different configurations in the II L chromosome, it was concluded that the Lancetilla parent (which developed, of course, in the population cage) was a heterozygote for two different gene arrangements in this chromosome. If the two larvae proved identical, further larvae were examined from the same culture, and usually a second configuration was found (in one instance, in the ninth larva examined). In a few cases (less than ten) the cultures contained so few good larvae that fewer than five of them could be classified as to the gene arrangement; these cases are not included in the count. The successful determinations are as shown in the accompanying tabulation (the two letters showing the gene arrangements in the two II L chromosomes of a pair).

	A/D	A/B	C/D	A/C	A/A	D/D	Total
Females	101	23	20	1	2	3	150
Males	33	5	10	1	1	0	50
Total	134	28	30	2	3	3	200

Only 8 out of the 200 adult flies tested (the A/C, A/A, and D/D classes) were homozygous for the gene arrangement in the basal part of the II L chromosome, and 192, or 96.0 per cent, were heterozygous for the subbasal inversion. This is slightly but barely significantly higher than the frequency found among the larvae raised under optimal conditions (the difference being 5.3 ± 2.7 per cent). It should also be noted that the submedian inversions occur equally frequently in combination with the gene arrangements in the basal part of the chromosome which do and which do not give a subbasal inversion in the hybrids between the strains from Lancetilla and from Palma (the A/B and C/D classes are about equal, namely, 28 and 30 flies out of 200).

Mortality of Eggs and Larvae.—An attempt was made to determine the stage of the life-cycle at which the differential mortality of the homozygotes occurs. Groups of about 50 adult flies from the experimental population were made to oviposit on the nutrient medium on paper spoons. The spoons were inspected daily, and the larvae that hatched were transferred by means of a needle to vials with food and left to develop at 25° C. Care was taken to use only the spoons which had not too many eggs (37–64). Similarly, not too few and not too many larvae were placed in any one vial (where possible, 20 larvae per vial).

Among the 1,135 eggs counted (on 23 spoons), 711, or 62.64 per cent, gave larvae. The egg mortality on different spoons was not uniform (chi square, 112.73), but on no spoon did all the eggs hatch. Among the 686 larvae which were allowed to develop, 623, or 90.8 per cent, gave adults. The larval survival was uniform (chi square, 23.6 for 22 degrees of freedom). In all, slightly more than 50 per cent of the eggs gave adult flies. In the absence of control experiments, it cannot be decided how many homozygotes were surviving among the flies that emerged. Since the egg hatch is rarely complete in normal laboratory cultures of *Drosophila*, some homozygotes were doubtless reaching the adult stage. Although most of the mortality occurs apparently in the egg stage, some larval and a small amount of pupal mortality also occur.

Comparison of Lancetilla with Other Populations.—The Lancetilla population of *D. tropicalis*, or at least its subpopulation in our experimental cage, approaches the status of a balanced lethal system. Close to one-half of the zygotes which these populations produce in each generation die on account of homozygosis for an unfavorable gene complex in the subbasal portion of the II L chromosome. Because of this differential mortality, the populations consist mostly of the heterotic inversion heterozygotes.

This amazing situation is certainly not characteristic of the species as a whole. Indeed, the sample of the Salvadorean population (see above) contained more than 50 per cent of homozygotes for the gene arrangements in the subbasal part of the chromosome. Although it is most likely that in this population the fitness of the structural homozygotes is lower than that of the heterozygotes, at least some of the homozygotes survive. Unfortunately, the progeny of the Salvadorean flies was discarded before its value was realized, and no experimental population of Salvadorean origin could be made. Townsend's data[4] suggest that, at least in Cuba, the heterozygosis for the subbasal inversion is not obligatory. And, finally, the Brazilian population lacks the subbasal inversion entirely and contains few inversions of any kind. Does it follow that the Brazilian population is most prosperous and that that of Lancetilla is on the verge of extinction?

The available evidence indicates that just the opposite is true. In equatorial Brazil *D. tropicalis* is a rare species, far outnumbered by other species of the *willistoni* group (*D. willistoni*, *D. paulistorum*, and *D. equinoxialis*). *Drosophila tropicalis* has been found to be common in only one locality in Brazil,[5, 6] and that locality represents an ecologically highly specialized habitat (patches of forest among the swamps of northeastern Marajo Island). In the West Indies *D. tropicalis* seems to be widespread and sometimes more common than its sibling, *D. willistoni*.[4] Mr. Heed very kindly put at our disposal his records of *Drosophila*-collecting in El Salvador and Honduras. In El Salvador he found flies of the *willistoni* group to be fairly common in a variety of habitats, chiefly on various fruits. At Lancetilla Mr. Heed found the *willistoni* group to be predominant in the rain forest (56 per cent of the total catch of *Drosophila*) and fairly common in man-modified habitats. The *willistoni*-like flies which he sent us were classified as to species usually by examination of the chromosomes in their progeny and by inspection of the male genitalia, but sometimes by just one of these two methods (the examinations of the genitalia were kindly made and recorded for us by Mr. B. Spassky). The relative frequencies (in per cent) of the four sibling species in the samples were as shown in the accompanying table.

Locality	*tropicalis*	*willistoni*	*equinoxialis*	*paulistorum*	Flies Examined
Lancetilla	54.8	11.1	34.1	0	135
La Lima	85.7	0	14.3	0	7
El Salvador	17.9	35.4	44.7	2.0	246

At Lancetilla, *D. tropicalis* appears to be the most successful species of *Drosophila*. In El Salvador it surrenders the dominance to other species. In Brazil the species is rare. This certainly does not suggest that the loss of close to one-half of the zygotes produced has jeopardized the species at Lancetilla. On the contrary, the heterosis which has developed in the inversion heterozygotes has apparently conferred a high adaptedness on the population as a whole and has permitted it to attain ecological dominance in the environments which it has mastered. Balanced polymorphism does raise the fitness of the Mendelian population in which it occurs.

Wright[7] found by mathematical analysis that the spread of translocations which produce disturbances of the disjunction of chromosomes at meiosis in heterozygotes will be opposed by natural selection. This demonstration led other writers to cast doubts on the evidence that translocations play a part in the chromosomal evolution of sexual populations, such as those of *Drosophila*. Wright's analysis was, however, explicitly based on the assumption that the translocation heterozygotes have no intrinsic advantages over the homozygotes. The Lancetilla population of *D. tropicalis* shows that this assumption, though valid in most cases, cannot be simply taken for granted, as Wright has clearly pointed out in his work. In this connection it is useful to keep in mind that the situation found in the Lancetilla population is not quite unique. Berrie and Sansome[8] found that 40 individuals of *D. funebris* from Manchester, England, were heterozygous for a triple inversion in one of the chromosomes, and Buzzati-Traverso[9] has stated that inversion homozygotes are infrequent in populations of *D. subobscura* from several European countries. The permanent heterozygosis in many strains of *Oenothera* is a classical case in the plant kingdom (see Stebbins[10] for review).

Summary.—A population of *D. tropicalis* from Lancetilla, Honduras, consists chiefly of heterozygotes for a certain inversion in the second chromosome. Inversion homozygotes are formed in every generation, but they die off chiefly in the egg stage and partly as larvae and pupae. The population thus sacrifices about one-half of its zygotes in every generation. The fitness of the heterotic heterozygotes is, however, so high that the population is a flourishing one. In certain other populations of the same species the inversions are less common or even altogether absent. These populations appear to be less prosperous than that of Lancetilla.

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*THE EFFECT OF CHRONIC GAMMA RADIATION ON THE PRODUCTION OF SOMATIC MUTATIONS IN CARNATIONS**

BY ALAN RICHTER AND W. RALPH SINGLETON

CORNELL UNIVERSITY, ITHACA, NEW YORK, AND BIOLOGY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY, UPTON, NEW YORK

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Introduction.—Chronic gamma radiation from a Co^{60} source has been found to be effective in producing gametic mutations of endosperm characters in maize. Also, the mutation rate increased more sharply than the increase in dose rate, giving an exponential relationship when mutation rate was plotted against dose rate.[1]

It was desired to test this finding with another organism and also to study mutations in somatic rather than germinal tissue. Carnations were chosen for this study. It was thought that somatic mutations in flower color could be easily