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INTERRACIAL HYBRIDIZATION AND BREAKDOWN OF COADAPTED GENE COMPLEXES IN DROSOPHILA PAULISTORUM AND DROSOPHILA WILLISTONI

BY THEODOSIUS DOBZHANSKY AND OLGA PAVLOVSKY*

DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY, NEW YORK CITY

Certain local populations, or races, of the three sibling species, *Drosophila tropicalis*, *D. paulistorum*, and *D. willistoni* possess an interesting genetic structure.[1,2,3] More than half the individuals in these populations are heterozygotes for the same inverted section in one of their chromosomes, and fewer than half are homozygous. In a panmictic population at equilibrium, such a state of affairs is possible only if greater proportions of the homozygotes than of the heterozygotes are removed by a differential mortality. Natural selection then establishes a situation known as "balanced polymorphism"; the Mendelian population in which it occurs may possess a high fitnees, since the hybrid vigor (heterosis) in the heterozygotes compensates for the low adaptive value of the homozygotes.[4]

In *D. tropicalis*, a population in which 70 per cent of the individuals were heterozygous for a certain inversion, was encountered at Lancetilla, Honduras; elsewhere in Central America and in the West Indies the same chromosomal inversion had frequencies below 50 per cent, while in South American populations it was rare or altogether absent.[1,3] In *D. paulistorum*, the sample from Urubamba, Peru, contained significantly more than 50 per cent of heterozygotes, while in two other localities on the eastern slope of the Andes in Peru the heterozygotes amounted to less than 50 per cent.[3] We have no evidence to show whether in these species the excesses of the heterozygotes are widespread or occur only in some small populations of isolated localities. In *D. willistoni* the situation is a little clearer. In at least three localities in northeastern Brazil the population samples contained more than 50 per cent of heterozygotes for a certain inversion (J, in the third chromosome), and in one of these localities samples taken on two successive years both showed this condition.[3] Elsewhere in South and Central America this chromosomal inversion is heterozygous in 50 or less per cent of the individuals, but another inversion (E, in the right limb of the second chromosome) reaches frequencies higher than 50 per cent of the heterozygotes in the Brazilian state of Ceará.[3]

The experiments reported below were designed to elucidate the nature of the genetic difference between populations in which the incidence of the heterozygotes is above and below 50 per cent. Our working hypothesis has been that this difference is quantitative rather than qualitative. Under balanced polymorphism, the incidence of the homo- and heterozygotes in a population at equilibrium is deter-

mined by their relative adaptive values. Furthermore, the superior fitness of the heterozygotes may be of different origins. If greater proportions of homozygotes than of heterozygotes are eliminated by differential mortality, there will be observed in the population a disturbance of the genotypic ratios demanded by the binomial square rule; on the other hand, the high Darwinian fitness of the heterozygotes may be due to their greater fecundity or a stronger sexual drive, in which case the Hardy-Weinberg ratios will remain unmodified.

Drosophila tropicalis.—As described previously,[1] the population of this species from Lancetilla, Honduras, contained 70.3 ± 5.3 per cent of heterozygotes for a certain chromosomal inversion. This incidence of the heterozygotes was observed among the larvae in the progenies of wild flies. These progenies were used to set up an experimental population in a "population cage." About 4 months later (corresponding, roughly, to six fly generations at the temperature of 25° C., at which all the experimental populations were kept), 90.7 ± 2.2 per cent of the larvae and 96.0 ± 1.4 per cent of the adult flies in the experimental population were inversion heterozygotes. The heterozygotes were evidently heterotic both in the natural and in the experimental environments, and their survival rates were higher than those of the homozygotes. Unfortunately, no experimental populations were made with flies from localities in which the incidence of the heterozygotes in the natural populations was below 50 per cent.

Drosophila paulistorum.—In the progenies of 29 wild females collected in the Urubamba Valley, Peru, 79.3 ± 7.6 per cent of the larvae were heterozygous for a certain inversion. In the progenies of 78 females from Tingo Maria, Peru, only 33.3 ± 5.3 per cent were heterozygous for the same inversion.[3] The distance between the two localities is roughly 400 km.

On February 6, 1956, a population cage, No. 165, was started with a foundation stock of about 4,045 flies, derived about equally from the 29 strains of Urubamba origin. On February 16 of the same year, experimental population No. 167 was started with about 4,220 flies derived from 66 strains of Tingo Maria origin. In late January, 1957, samples of the eggs deposited by the flies in the population cages were taken on several successive days, and larvae which hatched from these eggs were allowed to develop under optimal conditions. Examination of the chromosomes in the salivary glands of these larvae disclosed the following situation:

LARVAE	URUBAMBA, No. 165		TINGO MARIA, No. 167	
	Heterozygotes	Homozygotes	Heterozygotes	Homozygotes
Female	55	51	28	75
Male	52	42	28	69
Total	107	93	56	144

In the experimental population of Urubamba origin, 53.5 ± 3.5 per cent of the larvae were heterozygous for the inversion. This is significantly lower than the corresponding figure for the natural population (70.3 ± 7.6), but higher than that for the experimental population of Tingo Maria origin, 28.0 ± 3.2 per cent. The natural and the experimental populations from Tingo Maria appear to be alike in the incidence of the heterozygotes. The cause of the drop in the frequency of heterozygotes in the Urubamba experimental population is unclear. It is possible that one of the two homokaryotypes which must be present in these populations is less handicapped in relation to the other in the experimental than in the natural

environments. *Drosophila paulistorum* is a difficult material for cytological study, and we were unable to determine the incidence of the two kinds of the inversion homozygotes. However that may be, the Urubamba population is clearly different genetically from that from Tingo Maria, and this permits another interesting experiment to be made.

On December 12 and 19, 1956, population cages Nos. 170 and 171 were started with about 1,000 flies in each as the foundation stocks. In No. 170, the females (virgins) were derived from the Tingo Maria cage, No. 167, and males from the Urubamba cage, No. 165. In No. 171, the reciprocal cross was made. The populations of hybrid racial origin were kept for over 10 months, breeding freely; in late October, 1957, egg samples were taken in the population cages, and the chromosomes were examined in the larvae grown from these samples. The following conditions were found:

LARVAE	TINGO MARIA × URUBAMBA No. 170		URUBAMBA × TINGO MARIA No. 171	
	Heterozygotes	Homozygotes	Heterozygotes	Homozygotes
Female	6	102	2	104
Male	4	88	3	91
Total	10	190	5	195

A precipitate drop in the frequency of the inversion heterozygotes has evidently occurred in the experimental populations of hybrid origin. The populations have become virtually homozygous for one of the two gene arrangements which were present in both Urubamba and Tingo Maria populations. Hardly more than 15 or 16 fly generations have lived in the hybrid populations during the time interval between the start and the taking of the samples. Assuming that the initial frequency of the inversion heterozygotes was some 41 per cent (an average between the Urubamba and Tingo Maria populations, see above), the final frequency of about 4 per cent (averaging populations Nos. 170 and 171) indicates an intense selection in favor of one of the homozygotes.

Drosophila willistoni.—The experiments with this species have yielded results qualitatively similar to those described above for *D. paulistorum*. However, since *D. willistoni* is the more favorable of the two species for cytological study, the analysis of the results can be carried further.

The starting material consisted of 51 strains derived from flies collected at Recife, Pernambuco, Brazil, and 49 strains collected at Guaramiranga, Ceará, Brazil, in April, 1956. These localities are about 600 km. apart. Two inversions are of interest in these populations; inversion J in the third chromosome had the incidence of 60.7 ± 4.4 per cent in the Recife population and 54.9 ± 5.2 in that from Guaramiranga; inversion E in the right limb of the second chromosome had the incidence of 16.4 ± 3.3 per cent at Recife and 64.8 ± 5.0 at Guaramiranga.[3] On August 29 and 30, 1956, two experimental populations, Nos. 168 and 169, were started; the foundation stock in No. 168 consisted of about 3,250 flies from the 51 Recife strains, and in No. 169 of about 2,990 flies from the 49 Guaramiranga strains.

Some $4^1/_2$ months later, between January 14 and January 24, 1957, egg samples were taken in the populations, and the chromosomes were examined in the salivary glands of the larvae. The results were as follows (the sexes of the larvae not recorded):

	RECIFE, No. 168		GUARAMIRANGA, No. 169	
	Heterozygotes	Homozygotes	Heterozygotes	Homozygotes
Inversion III—J	123	77	85	115
Inversion II R—E	60	140	107	93

It can be seen that the frequency of heterozygotes for inversion J is the same in the natural and in the experimental population of Recife origin but that it has declined slightly in the experimental population of Guaramiranga origin; the frequency of heterozygotes for inversion E has risen in the experimental population of Recife origin, and perhaps declined in that of Guaramiranga origin. The examination of the experimental populations was repeated in late October to early November, 1957, when these populations were some 14 months old from the start. An attempt was made to distinguish not only the inversion heterozygotes from the homozygotes but also the two kinds of the homozygotes. For this purpose, adult flies which hatched in the food containers in the population cages were outcrossed, in individual cultures, to flies from a strain known to be homozygous for the "standard" gene arrangement in both the second and the third chromosomes. From each outcross, nine larvae were taken, and their salivary glands were stained with acetic orceine. Examination of the preparations disclosed whether the flies tested were hetero- or homozygous for the gene arrangements in their second and third chromosomes and, if they were homozygous, whether they carried the "standard" gene arrangement (denoted by a capital letter) or that modified by an inversion (denoted by a small letter). In the Recife population (No. 168), only the third chromosomes were recorded, and the following frequencies of the heterozygotes (Jj) and of the two kinds of homozygotes (JJ and jj) were found:

Recife, No. 168	Jj	JJ	jj
Adult females	64	28	8
Adult males	55	33	12
Total	119	61	20

The frequency of the Jj heterozygotes among the adult flies (59.5 per cent) is obviously close to that among the larvae in the same experimental population (61.5 per cent) and that in the natural population (60.7 per cent, see above). The data show, however, that one of the homozygous classes (JJ) is about three times as common as the other (jj). The observed frequencies of the three karyotypes differ appreciably from those demanded by the binomial square rule, as follows:

Recife, No. 168	Jj	JJ	jj
Observed	119	61	20
Expected	95.8	72.6	31.6
Difference	+23.2	−11.6	−11.6

Some differential mortality favoring the heterozygotes over the homozygotes evidently takes place in the Recife population. In the experimental population of Guaramiranga origin, both the second and the third chromosomes were recorded, with the following results:

GUARAMIRANGA No. 169	SECOND CHROMOSOME			THIRD CHROMOSOME		
	Ee	EE	ee	Jj	JJ	jj
Adult females	60	28	12	51	39	10
Adult males	59	21	20	56	34	10
Total	119	49	32	107	73	20

The frequencies of the heterozygotes Ee and Jj among the adult flies from the experimental population of Guaramiranga origin are 59.5 and 53.5 per cent, respectively, which compares with the values 64.8 and 54.9 per cent found in the natural population of that locality (see above). This is a good agreement. However, as in the Recife population, we find the two classes of the homozygotes very

unequal in frequencies, and the proportions of the classes deviate from those expected if the Hardy-Weinberg equilibrium were realized.

GUARAMIRANGA No. 169	—————Second Chromosome—————			—————Third Chromosome—————		
	Ee	EE	ee	Jj	JJ	jj
Observed	119	49	32	107	73	20
Expected	99.3	58.9	41.9	121.0	80.0	27.0
Difference	+19.7	−9.9	−9.9	+14.0	−7.0	−7.0

In the Guaramiranga population, as in that from Recife, there evidently is some differential mortality which favors the heterozygotes and discriminates against the homozygotes. However, hybridization of these populations shows that they differ in genetic structure. Two experimental populations, Nos. 178 and 179, were started on February 20, 1957, each with a foundation stock of about 1,000 flies, among which one sex was derived from Guaramiranga and the other from Recife. In No. 178 the cross was Guaramiranga (No. 169) ♀ ♀ × Recife (No. 168) ♂ ♂, and in No. 179 the reciprocal. About 10 months later, in December, 1957, egg samples were taken, and the proportions of the hetero- and homozygotes were determined in the larvae which grew from them. The results were as follows:

POPULATION No.	—————Second Chromosomes—————		—————Third Chromosomes—————	
	Heterozygotes	Homozygotes	Heterozygotes	Homozygotes
178, larvae	55	145	90	110
179, larvae	68	132	76	124
Total	123	277	166	234
Per cent	30.75	69.25	41.5	58.5

The frequency of heterozygotes for the inversion J in the third chromosome is significantly lower in the hybrid populations than in the parental Recife population and equal to or lower than that in the Guaramiranga population. The inversion E heterozygotes are less frequent in the hybrid populations than at Guaramiranga, and about as frequent as in the experimental population of Recife origin. A further check on this decline in the frequencies of the heterozygotes in the hybrid populations is provided by an analysis of the zygotic constitution of the flies in the hybrid populations, made in March, 1958. The determinations were made by outcrossing the adult flies from the population cages to a standard strain in individual cultures and examining the chromosomes in up to 9 larvae in each progeny. The following results were obtained:

POPULATION No.	—————Second Chromosome———			———Third Chromosome———		
	Ee	EE	ee	Jj	JJ	jj
178, adult ♀ ♀	15	20	5	17	15	8
178, adult ♂ ♂	12	25	3	11	28	1
179, adult ♀ ♀	15	22	3	11	26	3
179, adult ♂ ♂	12	26	2	13	26	1

In both populations combined, the frequency of adults heterozygous for the second chromosome inversion, Ee, is now 33.8 per cent, which agrees well with the figure 30.75 per cent found some months earlier among the larvae from the same populations. The corresponding figure for the third chromosome inversion heterozygotes are 32.5 and 41.5 per cent, i.e., the heterozygotes seem to be even less frequent among the adults than among the larvae (since the larval and the adult samples were not taken simultaneously, this frequency difference cannot be considered established).

A comparison of the observed frequencies of the homo- and heterozygotes in

populations Nos. 178 and 179 with those expected according to the Hardy-Weinberg rule shows the following:

POPULATION Nos. 178 AND 179	Second Chromosome			Third Chromosome		
	Ee	EE	ee	Jj	JJ	jj
Observed	54	93	13	52	95	13
Expected	60.0	90.0	10.0	59.0	91.5	9.5
Difference	−6.0	+3.0	+3.0	−7.0	+3.5	+3.5

The chi-squares are 1.17 for the second chromosome and 2.25 for the third; the observed values agree well with the expected ones. This is in contrast with the situation in populations of geographically pure Recife and Guaramiranga origin (Nos. 168 and 169, see above), in which significant excesses of heterozygotes were found. Wallace[5] has recently emphasized that the adaptive values of genotypes cannot be safely inferred from data on relative frequencies of homo- and heterozygotes in populations not at equilibrium. This stricture does not apply to our data, since we examined the experimental populations when they were more than a year old, at which time such populations usually approach an equilibrium status fairly closely. The differential mortality which favored the heterozygotes in populations of geographically pure origin is not observed in those of geographically mixed origin. It does not necessarily follow that in the latter the heterozygotes have no adaptive advantage, but, if they have such an advantage, it resides in traits other than differential survival in preadult stages. These populations are being continued, and we hope to report on their further behavior in due time.

Discussion.—Classical genetics liked to think of the genotypes of populations, races, and species as aggregates of genes with more or less autonomous effects, each gene selected on its own merits as adaptive in a given environment. The fact that adaptively coherent genotypes are, at least in higher organisms, integrated systems of genes is slowly gaining recognition. Studies on natural populations of *Drosophila* have supplied some of the clearest evidence. Many populations are polymorphic for variant gene arrangements which differ in inversions of blocks of genes in some of their chromosomes. The polymorphism is usually balanced, owing to the inversion heterozygotes being heterotic over most of the range of the environments which the population normally encounters. It has, however, been shown[4] that the fitness of an inversion heterozygote or a homozygote depends upon the geographic origin of the chromosomes which it carries. As a rule, heterozygotes whose chromosomes are derived from the same population are highly fit; heterozygotes which carry chromosomes of different geographic origins may or may not be. The rapid decline in the frequencies of the heterozygotes in the hybrid populations of *D. paulistorum* described above is a striking demonstration of this fact.

The heterosis is not a necessary consequence of being heterozygous for chromosomes with a given pair of gene arrangements; it is determined by the gene complexes which these chromosomes contain. Natural selection acts to adjust mutually, or coadapt, the gene contents of the chromosomes found in a given Mendelian population to yield high fitness in heterozygotes. The adaptive importance of the inversions, which enable us to distinguish under the microscope the chromosomes with the different gene complexes, is to guard the integrity of these complexes by means of suppression of the crossing over between the chromosomes which carry them. There is no selection pressure toward coadaptation of the

chromosomes found in different geographically separate and non-interbreeding populations. The evidence reported in the present article indicates that the chromosomal inversions present in the original populations produced heterosis in the natural as well as in the experimental environments. The behavior of the experimental populations of hybrid origins shows, however, that cytologically similar chromosomes were not identical in the gene contents in different populations.

Wallace[5] set forth an ingenious hypothesis, that variant gene arrangements maintained by natural selection in the same population are likely to be such that the integrity of the coadapted gene complexes will be protected from disintegration by crossing over. He pointed out the rarity in natural populations of so-called triads of versions, in which blocks of genes may be exchanged by crossing over in heterozygotes which carry certain combinations of the chromosomes. The populations of *Chironomus* studied by Acton[6] are in accord with Wallace's rule; rules of this kind are, however, not expected to hold except in a statistical sense; and Levitan and Carson[7] stressed the existence in *D. robusta* of situations not in accord with it. Although our experiments were not concerned with triads of inversions, their results have a bearing on Wallace's basic assumption. In the experimental populations of geographically uniform origins, heterosis is maintained, since the gene complexes coadapted in the natural population by natural selection continue to be protected by the inversion heterozygosis from breaking up. However, in the experimental populations of geographically mixed origins, there are two kinds of chromosomes with identical gene arrangements but containing different constellations of genes. Crossing over between such chromosomes yields new gene combinations, most or all of which never existed in nature because the geographic isolation of the parental populations prevented their hybridization. The evidence shows unambiguously that the recombination weakens or even destroys the heterosis conditioned in the geographically uniform populations by the coadapted gene complexes.

The agreement between our results and those of Vetukhiv[8] should be noted. This investigator found that hybridization of populations of *D. pseudoobscura* from different geographic regions results in a transitory heterosis in the F_1 and in a more or less pronounced breakdown of fitness in the F_2 generation. Some species of *Drosophila* are evidently differentiated geographically into local populations or races, which, although they are quite similar in the externally visible morphology, are distinct enough genetically that gene exchange between them leads to a loss of fitness. This conclusion evidently cannot be generalized to apply to races in other organisms, and particularly not to human races; it cannot legitimately be used to give aid or comfort to racism or to racists.

Summary.—The incidence of individuals heterozygous for certain chromosomal inversions exceeds 50 per cent in some local populations or races of *D. tropicalis, D. paulistorum*, and *D. willistoni*. In other populations of the same species the frequencies of the same inversion heterozygotes are below 50 per cent. Experimental populations made with flies of a given geographic origin generally retain the characteristics of the local race from which the founders of the population were derived. The chromosomal polymorphism is maintained in the populations because the inversion heterozygotes are superior in fitness to the homozygotes.

Experimental populations were set up, the founders of which were hybrids be-

tween races having more and fewer than 50 per cent of heterozygotes for certain inversions.   In these experimental populations of mixed geographic origins the frequencies of the heterozygotes fell to or below the frequency levels in the parental races having fewest inversions.   The superior fitness of the inversion heterozygotes depends upon coadaptation of the gene complexes which the chromosomes carry. In hybrid populations these gene complexes are broken up by crossing over, with a consequent loss of the heterosis.

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