FACTORS INFLUENCING FITNESS IN CHROMOSOMAL STRAINS IN *DROSOPHILA SUBOBSCURA*

MARVIN WASSERMAN

*Biology Department, Queens College of the City University of New York,
Flushing, New York 11367*

Manuscript received March 17, 1972
Revised copy received August 10, 1972

ABSTRACT

Chromosomal polymorphism can be maintained by means of genic dominance, genic overdominance, recombination effects and karyotype selection. Experiments were carried out which allow us to distinguish among these factors as they effect egg hatch and larva-to-adult survival in *D. subobscura*. A strong karotype selection was observed. A small but highly significant recombination effect was also observed despite the fact that the material used in the experiment consisted of inbred, related strains and was therefore less likely to show such an effect than strains randomly chosen from a population. On the other hand, these strains, being inbred, were more likely to show genic dominance or genic overdominance than randomly picked samples. However neither genic dominance nor genic overdominance was detected.

GENETIC polymorphism has been attributed to mutational pressure, neutral traits, transient changes and balanced polymorphism (FORD 1940). In *Drosophila* the evidence is overwhelming that chromosomal—particularly inversion—polymorphism is not maintained in natural populations by mutational pressure, nor are inversions neutral characters. A certain amount of chromosomal variability may be transient wherein one gene sequence is in the process of being replaced by another, better fit, one. Indeed the history of practically every *Drosophila* species group studied shows that there have been changes in the gene orders, and fixation of new unique gene sequences specific to species are common occurrences (WASSERMAN 1963; STALKER 1965; KASTRITSIS 1966; CARSON *et al.* 1970; WARD and HEED 1970). However, it is generally accepted that most of the inversion polymorphism is of the balanced type. Balanced polymorphism can be preserved if the species occupies a heterogeneous environment (LEVENE 1953; LEVINS and MACARTHUR 1966; MAYNARD SMITH 1966); if there is frequency-dependent selection (SOKAL and KARTEN 1964; LEWONTIN and MATSUO 1963; HUANG, SINGH and KOJIMA 1971); or if the heterozygotes are heterotic. It is of considerable interest to determine which factor—or combination of factors—is operating.

With chromosomal variability, we should also be interested in the level of organization of the genetic material under consideration, since selection may be operating at the genic level, at the chromosome level, or at the supergene level.

With genic selection, the gene orders (e.g., Arrowhead, Standard, Santa Cruz in *Drosophila pseudoobscura*) have no selective values, *per se*, but rather act as neutral vehicles of the individual alleles which happen to be present in these gene sequences: the selective value of the gene orders is determined by some function of the selective values of the individual alleles present in that gene order at that particular time. For example, chromosomal polymorphism might be maintained in a population if there is genic heterosis of either the overdominance or the dominance type. If one of the alleles of a heterotic locus were to be limited to one gene order while the other allele was limited to the other gene order, the overdominance of the locus would be conferred upon the heterokaryotype. Genic dominance heterosis might arise if each gene order were to have its own specific recessive deleterious alleles present at high frequencies. This might occur if there were a certain amount of inbreeding. The heterokaryotype would be heterotic because each gene order would carry the dominant normal alleles of the deleterious recessive alleles present in the other gene order. Recent references dealing with models are to be found in FRANKLIN and LEWONTIN (1970).

With chromosomal selection all chromosomes with a given gene order (e.g., the family of chromosomes with a gene order Arrowhead) have similar alleles, and these alleles differ from those found on chromosomes that carry other gene orders (e.g., the family of chromosomes called Standard). These gene order-specific alleles cause a karyotype selection because they control the adaptation of their carriers to specific parts of the environment. Individuals have fitness values which are definable on the basis of their karyotypes. With karyotype selection, polymorphism can be maintained if the species occupies a heterogeneous environment or if there is frequency dependent selection against the better fit homokaryotype. STRICKBERGER and WILLS (1966) demonstrated that different gene sequences may be adapted to different components of the environment. Moreover a wealth of information, summarized by DOBZHANSKY (1970), concerning geographical distributions, clines, seasonal changes and long-term changes in the frequencies of chromosomal rearrangements is strong circumstantial evidence for karyotype selection. The distinction between genic and karyotype selection which we are making here must be clearly understood. With genic selection, the heterokaryotypes are heterotic because they have captured heterotic loci which help to buffer them against the environment; the homokaryotypes are less fit because they are homozygous for deleterious recessive alleles or are less buffered against environmental variation. With karyotype selection each chromosome is adapted to one or several aspects of the environment. The fitnesses of the karyotypes can be defined under given environmental conditions and would change if and when these environmental conditions are altered. In both genic and karyotypic selection, and also the supergene selection to be described below, one would expect the different alleles of several loci to be essentially limited to one of the gene orders. The discovery of such a phenomenon by PRAKASH and LEWONTIN (1968) therefore does not allow us to distinguish from among the three types of selection.

Supergene selection differs from the above in that it requires that the organi-

zation of the genes along the length of the chromosome be important. There must be some "cooperation" between alleles along the length of the single homologue which is not present when the same alleles are distributed at random between the two homologues. In this way our model differs from that of FRANKLIN and LEWONTIN (1970) which does not require coadaptation of linked alleles, but does require heterosis. In our model, each family of gene orders would be represented by a large number of different supergenes in each population. Recombination between different supergenes of the same family of chromosomes would destroy the good supergenes and would thereby produce a frequency-dependent selection against the more common homokaryotype. This, in turn, may result in heterokaryotype heterosis where none existed before (WASSERMAN 1968). This is an important segment of the study presented here and it is therefore necessary to expand upon this recombination-induced-heterosis.

In brief: during the meiosis of heterokaryotypes, i.e., individuals heterozygous for such chromosomal mutations as inversions and centric shifts, the frequency of recombination within the heterozygous segment of the chromosomes is greatly reduced. As a result of this, the offspring of these heterokaryotypes inherit unrecombined pieces of chromosomes as single units. These blocks of genes, or supergenes, can preserve their integrity from generation to generation as long as they are carried along in heterokaryotypic individuals. The number of blocks of genes between which little or no recombination is possible is equal to the number of different gene orders present in the population. However, each gene order undoubtedly has a large number of representatives differing in the specific alleles present at the various loci within the supergene. It follows that selection acting upon favorable mutations and recombinations would tend to develop internally balanced cooperating alleles within the blocks. It also follows that an individual which is structurally homozygous for an inversion is probably not homozygous for the specific alleles contained within that gene block. Thus, recombination can occur between these alleles during gametogenesis of the homokaryotypic individual. This is an important, though a rarely emphasized fact.

Individuals which survive and reproduce usually carry better blocks of genes than those which do not. Although both homokaryotypes and heterokaryotypes carry two good pre-selected supergenes, they differ in the type of chromosome which they can transmit to their offspring. The offspring of a heterokaryotype will inherit one of the pre-selected supergenes unchanged except for mutations, while the offspring of a homokaryotype will inherit recombined supergenes. If one assumes that recombination between two well-adapted homologous gene blocks destroys the internal balance which produces the superior fitness, then the carriers of such recombinant gene blocks, on the average, will be less fit than their parents. Therefore if crossing over occurs during meiosis the offspring of homokaryotypes should be less fit than those of heterokaryotypes because of this recombination effect. Computer-run simulated experiments tested this recombination effect in various models differing in their defined karyotypic fitnesses (WASSERMAN 1968). It was found that not only is polymorphism maintained in those populations where the structural heterozygotes were initially defined as

being heterotic, but also at certain levels of selection and recombination, polymorphism is maintained even if the fitness value of one of the homokaryotypes is initially defined as being the best. This occurs because the recombination effect acts antagonistically against the more frequent (better) homokaryotype, and it can induce an overall heterosis where one previously did not exist. Only heterotic populations remained polymorphic; all others became monomorphic.

Unfortunately there is a lack of experimental data which would allow us to clearly recognize genic selection, chromosomal selection and selection at the supergene level. In this paper are described the results of some experiments which were performed in order to measure the relative importance of these factors. Our approach was to examine the relative fitnesses of Inbred lines, of genetically heterozygous homokaryotypes and of heterokaryotypes in order to determine whether there was heterosis or some other type of selection operating. Secondly, the data were analyzed so that it would be possible to determine whether selection was operating on the genic level (e.g., genic heterosis irrespective of the karyotype of the individual) or the chromosome level (e.g., the individuals had a fitness defined by their karyotypes). And finally, tests were run to determine the effect of recombination on the viability of the offspring of genetically heterozygous homokaryotypes since this is an obvious way one can determine whether there is a recombination effect: the recombination effect being indicative of chromosomal organization into supergenes.

In these experiments, *Drosophila subobscura* was used. This species is polymorphic for more inversions than any other *Drosophila* species (JUNGEN 1968). Perhaps the most striking feature of the European distribution of the *subobscura* inversions is the presence of north-south clines. According to Götz (1967), the character of the polymorphism varies from chromosome to chromosome. In the U chromosome, for example, two gene orders tend to dominate in any particular locality. In this study, strains homozygous for U standard or U_{1+2} were used. Inversions U_1 and U_2 overlap each other so that in the U-standard/ U_{1+2} heterokaryotype approximately $\frac{2}{3}$ of the U chromosome, or about 13% of the total euchromatin, is tied up within the double inversion loop (KUNZE-MÜHL and MÜLLER 1958). U-standard and U_{1+2} are the dominant gene orders for this chromosome in the Zurich area where they were collected. In the U chromosome, U-standard is a northern arrangement while U_{1+2} is more southerly centered. BURLA and GÖTZ (1965) have shown that for three different years there was a seasonal change in the frequency of the U chromosome gene orders in Switzerland. Each specific locality and year differed in detail. However, the overall picture was that each May U-standard had a frequency of about 75% but by September this dropped to about 50%, while U_{1+2} , the other dominant gene order, increased in frequency.

The components of fitness examined in this experiment were egg hatch (a measurement of both fertility and embryonic viability) and larva-to-adult survival; the former at 18°C, and the latter at two temperatures (18°C, and 23°C), and at two food concentrations for each temperature (0.6 mg of yeast/larva and 0.3 mg of yeast/larva).

TABLE 1

Crosses to produce the inbred lines, homokaryotypic F_1 and heterokaryotypic F_1

Female parent	Male parent	Inbred lines	Male parent	Homokaryotypic F_1	Male parent	Heterokaryotypic F_1
S_1	S_1	S-IN ₁₁	S_2	S-HO ₁₂	I_4	HE ₁₄
S_2	S_2	S-IN ₂₂	S_3	S-HO ₂₃	I_5	HE ₂₅
S_3	S_3	S-IN ₃₃	S_1	S-HO ₃₁	I_6	HE ₃₆
I_4	I_4	I-IN ₄₄	I_5	I-HO ₄₅	S_2	HE ₄₂
I_5	I_5	I-IN ₅₅	I_6	I-HO ₅₆	S_3	HE ₅₃
I_6	I_6	I-IN ₆₆	I_4	I-HO ₆₄	S_1	HE ₆₁

MATERIALS AND METHODS

The six *D. subobscura* strains used in this study were collected in the Zürich area and were most kindly provided to the author by Professor H. BURLA. Three strains—1, 26 and 29 (designated S_1 , S_2 , and S_3 respectively) are homozygous for the U-standard sequence; whereas the other three—2, 3 and 31 (I_4 , I_5 , and I_6 respectively) are homozygous for U_{1+2} . S_1 is derived from a single female collected at Küsnacht, near Zürich. Strains S_2 and S_3 were both obtained from the offspring of females from S_1 crossed to single U-standard males collected in Effretikon, near Zürich. Strains I_4 and I_5 were both derived from single females collected in Zürichberg. Strain I_6 resulted from a cross between females of strain I_4 and a U_{1+2} Effretikon male. All strains had been maintained in the laboratory in half-pint bottles at 18°C; S_1 , collected in 1949, for 19 years prior to the start of the experiment; I_4 and I_5 for 4 years; and S_2 , S_3 and I_6 for less than 1 year.

Measurements were made on the components of fitness of (a) the Inbred lines, i.e., the strains themselves, both U-Standard (S-IN) and Inversion strains (I-IN); (b) the homokaryotypic F_1 s produced by crosses between strains of like chromosomes, both U-Standard (S-HO) and U-inversion (I-HO) F_1 ; and (c) the heterokaryotypic F_1 hybrids produced by crosses between different chromosomal types (HE).

The mating schedule is shown in Table 1. Each strain contributes equally in the production of Inbreds, homokaryotypic F_1 and heterokaryotypic F_1 , both as female and male parents.

The recombination effect was measured by comparing the results obtained from reciprocal crosses between homokaryotypic and heterokaryotypic F_1 s (Double- F_1 crosses).

e.g., S-HO₁₂ females × HE₃₆ males → Recombinants
 HE₃₆ females × S-HO₁₂ males → Non-recombinants
 e.g., I-HO₄₅ females × HE₆₁ males → Recombinants
 HE₆₁ females × I-HO₄₅ males → Non-recombinants

Since crossing over does not occur in *Drosophila* males, and is greatly reduced in the structurally heterozygous region of the heterokaryotype, the offspring of a cross between an HE female and an HO male will receive unrecombined, unchanged chromosomes. The offspring of a cross between an HO ♀ and an HE ♂ will, however, receive a recombinant chromosome from their mother. Thus, in each set of Double- F_1 reciprocal crosses, the offspring differ consistently only in the presence or absence of recombinant chromosomes; it is here that we will look for a recombination effect. The offspring also differ as to their sex chromosomes, but not in any consistent systematic way. Moreover the crosses have been made in such a manner that any effect from the sex chromosomes would be averaged out among the recombinant and non-recombinant offspring.

The actual regimen used was as follows: Each strain, combining substrains where available, was placed in its own population cage and maintained there at 18°C for 2½ months (approximately 2 generations). Adults were removed from the cages and allowed to oviposit in egg collecting chambers. For each strain 22–24 half-pint bottles were inoculated with 300 eggs. The

flies emerging from these eggs, with a history of 3 generations of crowded conditions, and therefore fairly strong selection, were used as the parental generation of the experiment.

Virgins were collected, aged 1–7 days, mated in mass for 4 days, and then allowed to oviposit on freshly made media consisting of 2% agar with a heavy suspension of yeast sprayed on the surface. Each day, for up to 4 days, the food containing the egg sample was replaced with fresh media. Larvae were collected from each sample approximately 48 hr after the adults were first exposed to the food. Although each egg sample consisted of eggs laid over a 24 hr period, it was found that most of the larvae hatched within a relatively short period and it was rather easy to pick newly hatched larvae of about the same size. These were deposited in vials containing 8 ml of an agar, buffer and mold-inhibiting medium (Mr. ALEC KEITH, personal communication from Dr. H. BURLA): agar, 20.0 gm; citric acid, 7.95 gm; Na_2HPO_4 , 2.06 gm; Tegasept, 6 ml of 10%; Distilled water, 1000 ml. To this had been previously added a sterile piece of paper, to supply a suitable place for pupation, and a weighed amount of live, dried yeast, either 0.3 mg or 0.6 mg per larva. The yeast Engedura, purchased from the Royal Netherlands Fermentation Industries, Ltd., in Delft, Holland, supplied the food for the larvae, but could not grow since there were no nutrients in the media. The emerging virgins were scored daily and, if needed, mated to produce a subsequent generation for the recombination test.

Four or five days after oviposition, the eggs were examined to determine the egg hatch frequency. For statistical purposes, angular transformations were used on the egg hatch percentages.

RESULTS

The crosses to yield the Inbred lines and the F_1 data were run simultaneously. Therefore although, in the analysis, the Inbreds and the F_1 s are treated as separate generations, the environmental conditions such as food, temperature, light, etc. were essentially identical for these crosses and these results are directly comparable. The data are given in Table 2. Under specific environmental conditions, one can measure relative fitness values for different genotypes if they compete against each other in the same "population cage," or if each competes separately against a particular control genotype. When comparing different "populations" each consisting of genetically pure homogeneous populations and where there is no common control genotype, the problem of comparing relative fitnesses becomes important. For example, let us compare two cultures, A and B, under identical conditions—number of eggs present, amount of food, etc. If the egg hatch of A is higher than that of B, the amount of food available per larva will be different and further comparisons between these two cultures are invalid and could result in misleading conclusions. We see then, that under these circumstances egg-to-adult survival is an invalid criterion to use because it consists of two components of fitness, egg hatch and larva-to-adult survival (WASSERMAN 1970). In our experiment the egg hatch and larvae-to-adult survival were, therefore, measured and treated separately.

Inbred and F_1 : Egg hatch: A thousand eggs were examined for each cross of the two sets of Inbreds and their F_1 s. The results are shown in columns 3 and 4 of Table 2. Angular transformations were used on the egg hatch percentages in the statistical tests.

CLARK *et al.* (1960) observed a strong maternal inheritance in the development rate of *D. subobscura* eggs. Because reciprocal crosses were not made, we have no direct measurement for a maternal inheritance. However, an indirect method is

TABLE 2
Inbreds and F₁s: egg hatch and larva-to-adult survival

Class of cross	Offspring	Hatch from 1000 eggs	Egg hatch Class mean	18° survival			Class mean	Number of replicas	23° survival			Class mean
				Mean	Variance	Number of replicas			Mean	Variance	Number of replicas	
S-IN	S ₁ S ₁	878		35.8	652.2	5			53.6	387.3	5	
	S ₂ S ₂	939	923	18.2	261.2	5	29.2		42.2	248.2	5	43.7
	S ₃ S ₃	959		33.6	693.8	5			35.2	483.2	5	
I-IN	I ₁ I ₁	932		46.4	928.3	5			63.8	40.7	5	
	I ₅ I ₅	901	917	44.0	119.0	3	50.3		50.5	72.3	4	55.8
	I ₆ I ₆	919		60.6	34.3	5			53.0	750.5	5	
S-HO	S ₁ S ₂	906		31.0	337.8	10			37.6	129.4	10	
	S ₂ S ₃	964	937	33.3	161.6	10	34.4		43.4	227.2	10	38.1
	S ₃ S ₁	941		38.8	822.6	10			33.2	263.7	10	
I-HO	I ₁ I ₅	886		49.4	145.6	7			48.6	130.3	7	
	I ₅ I ₆	881	897	37.7	224.0	10	45.0		47.0	302.7	10	46.7
	I ₆ I ₄	923		47.8	204.6	10			44.5	185.6	10	
HE	S ₁ I ₁	914		52.2	179.5	10			47.5	206.5	10	
	I ₄ S ₂	881		43.2	186.2	10			45.0	161.8	10	
	S ₂ I ₅	865	882	36.2	193.5	10	44.4		44.9	120.1	10	47.8
	I ₅ S ₃	847		55.2	140.2	10			53.9	128.8	10	
	S ₃ I ₆	871		37.5	216.3	10			46.8	206.2	10	
	I ₆ S ₁	917		42.3	161.8	10			48.6	182.3	10	

FIGURE 1.—The general method used for calculating one of the twelve values of $(|F_1 - \text{female Inbred line}| - |F_1 - \text{male Inbred line}|)$ is shown. In actual tests angular transformations were used on the egg hatch percentages.

possible. In maternal inheritance the phenotypes of the offspring are determined by their female parents. Therefore, if there were a maternal inheritance the egg hatch of the F_1 hybrids should, on the average, be closer to the egg hatch of the strain from which their maternal parents came rather than that of the strain from which their paternal parents came. Figure 1 shows the method used for determining maternal inheritance. For each cross the absolute differences in egg hatch between the hybrids and their maternal Inbred lines $(|F_1 - \text{♀ IN}|)$ should be less than those between the hybrids and their paternal Inbred lines $(|F_1 - \text{♂ IN}|)$. The F_1 offspring did resemble their female parent line more closely than their male parent line, but this was not significant; the mean of the $(|F_1 - \text{♀ IN}| - |F_1 - \text{♂ IN}|) = 0$ ($t = 0.33$; 11 degrees of freedom; $.7 < P < .8$). There was, then no evidence for a maternal inheritance in the egg hatch data. Moreover, our mating schedule calls for each strain to be used once each as both the female and male parent for each of the three different types of crosses, and therefore a maternal inheritance, if present, would be averaged out in the comparisons and not be important.

By combining the five genotypic classes in various ways it is possible to examine the data for evidence of genic effects and chromosomal effects. If genic heterosis were important, increasing the amount of heterozygosity should lead to a higher egg hatch. The Inbred lines, although not isogenic, are the most homozygous; whereas the heterokaryotypic F_1 is the most heterozygous. If there are heterotic loci which are instrumental in preserving chromosomal polymorphism one would expect the heterokaryotypes to be clearly superior to both the Inbred lines and the homokaryotypes. Inbred- F_1 tests were made by direct comparisons between each F_1 and its midparent, the mean of the Inbred lines from which the F_1 parents came ($\bar{\text{IN}}$). The mean differences were found to be small and not significant (Table 3, lines 1 and 2). Mean differences between HO and the HE also proved to be small and not significant (Table 3, line 3). We see, then, that there is no evidence for genic heterosis in the egg hatch data.

With chromosomal selection, the gene orders, *per se*, might differ from each other in viability. The remainder of Table 3 (lines 4–6) gives the results of the

TABLE 3

Comparison among Inbred and F₁s: egg hatch

X	Comparison	Y	Percent X-Y	Degrees of freedom	t
			X		
$\overline{IN}$		HO	0.3	5	0.28
$\overline{IN}$		HE	4.1	5	2.21
HO		HE	2.5	10	1.63
S/S		S/I	5.2	10	3.27**
S/S		I/I	2.5	10	1.99
I/I		S/I	2.6	10	1.77

** $P < .01$

tests between the various karyotypes. The egg hatch of the heterokaryotypes, 88.25% is highly significantly lower than the 93.0% hatch of the U-standard homokaryotypes (S-IN and S-HO combined). The inversion homokaryotypes (I-IN and I-HO) are intermediate.

Control and F₁: Larva-to-adult survival: Larva-to-adult survival was tested at two temperatures, 18° and 23°, and at two food levels at each temperature. Each vial was inoculated with 100 larvae plus either 30 or 60 mg of live yeast. At 0.3 mg/larva, only 0.9% of the larvae developed into adults at 18° and 1.4% at 23°. At 0.6 mg/larva, 40.5% developed into adults at 18° and 46.6% at 23°. The lower concentration of food was obviously insufficient for our purposes and we will henceforth only consider the data obtained from the 60 mg vials.

Columns 5-12 of Table 2 list the results, giving the number of replica vials, means and variances of the crosses, as well as class means. As was done for the egg hatch data, tests for maternal inheritance were performed. The tests failed to show evidence for a maternal inheritance: (1) at 18°, $t = 0.34$; 11 degrees of freedom; $.7 < P < .8$; whereas (2) at 23°, $t = 0.58$; 11 degrees of freedom; $.5 < P < .6$.

Differences among the variances within the classes, in some cases, appear to be large (Table 2). However F-max tests for homogeneity of variances (SOKAL and ROHLF 1969) showed that only the variances found within the I-IN class were heterogeneous ($P < .05$ at both temperatures). Because there was this heterogeneity, all other comparisons on the larva-to-adult data were made with the Mann-Whitney U test, a nonparametric test (SOKAL and ROHLF 1969). A normal deviate is calculated by means of the following formula:

$$N.D. = (| (N_1 N_2 / 2) - U | - 1/2) / \sqrt{[(1/12) N_1 N_2 (N_1 + N_2 + 1)]},$$

where N.D. = the normal deviate; N_1 = the number of observations in sample 1; N_2 = the number of observations in sample 2; and U = the number of times a score in sample 1 is less than a score in sample 2.

Comparisons were made among the five genotypic classes at each temperature. The results of each of the 12 F₁ crosses were compared with those of its own

FIGURE 2.—Ranks of Inbreds and F_1 s according to larva-to-adult survival. Arrows connect classes which differ significantly from each other, the head of the arrow being the inferior class. * = $p < .05$; ** = $p < .01$.

parental Inbred lines and a normal deviate was obtained. In the S-IN to S-HO and the I-IN to I-HO tests, the normal deviates were combined to form the values used for our final comparisons. In the other tests, this was not possible and, therefore, all of the scores were grouped together and a single, overall normal deviate was calculated. This is a less sensitive method.

Figure 2 gives the ranks of the classes at both temperatures and the results of the statistical tests. The two rankings differ from each other only in minor ways. The reversal in the relative positions of S-IN and S-HO is not critical: the two do not significantly differ from each other at either temperature, and they are probably equal. The same is true for HE and I-HO.

Now let us look for evidence of genic heterosis and chromosomal selection. The lack of importance of genic heterosis is shown in Table 4, lines 1–3. At 18° there were no significant differences found. However, at 23° the HO are inferior to both the IN and the HE, a result which is inexplicable in terms of genic heterosis. Comparisons among the three karyotypes show that at both temperatures the U-standard homokaryotypes are inferior to individuals carrying at least one inversion chromosome (Table 4, lines 4–6).

TABLE 4

Mann-Whitney U tests Inbred and F₁ : larva-to-adult survival

Comparison X Y		18°		23°	
		Percent X-Y X	Normal deviate	Percent X-Y X	Normal deviate
$\overline{IN}$	HO	0.3	0.36	17.3	2.6**
$\overline{IN}$	HE	-11.8	0.48	4.1	1.65
HE	HO	11.8	1.64	12.7	2.33*
I/I	S/I	7.3	1.44	7.2	1.42
I/I	S/S	49.8	4.63***	25.3	4.76***
S/I	S/S	39.6	3.88***	16.9	2.46*

* = $P < .05$; ** = $P < .01$; *** = $P < .001$.

It is obvious, therefore, that no evidence for genic heterosis was found in our experiments. Survival is simply and consistently determined by the karyotype of the individual. Moreover it is seen that under our experimental conditions the inversion chromosomes, U_{1+2} , are dominant and superior to the U-standard chromosomes.

Double-F₁ crosses: The Inbred and F₁ data give us no information on the organization of the alleles along the length of the chromosome. It is the offspring of the F₁ generation which should demonstrate the recombination effect. Crosses between heterokaryotypic F₁ females and homokaryotypic F₁ males produce offspring which have no recombination in the inverted regions. The offspring of the reciprocal crosses are genetically identical, but may be carrying a chromosome which has had a cross over in the critical region. Comparisons between these reciprocal crosses allow us to measure the recombination effect. Table 5 gives the results of the Double-F₁ crosses. Reciprocal crosses are placed next to each other to make comparisons easier. The egg hatch and larva-to-adult survival will be treated separately.

Double-F₁: Egg hatch: The first section of Table 5 gives the egg hatch data. Columns 5 and 6 show that the eggs laid by the homokaryotypic females have a consistently lower percentage of egg hatch than do those laid by the heterokaryotypic females, i.e., the recombinant offspring have a lower egg hatch than do the non-recombinants. The bottom row gives the total of the egg hatch for the F₁ parents of these double-F₁ crosses; i.e., of the 12,000 S-HO, I-HO and HE eggs laid (see Table 2), 90% hatched. Comparisons by means of a test outlined by MAXWELL (1961, p. 77) between each type of double F₁ offspring with their F₁ parents and with each other (Table 6, columns 1-3), show that the 90.0% egg hatch of the F₁ parents is highly significantly lower than those of their offspring. Moreover, the 94.0% egg hatch of the recombinant offspring is highly significantly lower than the 97.7% of the non-recombinants. Selection during the F₁ generation had improved the egg hatch from 90.0% to 97.7%, approximately 8.6%. However, where present, recombination reduced this to 94.0%, a 4% reduction.

Double-F₁: Larva-to-adult survival: The larva-to-adult survival data for the

TABLE 5
Double- F_1 generation egg hatch and larva-to-adult survival

Female	Cross Male	Egg hatch		Percent egg hatch		Percent NR-R		Number of replicas	18° survival		Percent NR-R		Number of replicas	23° survival		Percent NR-R	
		Total eggs	Total hatch	Percent egg hatch	Percent NR-R	Percent NR-R	Percent NR-R		Total larvae	Percent emergence	Percent NR-R	Percent NR-R		Total larvae	Percent emergence	Percent NR-R	Percent NR-R
S_1S_2	S_1I_6	1000	971	97.1	1.5	—	—	20	1000	52.1	—	—	5	250	52.8	—	6.0
S_3I_6	S_1S_2	1000	986	98.6	—	—	—	20	1000	47.4	—	—	17	850	56.2	—	—
S_2S_3	S_1I_4	1000	944	94.4	3.2	—	—	16	800	53.0	—	—	19	950	58.4	—	1.0
S_1I_4	S_2S_3	1000	975	97.5	—	—	—	16	800	52.2	—	—	18	900	59.0	—	—
S_3S_1	S_2I_5	581	515	88.6	8.2	—	—	4	200	49.0	—	—	8	400	55.2	—	—
S_2I_5	S_3S_1	883	852	96.5	—	—	—	4	200	37.5	—	—	6	300	53.7	—	—
I_4I_5	I_3S_1	1000	959	95.9	1.9	—	—	18	900	62.8	—	—	11	550	59.6	—	3.6
I_3S_1	I_4I_5	1000	978	97.8	—	—	—	18	900	57.7	—	—	11	550	61.8	—	—
I_5I_6	I_1S_2	893	886	96.8	1.5	—	—	—	—	—	—	—	12	600	51.8	—	5.6
I_1S_2	I_5I_6	1000	983	98.3	—	—	—	—	—	—	—	—	11	550	54.9	—	—
I_3I_4	I_2S_3	1000	872	87.2	10.7	—	—	23	1150	51.7	—	—	16	800	52.0	—	13.0
I_2S_3	I_3I_4	1000	976	97.6	—	—	—	23	1150	55.2	—	—	12	600	59.8	—	—
Total recombinants		5474	5147	94.0	3.8	—	—	81	4050	54.4	—	—	71	3550	55.3	—	4.5
Total non-recombinants		5883	5750	97.7	—	—	—	81	4050	52.4	—	—	75	3750	57.9	—	—
Total F_1		12000	10796	90.0	—	—	—	117	11700	43.1	—	—	117	11700	45.0	—	—

R = Recombinants. NR = Non-recombinants.

TABLE 6

F₁s and double-F₁ : comparisons of egg hatch and larva-to-adult survival

Comparison		Egg hatch	Normal deviates 18° survival	23° survival
F ₁	Recombinants	5.54***	4.87***	5.02***
F ₁	Non-recombinants	11.87***	4.02***	6.20***
Recombinants	Non-recombinants	7.31***	1.27	1.96*

* = $P = .05$; *** = $P < .001$.

double- F_1 crosses are given in Table 5. Unfortunately, in the 18° line, it was impossible to obtain sufficient larvae for the $I_5I_6 \times I_4S_2$ cross and its reciprocal. At each of the two temperatures, Mann-Whitney comparisons were made between the two types of offspring with their F_1 parents and with each other (Table 6, columns 4 and 5). At both temperatures the F_1 are highly significantly inferior to their offspring. The improvement between generations was on the order of 25%, F_1 survival being about 44% as compared to 55% for their offspring.

At 23°, the non-recombinants are statistically significantly better than the recombinants ($P = 0.05$). However, at 18°, the trend is in the opposite direction: although they do not significantly differ from each other, it is the recombinant crosses which produce the most offspring. It is not clear, then, whether there is a real difference between the recombinants and the non-recombinants in the larva-to-adult survival of the double- F_1 crosses. However, there can be no doubt about the improvement of these offspring over their F_1 parents.

DISCUSSION

The selective value of a gene order is dependent upon the other gene orders present in the population. The presence of balanced polymorphism in natural populations and establishment of equilibria in population cages indicate that the two or more gene orders are in some way coadapted to each other (PAVLOVSKY and DOBZHANSKY 1966). In this paper we are attempting to examine the underlying mechanisms of this coadaptation; namely the type of selective forces involved and the level of organization of the genetic material which is being subjected to the selection.

The results of the crosses to produce an F_1 are relatively clearcut and a simple interpretation of our laboratory experiments is adequate. The three Inbred U-standard strains and, to a lesser extent, the three Inbred inversion strains have a high degree of genetic relationship, and one would suspect that they carry many of the same alleles. Moreover, considering that these Inbred lines were not isogenic, it is perhaps not too surprising that the homokaryotypic F_1 s are not superior to the Inbred lines and that there is no reduction in viability in the Inbred crosses. However, the heterokaryotypic F_1 s are superior to neither the Inbred lines nor to the homokaryotypic F_1 s. This lack of a heterokaryotypic heterosis demonstrates unequivocally that there was no genic heterosis present, and therefore no genic selection.

The only significant results obtained from the egg hatch data indicate that the U-standard homokaryotypes in both the Inbred and the F_1 were superior to the heterokaryotypes, the worst category. The larva-to-adult survival data could be understood if the U-standard homokaryotypes are taken to be inferior to both the heterokaryotypes and the U_{1+2} homokaryotypes, a single U_{1+2} chromosome being completely dominant to the recessive U-standard chromosomes. These are, of course, examples of karyotype selection, and indicate that the different karyotypes, *per se*, confer upon their carriers different fitnesses.

Further indirect evidence in support of the fact that chromosome selection was an important factor in our experiments can be inferred from the improvement found between the F_1 and the Double- F_1 generations. The average egg hatch rose from 90.0% to 96.2%, a 7% improvement; while the adult emergence went from 44% to 55%, a 25% improvement. The Double- F_1 individuals must have been better fit than the F_1 s since there was an additional generation of selection in their history. However, a 25% improvement in the larva-to-adult survival seems excessive and other factors may have contributed. One of these must have been facilitation. The Inbred and F_1 vials consisted of pure cultures of individuals which were relatively genetically homogeneous. The Double- F_1 vials, on the other hand, contained two types of individuals, homokaryotypes and heterokaryotypes. Such mixed cultures are often more productive than pure cultures presumably due to differential utilization of niches among the genotypes (BEARDMORE 1963). If the improvement is indeed due to facilitation, then it is clear that the two karyotypes are exploiting the environment in different ways: or the different karyotypes, *per se*, have different fitnesses.

In view of the small sample of chromosomes examined and the high degree of genic relationship present in this sample, one should not assume that the specific fitnesses found under our experimental conditions would hold for all U-standard and U_{1+2} inversions from the Zürich region. However our results are consistent with karyotype selection and not genic selection and this author feels that this is strong evidence pertaining to the mechanism by which inversion polymorphism is maintained in nature.

In our experiments, the recombination effect is to be observed in the double- F_1 cross. The egg hatch percentage of the heterokaryotypic F_1 females $\times$ homokaryotypic males was higher than that of the reciprocal crosses. This is the maternal effect predicted in 1968, but it is necessary to ascertain that it is due to recombination and not some other phenomenon.

CLARK, MAYNARD SMITH and TRAVERS (1960), using inbred strains, demonstrated a *maternal inheritance* for the rate of egg development in *subobscura*. BARNES (1968) and MORIWAKI and TOBARI (1963) found a maternal inheritance for an F_1 heterosis for fecundity in other species. In maternal inheritance, a character present in the female—or her ancestry—is passed on to her offspring. We have failed to find such a maternal inheritance for egg hatch in the crosses for the various F_1 . Now let us look for maternal inheritance in the egg hatch of the Double- F_1 hybrids. The relevant data are given in Table 7. The first two columns give the genotypes of the parents and the third column the egg hatch frequency

TABLE 7
Egg hatch frequencies

Double- F_1 cross		Offspring	Maternal strain	Maternal grandmother's strain	Mean of maternal grandparent's strain
Maternal parent	Paternal parent				
S_1S_2	S_3I_6	.971	.906*	.878	.9085
S_3I_6	S_1S_2	.986*	.871	.959*	.939*
S_2S_3	S_1I_4	.944	.964*	.939*	.949*
S_1I_4	S_2S_3	.975*	.914	.878	.905
S_3S_1	S_2I_5	.886	.941*	.959*	.9185
S_2I_5	S_3S_1	.965*	.865	.939	.920*
I_4I_5	I_6S_1	.959	.886	.932*	.9165*
I_6S_1	I_4I_5	.978*	.917*	.919	.8985
I_5I_6	I_4S_2	.968	.881	.901	.910
I_4S_2	I_5I_6	.983*	.881	.932*	.9355*
I_6I_4	I_5S_3	.872	.923*	.919*	.9255
I_5S_3	I_6I_4	.976*	.847	.901	.9300*

* Superior strain.

of the Double F_1 hybrids. For each reciprocal cross the egg hatch of the heterokaryotype mothers is greater than the egg hatch of the homokaryotype mothers. Column 4 gives the egg hatch of the mothers themselves. Comparing within reciprocal crosses one sees that four of the homokaryotypic mothers had higher egg hatches than the heterokaryotypic mothers; one heterokaryotypic mother was better than the homokaryotypic mother; and for one cross they were even. It is possible that the maternal inheritance might be delayed and one should look at the maternal grandparents of the strains for evidence of maternal inheritance. Column 5 gives the egg hatch of the maternal grandmother's strain: in four of the comparisons the homokaryotypic maternal grandmothers were superior and in the other two the heterokaryotypic maternal grandmothers were superior. Finally, column 6 gives the mean of the maternal grandparents: in two of the comparisons the homokaryotypic maternal grandparents were superior while in four the maternal grandparents of the heterokaryotypes were superior. It is clear then that in our experiment, *the consistent superiority of the offspring of the heterokaryotypic females is not a reflection of a concomitant superiority in the females themselves or in the female ancestry.*

Occasionally differences between reciprocal crosses have been found which are not due to maternal inheritance (McFARQUHAR and ROBERTSON 1963; ANDERSON 1968). These have been ascribed to *maternal effects* caused by genotypic-cytoplasmic interactions. They are unpredictable and apparently haphazard events which occur in some crosses but not in others. However, the results obtained in our experiments call for a consistent, underlying cause: the maternal effect is due to recombination. The U_{1+2} inversions cover approximately $\frac{2}{3}$ of the chromosome. The suppression of recombination perhaps extends still further, making a considerable segment of the chromosome into a single inherited unit, a single gene block. All F_1 adults which reproduce carry good pre-selected chromo-

somes. The offspring of the heterokaryotypic females inherit unchanged gene blocks. Those of the homokaryotypic females inherit recombined, altered chromosomes. STRICKBERGER (1965) showed that recombination can improve poor chromosomes. Our experiments show that recombination can lead to the deterioration of good chromosomes, a result less surprising than those of STRICKBERGER.

The recombination effect, although highly significant ($P \ll .001$), was small, being a reduction of about 4% in viability. The recombination effect should be most effective when the homokaryotypes are heterozygous for blocks of genes which are rather different. However, as we mentioned before, there was a high degree of genetic relationship among the U-standards and among the inversion strains used in our tests. Thus the material was not ideal for detecting a recombination effect; yet one was found.

The adult emergence data are not as clearcut as are those of the egg hatch. At 23°, there is a barely significant ($P = .05$) recombination effect with an overall reduction in fitness due to recombination of approximately 3%. However, at 18°, although not significant, the trend is in the opposite direction. In view of this, it might be best to suspend judgment on the recombination effect on adult emergence.

Other investigators have examined the effect of inversion heterozygosity in females on the viability of their offspring. However, having other problems in mind, these investigators used other methods and accumulated data which for the most part are not comparable to ours. X-ray induced inversions were used (TERZAGHI and KNAPP 1960) and non-reciprocal crosses producing genotypically different offspring were compared (RILES 1965; ANDERSON, DOBZHANSKY and KASTRITSIS 1967). The only data from reciprocal crosses between naturally occurring inversions were some of those obtained in *D. pseudoobscura* by TERZAGHI and KNAPP. Heterokaryotypic AR/ST females crossed to ST/ST homokaryotypic males did produce a higher, but not significantly higher, egg hatch than the reciprocal cross (97.0% compared to 95.9%). There had been no prior attempt to select for good chromosomes, so there was no obvious advantage for the suppression of recombination.

Most of the work was carried out during the Sabbatical leave supported by an N.S.F. Science Faculty Fellowship at the University of Zürich. It is with great pleasure that I acknowledge the help given me by Professor H. BURLA and his staff. Professor BURLA supplied the strains, technical assistance and vital suggestions concerning techniques. He also contributed his ideas freely in many discussions on the work and the manuscript. I am also very grateful to Drs. L. MARCUS and H. L. CARSON and to Mrs. H. R. KOEPFER for their constructive criticisms, and to Drs. L. MARCUS and HAMPEL for their aid in the statistical analyses. The author's research is supported by N.S.F. grant #GB 5187 and N.I.H. grant #5-SO5-RR-07064-03.

LITERATURE CITED

- ANDERSON, W. W., 1968 Further evidence for coadaptation in crosses between geographical populations of *Drosophila pseudoobscura*. Genet. Res., **12**: 317-330.
- ANDERSON, W. W., TH. DOBZHANSKY, and C. D. KASTRITSIS, 1967 On the relationship of structural heterozygosity in the X and 3 chromosomes of *Drosophila pseudoobscura*. Am. Naturalist **101**: 89-92.

- BARNES, B. W., 1969—Maternal control of heterosis for yield in *Drosophila melanogaster*. *Heredity* **23**: 563–572.
- BEARDMORE, J. A., 1963 Mutual facilitation and the fitness of polymorphic populations. *Am. Naturalist* **97**: 69–74.
- BURLA, H. and W. GÖTZ, 1965 Veränderlichkeit des chromosomalen Polymorphismus bei *Drosophila subobscura*. *Genetica* **36**: 83–104.
- CARSON, H. L., D. E. HARDY, H. T. SPIETH and W. S. STONE, 1970 The evolutionary biology of the Hawaiian *Drosophilidae*. pp. 437–543. In: *Essays In Evolution and Genetics. In Honor of Theodosius Dobzhansky*. Edited by M. K. HECHT and W. C. STEERE, Appleton-Century-Croft, New York.
- CLARK, J. M., J. MAYNARD SMITH and P. TRAVERS, 1960 Maternal effects on the rate of egg development in *Drosophila subobscura*. *Genet. Res.* **1**: 375–380.
- DOBZHANSKY, TH., 1970 *Genetics of the Evolutionary Process*. Columbia University Press, New York.
- FORD, E. B., 1940 Polymorphism and Taxonomy. In: *The New Systematics*. Edited by J. HUXLEY. Oxford University Press, London.
- FRANKLIN, I. and R. C. LEWONTIN, 1970. Is the gene the unit of selection? *Genetics* **65**: 707–734.
- GÖTZ, W., 1967 Untersuchung über den chromosomalen Strukturpolymorphismus in kleinasiatischen und persischen Populationen von *Drosophila subobscura*. *Mol. Gen. Genetics* **100**: 1–38.
- HUANG, S. L., M. SINGH and K. KOJIMA, 1971 Study of frequency-dependent selection observed in the esterase-6 locus of *Drosophila melanogaster* using a conditioned media method. *Genetics* **68**: 97–104.
- JUNGEN, H. E., 1968 Inversionpolymorphismus in tunesischen Populationen von *Drosophila subobscura*. *Arch. d. Julius Klaus Stiftung* **43**: 1–55.
- KASTRITSIS, C. D., 1966 Cytological studies on some species of the tripunctata group of *Drosophila*. *Univ. Tex. Publ.* **6615**: 413–474.
- KUNZE-MÜHL, E., and E. MÜLLER, 1958 Weitere Untersuchungen über die chromosomale Struktur und die natürlichen Strukturtypen von *Drosophila subobscura*. *Chromosoma* **9**: 559–570.
- LEVENE, H., 1953 Genetic equilibrium when more than one ecological niche is available. *Am. Naturalist* **87**: 331–333.
- LEVINS, R. and R. MACARTHUR, 1966 The maintenance of genetic polymorphism in spatially heterogeneous environment: variations on a theme by Howard Levene. *Am. Naturalist* **100**: 585–589.
- LEWONTIN, R. C. and Y. MATSUO, 1963 Interaction of genotypes determining viability in *Drosophila busckii*. *Proc. Natl. Acad. Sci. U.S.A.* **49**: 270–278.
- MAYNARD SMITH, J., 1966 Sympatric speciation. *Am. Naturalist* **100**: 637–650.
- McFARQUHAR, A. M. and F. W. ROBERTSON, 1963 The lack of evidence for co-adaptation in crosses between geographical races of *Drosophila subobscura*. *Genet. Res.* **4**: 104–131.
- MAXWELL, A. E., 1961 *Analyzing Qualitative Data*. Methuen & Co., Ltd., London.
- MORIWAKI, D. and Y. N. TOBARI, 1963 Maternal effects and heterosis in *Drosophila ananassae*. *Genetics* **48**: 171–176.
- PAVLOVSKY, O. and TH. DOBZHANSKY, 1966 Genetics of natural populations XXXVII. The co-adapted system of chromosomal variants in a population of *Drosophila pseudoobscura*. *Genetics* **53**: 843–854.

- PRAKASH, S. and R. C. LEWONTIN, 1968 Molecular approach to the study of genic heterozygosity in natural populations, III. Direct evidence of coadaptation in gene arrangements of *Drosophila*. *Proc. Natl. Acad. Sci. U.S.* **59**: 398-405.
- RILES, L., 1965 Inversion polymorphism and embryonic lethality in *Drosophila robusta*. *Genetics* **52**: 1335-1343.
- SOKAL, R. R. and I. KARTEN, 1964 Competition among genotypes in *Tribolium castaneum* at varying densities and gene frequencies (the black locus). *Genetics* **49**: 195-211.
- SOKAL, R. R. and F. J. ROHLF, 1969 *Biometry*. W. H. Freeman and Co., San Francisco.
- STALKER, H., 1965 The salivary gland chromosomes of *Drosophila micromelanica* and *Drosophila melanura*. *Genetics* **51**: 487-507.
- STRICKBERGER, M., 1965 Experimental control over the evolution of fitness in laboratory populations of *Drosophila pseudoobscura*. *Genetics* **51**: 795-800.
- STRICKBERGER, M. and C. J. WILLS, 1966 Monthly frequency changes in *Drosophila pseudoobscura* third chromosome gene arrangements in a California locality. *Evolution* **20**: 592-602.
- TERZAGHI, E. and D. KNAPP, 1960 Pattern of chromosome variability in *Drosophila pseudoobscura*. *Evolution* **14**: 347-350.
- WARD, B. L. and W. B. HEED, 1970 Chromosome phylogeny of *Drosophila pachea* and related species. *J. Heredity* **61**: 248-258.
- WASSERMAN, M., 1963 Cytology and phylogeny of *Drosophila*. *Am. Naturalist* **97**: 333-352.
- , 1968 Recombination-induced chromosomal heterosis. *Genetics* **58**: 125-139.
- , 1970 Egg-to-adult survival: an invalid measurement for determining preadult viability. *Am. Naturalist* **104**: 404-407.