FITNESS OF KARYOTYPES IN *DROSOPHILA PSEUDOOBSCURA*

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ABSTRACT

In the dynamics of the survival of chromosomal polymorphism selection may be operating at the genic level, at the chromosomal level or at the supergene level. Tests designed to distinguish between these levels were run on *Drosophila pseudoobscura*. There was no evidence for heterosis, a necessary requirement for gene-determined chromosomal polymorphism. A strong chromosomal selection was observed. No evidence was found for the presence within one locality of more than a single superallele for each supergene (= gene order). These results are compared to those found by others.

THE preservation of chromosomal variability in natural populations is undoubtedly due to balanced polymorphism. There is, however, some question as to the type of selection responsible for the polymorphism. Thus, it has been proposed that the balance is due to heterosis of the heterokaryotype (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY 1950; ANDERSON *et al.* 1968), frequency-dependent selection (EHRMAN and PETIT 1968; EHRMAN and SPIESS 1969; HUANG, SINGH and KOJIMA 1971; BUNDGAARD and CHRISTIANSEN 1972; COCKERHAM *et al.* 1972) or annidation, where a heterogeneous environment contains various niches for the several genotypes (LUDWIG 1950; LEVINS and MACARTHUR 1966; MAYNARD-SMITH 1966).

In addition to determining which of the above factors, or combination of the above factors, constitutes the balancing mechanism, it is of considerable interest to discover the level of organization of the genetic material upon which selection is operating. Selection may be acting at the genic level, at the chromosome level, or at the supergene level.

A number of models have been proposed for inversion polymorphism through polygenic effects. Basic to the polygenic models is the assumption that the gene orders, *per se*, have no selective value, but rather act as neutral vehicles of the individual alleles which happen to be present in the gene sequences in that population. These models have been investigated primarily by means of computer-simulated tests (see e.g., FRASER and BURNELL 1967). The results of such tests indicate that a stable inversion polymorphism is developed only if there are overdominant heterotic loci present. 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. Thus the heterokaryotype would be heterotic because of a genic effect involving a relational balance between alleles at the same locus of the two homologs. This kind of heterosis is the *sine qua non* of gene-determined balanced polymorphism; if such overdominant loci are lacking, polygenic effects cannot be the primary determining factor for such polymorphisms. The heterotic loci are believed to operate by increasing the individual's developmental homeostasis, i.e., by buffering the development of the individual against environmental fluctuations (LERNER 1954; CARSON 1959). A second type of result from the computer-simulated polygenic studies which is germane to our approach is the fact that even without crossover inhibitors such as inversions, as the number of genes put into their systems increased, linkage disequilibrium became stronger and selection appeared to act on the chromosome as a whole and not on the genes (FRANKLIN and LEWONTIN 1970; SLATKIN 1972). The presence of a strong linkage disequilibrium in a cytologically homogeneous population indicates that an inversion system is not contributing anything new, but merely tightening up the linkage.

A second level of organization is the chromosome as defined by the gene orders. In chromosome selection we are assuming that the gene order in some way *defines* the adaptive nature of its component alleles; it is not merely a neutral vehicle whose adaptive significance is determined by the nature of the alleles which happen to be present at any particular time. For example, the various Arrowhead (AR) chromosomes may be generally adapted to one aspect of the environment, while the Standards (ST) are adapted to a second part, and the Pikes Peaks (PP) to still a third. Inversional differences prevent recombination between gene sequences and allow each gene order to diverge genetically; e.g., AR chromosomes would tend to accumulate those alleles which best fit the carrier to the AR environment. A number of different AR chromosomes can coexist in a population, each differing qualitatively and quantitatively in the number of, and specific loci, of the "good" AR alleles. A mutational load and a changing AR environment could prevent fixation of alleles within the AR gene arrangement. Recombination between genetically heterozygous homokaryotypes would reshuffle the alleles and on the average produce no overall change in fitness. However, recombination coupled with selection could lead to improvement (STRICKBERGER 1965).

Most investigators of natural or laboratory populations examine the karyotype of the individuals. It is usually assumed that selection is operating at the chromosome level and a degree of success has been obtained in describing and explaining observations by conferring relative adaptive values to different karyotypes. Thus geographical clines and altitudinal clines in the frequencies of the various gene sequences, as well as cyclic seasonal changes, cyclic changes lasting several years, and directional changes of up to 17 years in duration in these frequencies have been described (DOBZHANSKY 1958; DOBZHANSKY, ANDERSON and PAVLOVSKY 1966). Implicit in all of this is that selection must take place at the chromosome level. Indeed, although it is often difficult to demonstrate, particular gene orders have been shown to be generally adapted to a particular type of environment

such as temperature or rainfall (MOHN and SPIESS 1963; STRICKBERGER and WILLS 1966; MAYHEW *et al.* 1966). Moreover, unlike genic selection, where only heterosis can ensure a balanced polymorphism, at the chromosome level, frequency-dependent selection, or annidation as well as heterosis is sufficient to produce a balanced polymorphism.

DOBZHANSKY (1959, 1970) has proposed that there is a level of organization along the length of the chromosome, so that alleles on a single homolog "cooperate" with each other. Therefore the balance is an internal balance and the cooperation involved is between alleles at different loci on the same chromosome. He considered this a supergene and equated the gene orders with supergenes. At any time level in each population, these supergenes are coadapted to each other in such a way that the heterokaryotypes are heterotic. Supergenes from different populations may not be coadapted and therefore interpopulation hybrids may not be heterotic (DOBZHANSKY and PAVLOVSKY 1958). The stress has always been to use the supergene in order to explain the relational coadaptation (= heterokaryotype heterosis) found within populations, but not necessarily between populations (DOBZHANSKY 1950). It is also possible that there are a number of well-adapted representatives of each gene order in a population (WASSERMAN 1960, 1968). If so, rather than there being only a single supergene for each gene order, there may be a number of internally balanced superalleles for each gene order. Parenthetically, previously we (WASSERMAN 1968, 1972) had used the term supergene as equivalent to superallele, but this is probably unnecessarily confusing since the general practice is to define the number of supergenes as being the number of different gene orders. Homokaryotypes which are heterozygous for internally well-balanced superalleles would be well-adapted for the environment. However, recombination would tend to disrupt the internal balance so that the offspring of well-adapted homokaryotypes would be less fit. Computer-simulated experiments have shown that this recombination effect creates a frequency-dependent selection whereby the overall fitness of the favored and most frequent homokaryotype is reduced. This can, at certain levels of selection, produce a balanced inversion polymorphism even when one of the homokaryotypes is the "fittest". When such a polymorphism exists, the heterokaryotypes assume an overall heterosis.

Although there is a tremendous amount of information on chromosomal variability, there is, unfortunately, a lack of experimental data which would allow us to clearly recognize and distinguish among genic, chromosomal and supergene selection. WASSERMAN (1972) reviewed the pertinent data and presented the results of a study on an inversion system in *Drosophila subobscura*. Egg hatch and larva-to-adult survival were examined and a strong chromosomal selection was observed. A recombination effect was also found in the egg hatch data, indicating that selection could differentiate between different superalleles. However, no evidence of any genic effects was uncovered. In this paper we are presenting the results of our tests on *Drosophila pseudoobscura*.

MATERIALS AND METHODS

The studies were carried out on eight *D. pseudoobscura* strains homozygous for their third chromosome gene order: four strains carrying Arrowhead (AR) and designated A₁, A₂, A₃ and A₄, and four strains carrying Pikes Peak (PP) and designated P₅, P₆, P₇, and P₈. The technique of SPASSKY *et al.* (1960) with the marker strain dominant Lobe, recessive orange/dominant Blade, dominant Scute, recessive purple (*L, or/Bl, Sc, pr*) was used to develop the strains from wild males collected in July 1970 in the Black Forest, ten miles north of Colorado Springs, Colorado. This involved the crossing of wild males collected in the Black Forest individually to the *L, or/Bl, Sc, pr* females. A single F₁ *L, or/+* male from each cross was then backcrossed to the *L, or/Bl, Sc, pr* females. From among the offspring, *L, or/+* females were crossed to *Bl, Sc, pr/+* males. Strains were established from the wild-type offspring resulting from this cross. These strains were each homozygous for one of the third chromosomes present in the original wild male. Cytological examination determined the particular third chromosome gene order present in the strain.

Egg-to-adult survival was measured on five classes of flies: (a) Inbred lines, i.e., the homozygous strains themselves; (b) two classes of genetically heterozygous homokaryotypes, those carrying recombinant third chromosomes and those carrying non-recombinant third chromosomes; and (c) two classes of heterokaryotypes, those with recombinant third chromosomes and those with unrecombined third chromosomes. The recombinant and non-recombinant AR homokaryotypes were also tested for differences in mating propensity.

The basic techniques for determining egg-to-adult relative viability were those of SPASSKY *et al.* (1960), with some minor modifications. In general, relative viability was determined in a test generation comprising four phenotypes which, with equal viability, should occur in equal frequencies: wild-type flies, Blade, Scute flies, Lobe flies, and Blade, Scute, Lobe flies. Comparisons were made between the wild-type flies and the Blade, Scute, Lobe flies since these latter could serve as a control for all experiments. In all cases, the test generation was produced by crossing eight *L, or/+* females with eight *Bl, Sc, pr/+* males at 25°. The flies were transferred to fresh food every two days for a total of eight transfers. Vials 1, 3, 5 and 7 were raised at 25° while vials 2, 4, 6 and 8 were placed at 15° as soon as the adults were removed. Counts were made on only the first three vials at each temperature unless it was necessary to discard a vial because of mold or mishap, in which case the fourth vial was used.

For the measurement of Inbred viability, females of each strain were mated to males of the *Bl, Sc/L* stock and yielded both Lobe and *Bl, Sc* offspring. The mating of Lobe females to their Blade, Scute brothers resulted in the test generation.

Figure 1 gives an example of the types of crosses used for the homokaryotype tests. The female parent is placed to the left (e.g., 5/5 × 6/6 means PP₅/PP₅ females crossed to PP₆/PP₆ males). Similarly, in noting a karyotype, the chromosome to the left of the slash is derived from the female parent (e.g., 5/6 indicates a PP₅ chromosome from the mother and a PP₆ chromosome from the father). Recombinant chromosomes are underlined. The G_I females, I₁ and I₃, are heterozygous homokaryotypes, carrying non-recombinant chromosomes. Their offspring however will be carrying recombinant chromosomes. The G_I males, I₂ and I₄, will of course pass on their chromosomes unaltered to the G_{II} generation. No further recombination is possible in succeeding generations since *Bl, Sc* is on a Standard gene arrangement, while *L* is on a Santa Cruz gene order. Crosses for the G_{III} generation were done in such a manner as to produce individuals which were completely heterozygous for their third chromosomes. By comparing the G_{III} generations produced by cross (A) and cross (B) it is possible to determine the effects of recombination. All G_{III} individuals from cross (A) will have received a recombined 5-6 chromosome from their mother and a recombined 7-8 chromosome from their father. In contrast, G_{III} individuals from cross (B) will receive either an unaltered 5 or an unaltered 6 from their mother, and an unaltered 7 or an unaltered 8 from their father. The specific example shown in Figure 1 gives the crosses performed for the (5 × 6) + (7 × 8) PP test. Tests were also run on (5 × 7) + (6 × 8), and also (5 × 8) + (6 × 7) PP tests, as well as reciprocal crosses at the G₀ generation for each test.

FIGURE 1.—An example of the mating schedule used to produce recombinant and non-recombinant homokaryotypes.

An identical series of tests was run for the AR homokaryotype experiments. The experiments to detect the effect of recombination on the heterokaryotypes were done in the same manner, except that the crosses to produce the G_{III} were made between AR- G_{II} and PP- G_{II} individuals.

The recombinant and non-recombinant Arrowhead homokaryotypes were tested for differences in mating propensity in the Elens-Wattiaux mating chamber. Forty flies were used per chamber, ten males and ten females of each type. In each chamber flies of one type had a small notch in the margin of the left wing as a means of identification. Six replicate chambers were run for most crosses; in three of the chambers one type was notched and in the remainder the alternate type had a notch. The flies were seven to ten days old when introduced into the chambers, which were run for one hour and observed constantly. Temperature and lighting conditions did not vary significantly.

All these experiments were completed within eighteen months of the original collection.

RESULTS

We wish to compare the viabilities of the phenotypically wild-type flies from experiment to experiment. The only phenotype which is present in all tests, which has a consistent genotype, and therefore which can be used as a control, is Blade, Scute, Lobe. Both the Blade flies and the Lobe flies vary as to the origin of the wild-type chromosome present. Therefore all of our tests examine the relative viabilities of the wild flies compared to that of the Blade, Scute, Lobe flies.

The results for the Inbred crosses are given in Table 1. Chi-square tests and Cochran's critical ratio test as described by MAXWELL (1961, pp. 77-79) failed to show significant differences in relative survival of the Inbreds at the different temperatures, nor were there any significant differences between the AR and PP within temperatures.

Tables 2 and 3 summarize the viability results of the homokaryotypes and heterokaryotypes. The raw data were analyzed using chi-square tests and Cochran's critical ratios to determine whether differences existed between Recombinant and Non-recombinant flies. No significant differences were found between these two types, and in all of the subsequent analyses of the survival data, the Recombinants and Non-recombinants were combined. Comparisons were made to determine whether temperature had an effect on the relative survival values. Chi-square tests indicated that the relative survival of both the AR homokaryo-

TABLE 1
Relative viabilities of Inbred lines

Temperature	Karyotype	No. of replicas	Phenotypes				Total
			Bl,Sc/L	Wild	Bl,Sc	L	
15°	AR	12	1.000	.710	.845	.907	2745
	PP	14	1.000	.732	.845	.920	2748
	TOTAL	26	1.000	.721	.845	.913	5493
25°	AR	12	1.000	.700	.876	.861	2863
	PP	14	1.000	.646	.793	.813	3470
	TOTAL	26	1.000	.669	.829	.834	6333

TABLE 2

Relative viabilities of homokaryotypes and heterokaryotypes at 15°

Karyotypes	Classes	No. of replicas	Phenotypes				Total
			Bl,Sc/L	Wild	Bl,Sc	L	
AR-HO	Recombinants	33	1.000	1.187	1.113	.873	7557
	Non-recombinants	34	1.000	1.158	1.068	.882	7704
	Total	67	1.000	1.172	1.090	.878	15261
PP-HO	Recombinants	35	1.000	.810	.952	.834	8210
	Non-recombinants	28	1.000	.740	.879	.802	6202
	Total	63	1.000	.779	.920	.820	14412
HE	Recombinants	18	1.000	.866	.890	.943	3785
	Non-recombinants	17	1.000	.907	.997	.914	3776
	Total	35	1.000	.886	.943	.929	7561
TOTAL	Recombinants	86	1.000	.955	.997	.870	19552
	Non-recombinants	79	1.000	.943	.980	.858	17682
	Total	165	1.000	.949	.989	.864	37234

TABLE 3

Relative viabilities of homokaryotypes and heterokaryotypes at 25°

Karyotypes	Classes	No. of replicas	Phenotypes				Total
			Bl,Sc/L	Wild	Bl,Sc	L	
AR-HO	Recombinants	33	1.000	.857	1.018	.935	7317
	Non-recombinants	34	1.000	.904	1.049	.880	8065
	Total	67	1.000	.881	1.034	.906	15382
PP-HO	Recombinants	35	1.000	.776	.933	.836	9902
	Non-recombinants	28	1.000	.778	.886	.821	7628
	Total	63	1.000	.777	.912	.830	17530
HE	Recombinants	18	1.000	.804	.996	.853	3909
	Non-recombinants	17	1.000	.853	.976	.842	3920
	Total	35	1.000	.828	.986	.848	7829
TOTAL	Recombinants	86	1.000	.808	.973	.872	21128
	Non-recombinants	79	1.000	.842	.968	.848	19613
	Total	165	1.000	.824	.970	.861	40741

types ($p < .001$) and the heterokaryotypes ($p < .01$) were better at 15° than at 25°.

Figure 2 gives the ranks of the karyotypes at both temperatures and the results of chi-square statistical tests. The two rankings differ from each other only in that the relative positions of PP-IN and AR-IN are reversed. This is certainly not meaningful since in neither instance do the Inbreds differ from each other significantly, and they are therefore probably equal to each other. The Inbreds are homozygous for their third chromosomes, and show a relatively low survival ability (Figure 3), being highly significantly less viable than the homokaryotypes and the heterokaryotypes. This is a genic effect and it is consistent with data of this nature, merely indicating the extent of the genetic load in this sample of chromosomes. Our data do not allow us to determine whether this is a mutational load

FIGURE 2.—Egg-to-adult survival: Ranks of karyotypes. Arrows connect types which differ significantly from each other, the head of the arrow indicating the inferior type. ** = $p < .01$; *** = $p < .001$.

or a segregational load. However, in either case, it is not really important in determining the mechanism for the survival of chromosomal polymorphism. Most individuals in natural populations are genetically heterozygous whether they are structural homokaryotypes or heterokaryotypes. In order to gain some insight into chromosomal polymorphism one must compare the heterokaryotypes with genetically heterozygous homokaryotypes, and not with individuals which are homozygous for their chromosomes. Our tests were designed to ensure the greatest amount of genetic variability among the flies tested.

At both temperatures, the heterokaryotype is intermediate between the poorer, PP-homokaryotype and the better, AR-homokaryotype (Figure 2). At 15° the differences between the three types are highly significant, whereas at 25° the AR are highly significantly superior to the PP, with the heterokaryotypes being

FIGURE 3.—Egg-to-adult survival: Ranks of combined Inbreds, combined homokaryotypes and heterokaryotypes. Arrows indicate significant differences between adjacent classes, the head of the arrow indicating the inferior class. ** = $p < .01$; *** = $p < .001$.

intermediate. The heterokaryotypes do not show heterosis when compared to the homokaryotypes combined (Figure 3). The data are therefore unequivocal: there is no evidence for genic effects nor for heterosis. The survival of individuals is determined by the chromosomes they carry: At 15° the more AR chromosomes an individual is carrying, the better it is for that individual's egg-to-adult relative viability, whereas at 25° the AR homokaryotypes are better, but not significantly better than the heterokaryotypes, and both are better than the PP homokaryotypes. This is clearly a chromosomal effect, and shows karyotypic selection.

The recombinant and non-recombinant Arrowhead homokaryotypes were tested in Elens-Wattiaux mating chambers. The mating indices developed by LEVENE in EHRMAN and PETIT (1968) were applied to our data on more than 1000 matings. No significant differences were found in male and female sex drive or in discrimination.

DISCUSSION

We wish to gain some insight into the genetic factors involved in preserving chromosomal polymorphism in natural populations. Although an artificial system was investigated, the results of our laboratory experiments should generally reflect the natural situation by indicating the type of selection which is operating and the level of organization at which the selection is taking place. Thus if the heterokaryotypes are heterotic because they are heterozygous for heterotic loci which buffer the development of the individual against environmental fluctuations, one would expect such heterosis to be manifest under the environmental conditions found in the laboratory. Similarly, if internally balanced alleles are cooperating to produce superalleles, recombination should have an effect on survival whether the test is performed in a natural population in Mather, California, or an artificial one in a laboratory vial. And finally if the selection is operating at the chromosome level, one should be able to define an adaptive value for the karyotypes under given environmental conditions. The results of our experiments are clear-cut and unambiguous.

The *pseudoobscura* Inbred strains were homozygous for third chromosomes which had been isolated from wild males. Presumably well suited in various combinations with other third chromosomes for the natural conditions, these chromosomes were immediately tested in our system. Here they had somewhat lower egg-to-adult viabilities than other, similarly tested laboratory-stock chromosomes which had had some opportunity to "acclimatize" (SPASSKY *et al.* 1960; STRICKBERGER 1972). In our experiments outbreeding produced homokaryotypes and heterokaryotypes both of which were highly significantly superior to the Inbred lines. Outcrossing highly inbred or partially inbred lines often produces heterosis (VETUKIV 1954; LERNER 1954). This heterosis is clearly an example of a genic effect, whereby the F_1 hybrid vigor is either due to normal dominant alleles masking deleterious recessive alleles or due to overdominance genes. Although interesting in relation to genetic load, these results shed little light on the mechanisms for the preservation of chromosomal polymorphism in nature. Under natural conditions selection operates on genetically heterogeneous

homokaryotypes and heterokaryotypes, and not on isogenic homozygotes. Comparisons between genetically homozygous homokaryotypes and genetically heterozygous heterokaryotypes are useless in helping us to understand how chromosomal polymorphism is maintained in nature.

Looking at the critical data, in egg-to-adult viability at 15° the Arrowhead homokaryotypes are highly significantly superior to the heterokaryotypes, which in turn are highly significantly superior to the Pikes Peak homokaryotypes, whereas at 25° the Arrowhead homokaryotypes are highly significantly superior to the Pikes Peak homokaryotypes, while the heterokaryotypes are intermediate. There is no evidence of heterosis. Since heterosis is the *sine qua non* for genically determined chromosomal polymorphism, we can conclude that we have no evidence that genic selection is involved in chromosomal polymorphism. Several other investigations have also failed to yield heterosis when comparing homokaryotype and heterokaryotype viability (WASSERMAN 1972; STRICKBERGER 1972; NICKERSON and DRUGER 1973). Superior homeostatic properties have been demonstrated for heterokaryotypes (Moos 1955). However, these heterokaryotypes were not heterotic, but were inferior to one of the homokaryotypes. Heterokaryotype heterosis has been used to explain a number of different phenomena: the attainment of balanced polymorphism in experimental population cages, the fact that populations living in marginal regions are less variable than those found in central heterogeneous environments (CARSON 1959), and the fact that the number of gene orders within a locality is correlated with the number of available ecological niches (DA CUNHA *et al.* 1959). These, however, could also be due to other types of selection such as frequency-dependent selection or annidation. Even when heterokaryotype heterosis has been found, its importance has not been unequivocally demonstrated. WALLACE (1948) showed that females heterozygous for sex ratio in *pseudoobscura* are heterotic. However, despite this, unless the sex ratio males were superior to the standard males, sex ratio was lost in population cages. Heterokaryotype heterosis has been found by ANDERSON *et al.* (1968) in population cages and by SPIESS for mating behavior (e.g., SPIESS and SPIESS 1967). However, frequency-dependent selection, which may be more important than heterosis, has also been found in cage experiments (PAVLOVSKY and DOBZHANSKY 1966; ANDERSON *et al.* 1968) as well as in mating chambers (EHRMAN and SPIESS 1969). The minority effect on mating propensity may, in itself, be sufficient to maintain a balanced polymorphism in population cages, if not under natural conditions. On more theoretical grounds, arguments can be presented against genic effects being instrumental in determining chromosomal polymorphism. The primary action of heterotic loci is to buffer the development of the individual against environmental fluctuations (LERNER 1954; CARSON 1959). A considerable amount of genetic polymorphism has been found in *pseudoobscura* (HUBBY and LEWONTIN 1966; LEWONTIN and HUBBY 1966). The geographical distribution of these polymorphisms indicates that selection is operating to maintain the polymorphism (PRAKASH, LEWONTIN and HUBBY 1969). The presence of this type of polymorphism has been explained by assuming that these loci are heterotic (SVED, REED and BODMER 1967; KING

1967; MILKMAN 1967). The genetic polymorphisms are distributed throughout the genome, yet the vast majority of the chromosomal rearrangements are limited to a single chromosome, chromosome 3. If heterotic loci are distributed throughout the genome, why are all inversions, except sex ratio, found on chromosome 3? Moreover, an examination of the polymorphic loci associated with the inversion systems of *pseudoobscura* does not uncover strong evidence for heterotic loci as a basis for inversion-balanced polymorphism. Both *Pt-10* and *Amy*, third chromosome genes, show clear differentiation between the Standard phylad and the Santa Cruz phylad (PRAKASH and LEWONTIN 1968). But as the authors point out, "There is no correlation between genic heterozygosity on the third chromosome and chromosomal heterozygosity. . .". The Standard phylad has, basically, a different geographical distribution than the Santa Cruz phylad. On the other hand, the *Pt-12* locus tends to differentiate the Standard sequence from the other sequences (PRAKASH and LEWONTIN 1971), while two loci on the X chromosome differentiate sex ratio from Standard (PRAKASH and MERRITT 1972). At the present time it is not certain whether these genes are heterotic and are conferring heterosis upon the heterokaryotypes as suggested by PRAKASH and MERRITT (1972), or whether they are examples of the differences in the allelic contents which help to determine the adaptive values of the gene sequences. Since Standard is polymorphic for the *Pt-12* locus, it would be informative to learn whether the *Pt-12* heterozygotes are heterotic amid a population of Standard homokaryotypes. Finally, a heterokaryotype heterosis based on heterotic loci, broadly distributed as are *Pt-12* and the sex ratio loci, just cannot explain the coadaptive nature of the heterosis found by DOBZHANSKY (1950) and by DOBZHANSKY and LEVENE (1951).

The results reported here show that under our experimental conditions and at both temperatures the AR homokaryotypes are the best genotypes, the PP homokaryotypes are the worst, and the heterokaryotypes are intermediate. Parenthetically the AR sequence was also the dominant form in the Black Forest, Colorado population from which these chromosomal strains were derived (WASSERMAN, KOEPFER and GELLER 1971). Of 100 chromosomes sampled from collected males, 60 were AR, 35 were PP, 2 were ST, 2 were TL and 1 was OL. Our experimental laboratory differences clearly indicate chromosome selection, and add to the wealth of data showing chromosome selection cited above and by DOBZHANSKY (1970). Our experimental design gives us no information on the type of chromosomal selection (i.e., annihilation or frequency-dependent selection) which may be operating in nature.

Supergene selection differs from chromosomal selection in that in the former the genes along the length of the chromosome are believed to "cooperate" with each other to produce a well-integrated supergene. The fact that different gene orders from the same locality tend to produce a balanced polymorphism in population cages has indicated to DOBZHANSKY (1959, 1970) that the supergenes are coadapted to each other, and that the coadaptation is due to the formation of heterotic heterokaryotypes. Cages initiated with flies from different localities often do not yield an equilibrium; these supergenes are not coadapted to each

other because no heterosis exists. Whatever the nature of the coadaptation, i.e., whether it is heterosis or not, it is clear that the Arrowhead supergene in one locality is not the same as the Arrowhead supergene from a second locality. In other words, there is a geographical distribution of superalleles for each supergene. In our experiment, we have tried to determine whether there was more than only a single superallele in the locality tested. Our original premise was that the chromosomes which had been isolated from nature would be "good" superalleles since the carriers had been able to survive to maturity under natural conditions (WASSERMAN 1968). Recombination between good superalleles could result in a break-up of the cooperating loci and should lead to a reduction in the fitness of the recombinant offspring. Such a reduction in fitness was found in egg hatch data of *subobscura* and thus the presence of more than one superallele in the sampled locality was detected (WASSERMAN 1972). However, recombination had no detectable effect upon egg-to-adult survival in *pseudobscura*. The apparent inability of selection to differentiate between the various Arrowhead chromosomes and their recombinant products and between the Pikes Peak chromosomes and their recombinant products may, at least in part, be due to the fact that the parental chromosomes were poorly adapted to our laboratory conditions. Recombination between such poorly adapted chromosomes did not produce any further observable loss in fitness.

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