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Studies on Selection in Natural and Experimental Populations of Drosophila Pseudoobscura

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STUDIES ON SELECTION IN NATURAL AND EXPERIMENTAL POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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PREFACE

The purpose of the research reported in this thesis is to clarify some of the genetic mechanisms which operate in evolution. For the privilege of studying at The Rockefeller University, I am deeply grateful to President Detlev W. Bronk. I am grateful to Dean Frank Brink for his encouragement of my work and for his generous support of it. To Professor Th. Dobzhansky I owe the greatest debt of gratitude for his guidance and encouragement in every phase of this work. Every person in Professor Dobzhansky's laboratory has helped me; in particular, I am grateful to Dr. Lee Ehrman, Mrs. Olga Pavlovsky, Dr. Victor Salceda, and Mr. Boris Spassky. Mr. and Mrs. George Bradt, Dr. Wilson Crumpacker, Dr. Marvin Druger, Dr. Costas Kastritsis, Dr. W. D. Scowcroft, Miss Helen Stavrou, and Dr. Christopher Wills aided in the collections.

ABSTRACT

Two genetic characters in Drosophila pseudoobscura were utilized in an investigation of some of the genetic mechanisms in evolution. The characters are (1) body size, which is a continuously-varying, polygenic trait, and (2) the arrangement of genes along the third chromosome, which is a Mendelizing, discrete trait. Collections of Drosophila pseudoobscura were taken in many localities in the American West. The two characters vary regularly with the physiographic division of the West. This variation is evidence that the frequencies of the genes controlling each character are strongly regulated by selection; such variation is the first stage in the genetic divergence which leads to the formation of new species. The frequencies of the gene arrangements on the third chromosomes are contrasted with those obtained in previous samples dating back as far as thirty years. A consistent pattern of change is apparent. The agent of selection responsible for these changes cannot be decided at present, although several possibilities are discussed. The system of inversions on the third chromosome is shown to be independent of that on the X-chromosome.

One of the commonest geographic variations of insects is that of body size with temperature, the genetically larger strains coming from the cooler regions. Body size was studied in six experimental populations of Drosophila pseudoobscura which had been exposed to different temperatures. These populations were genetically identical at their inception but were maintained thereafter at different temperatures. After six years a striking genetic divergence in body size was found. The populations kept at the lower temperature had genetically larger flies than those kept at the higher temperatures. Crosses between the populations showed that the genes for larger size are partially dominant. The temperature-directed selection for body size in these experimental populations may well be similar to that which has produced the temperature-oriented gradients for body size in natural populations of several species of Drosophila.

Eleven experimental populations were derived from the samples of natural populations ranging from Canada to Mexico. Each population was begun, as far as practicable, with the same chromosomal constitution as had the sample from the locality in nature. These populations were crossed

to yield F_1 and F_2 hybrids. The F_1's varied irregularly, while the F_2's showed a consistent "breakdown" of size, the F_2's being significantly smaller than their F_1 parents. The natural populations have coadapted, or internally balanced, genetic systems, with genes mutually adjusted by selection for favorable interactions. Recombination disrupted the balanced genic complexes to give the F_2 breakdown. The frequencies of the inversions were followed in these same experimental populations for two years. The equilibrium frequencies established in the laboratory populations were quite different from those in the natural populations which were the ancestors of the laboratory ones. These results with Drosophila pseudoobscura stand in interesting contrast with those obtained by European workers who studied Drosophila subobscura. They lend additional support to the hypothesis that Drosophila pseudoobscura is genetically flexible, while Drosophila subobscura is genetically rigid. The contrast between the mode of genetic adjustment in the two species illustrates the very different pathways evolution may take in adapting similar organisms to similar environmental stresses.

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I. GENERAL INTRODUCTION

Just a little over one hundred years ago, in 1859, Charles Darwin published On the Origin of Species, documenting the theory of evolution by natural selection. We know today that Darwin was correct in most of the major points of his theory, with one important exception. He never uncovered the means by which parents transmit their characters to their offspring. The assimilation of genetic knowledge into evolutionary theory dates to the work of two mathematically-minded biologists and one biologically-minded mathematician, Haldane (1932), Wright (1931), and Fisher (1930). Simultaneously these three studied the consequences of particulate genes in populations. They found that evolutionary changes could be accounted for by their mathematical models. The mathematical investigations stimulated experimental biologists to attempt verification of the mathematical theory. Then began a period of synthesis, of assimilation of all biology into evolution, that continues to the present day. The result is the biological theory of evolution, rooted in genetics and ramifying into every field of biology. I will briefly sketch the most important contributions. Dobzhansky (1937) related genetics to evolution and thereby laid the cornerstone for the wide-ranging synthesis that we have today. Huxley (1942) presented a general review of the literature, interpreted in evolutionary terms. Mayr (1942) surveyed zoology and systematics and fit them to the new evolution. Stebbins (1950) reviewed the botanical literature in relation to evolutionary mechanisms. Rensch (1959) ranged over systematics, morphology, and paleontology to show how higher systematic categories arise. Schmaulhausen (1949) showed how the studies of comparative morphology contribute to evolution, and examined the role of different types of selection. White (1945) related cytology and evolution, and Darlington considered how different cytogenetic systems arise. Simpson (1944, 1953) showed that paleontology is consistent with the modern theory of evolution, and that the changes in the past were of the slow, gradual types that evolutionary theory predicts. Up-to-date treatments are given by Dobzhansky (1951), Grant (1963), and Mayr (1963).

The attention of evolutionists is directed today toward the mechanism of evolution, toward the processes which cause evolutionary changes and species formation, rather than toward the products themselves. Our interest

is at the intra-species level, for it is here that the basic step of evolution occurs. The present experiments were designed to clarify some of the genetic mechanisms in evolution. For this purpose I have chosen two characters. The first is body size, a quantitative, polygenically-determined trait which is closely related to fitness and to adaptedness. The second is a Mendelizing genetic character, the structural types of a particular chromosome. Each character is particularly well suited to investigate certain aspects of the evolutionary mechanism and its genetic basis. Together, these two characters exemplify the two great classes of inheritance, the continuous and the discrete. Our aim is the same in studying each; only the methods are different. Each of the different structural karyotypes may be followed throughout the analysis, and a precise mathematical formulation of its role in the population is possible. For body size, however, our analysis will be statistical, the genetic entities being characterized at the level of populations by means and by variances. Both natural and experimental populations have been studied in order to correlate the changes in nature with those in the laboratory, where the selective agents in the environment may be controlled.

II. MORPHOLOGICAL VARIATION IN DROSOPHILA PSEUDOOBSCURA

A. Introduction

1. General In most organisms variation in body size is polygenic, that is, determined by the action of many genes with individually small effects. Most characters of evolutionary importance have polygenic bases (Falconer, 1960); for this reason, size has often been used in evolutionary genetic studies. The general evolutionary implications of size are discussed by Bonner (1965) in a fascinating new book.

The experiments reported below were conducted on Drosophila pseudoobscura. Body size in this species is known to be a polygenic character with a high heritability. The heritability of a continuously varying, polygenic trait is the fraction of the total variation in that character which is genetic. The heritability of body size in Drosophila pseudoobscura lies somewhere between 25% and 35% (Frahm and Kojima, 1966); that is, of

all the variation in size under the carefully controlled conditions in the laboratory, from one-fourth to one-third is genetic. Size is a character rather accessible to selection in this species. Body size is correlated with fitness in the sense of evolutionary success; Tantawy and Vetukhiv (1960) and Tantawy (1961) found that larger flies lay more eggs and live longer than do smaller ones.

The experiments to be reported below attempt to study selection at two levels. We shall consider first selection by agents in the external environment, the sort of selection which leads to geographic variation in morphological characters. Second we shall consider the selection which acts internally to each population, selection which mutually adjusts the effects of the genes in a population to achieve the greatest fitness.

2. Geographic variation Within any population of organisms there are differences among the individual members in many phenotypic characters, body size being just one example. Variation occurs between populations, also, and often the variation follows a regular pattern with the environment. This geographic variation can usually be shown to have an underlying genetic basis. It furnishes perhaps the best evidence of evolution at work. We infer that the genetic differentiation among the populations has been brought about by the varying selections imposed by the varying environment. The local genetic differentiation within species creates the subspecific and varietal categories of the taxonomist. The divergence of geographically separated populations ranges from minor to nearly total.

For many variations a selective value may be demonstrated. The peppered moth, Biston betularia, is a good example (see Kettlewell, 1956, and Ford, 1964). These moths rest on the trunks and boughs of trees. In unpolluted environments, where the trees are covered with light-colored lichens, the moths are themselves light in color. Near industrial centers, however, the lichens are absent and the trees blackened with soot. In such blackened forests the moths are melanic, blending with the trees. The color differences between the light and dark forms is genetic, depending on a major gene with modification by others. Observations on both color types released together in each environment showed that about twice as many "conspicuous" moths were eaten as were those which blended with the environment. Selection thus occurred through differential predation by

birds. Geographic variation, as in color for the moths, is important because the differences at this level may widen in time to yield distinct species.

All types of organisms--invertebrates, vertebrates, plants, and microorganisms--vary with the environment. The genetic nature of the variation is demonstrated in experiments where organisms from different environments are raised together in a common one. Warm-blooded animals from the cooler range of the species are often larger and have shorter appendages relative to body length, than do animals from the warmer range. This variation probably results from the heat conserved at low temperatures by a larger body with shorter appendages (see Rensch 1959, 1960 for many examples). Turesson's (1922) classic studies of ecological variation in plants uncovered an amazing variety of modifications in response to environmental agents such as wind. Gause (1947) and his associates found variations in the size of various protozoans corresponding to the salinity of the water from which they were taken. The extent of pigmentation in many beetles is correlated with humidity, races in warm, humid areas being darker than races in cool, dry localities (see Dobzhansky, 1933 for an example). The literature on geographic variation is immense; Mayr (1963) and Grant (1963) have recently summarized the literature for animals and plants, respectively. We shall turn our attention to the genus Drosophila, the subject of the experiments to be reported below.

Geographical gradients in body size have been described in several species of Drosophila. Flies of the same species from cooler regions tend to be genetically larger than flies from warmer regions (Ray, 1960). Stalker and Carson (1947, 1948) found in Drosophila robusta a trend to increased wing length with increasing latitude and with increasing altitude. Thorax length, like wing length an index of general body size, increased with altitude but not with latitude. For Drosophila subobscura, Prevosti (1955) and Misra and Reeve (1964) found positive correlations between body dimensions and latitude, flies from the cooler regions being larger. Sokoloff (1965) found no evidence of a general cline in size correlated with latitude or any other factor in Drosophila pseudoobscura. As Sokoloff noted, however, the complex, mountainous topography of the territory which this species inhabits may obscure such relationships. Tantawy and Mallah

(1961) found that Drosophila melanogaster from southern regions of the Far East were smaller than those from northern areas. Thus, we see that several species of Drosophila vary in size over their normal habitat, and that temperature is one of the important factors in the variation.

Two sets of experiments were designed to study geographic variation in Drosophila pseudoobscura and to clarify the mechanisms by which the variation originates. Natural populations from Canada to Mexico were sampled to see how size varies among localities. Experimental populations begun by Dr. M. Vetukhiv shortly before his death offered an opportunity to study the selective effects of temperature. These populations, genetically identical at the beginning, were maintained at three different temperatures for over seven years. I have studied them to determine whether the relationships of body size to environmental temperature found in some of natural populations of Drosophila might be paralleled in Vetukhiv's experimental populations.

3. Coadaptation of genotypes Genes are considered as separate entities in the simple models which population geneticists customarily set up to follow the fate of genes in populations. The situation in actual populations, however, is far more complex. Linkage and interactions between genes are two important factors which force us to consider the genotype as a whole. Only in the last few years have mathematical geneticists been able to incorporate these refinements into the theory of populations. Lewontin (1964) studied the role of linkage, and Kojima (1959, 1961), the role of genic interactions, or epistasis. The mathematical treatments suggest crucial roles for linkage and epistasis in evolution.

Let us consider a population of organisms, such as Drosophila. We have already seen that the organisms will vary quantitatively in many characters as we consider populations in different environments. The geographic variations result from selection to adapt the organisms to the specific local conditions. Within each population the genes will also be selected to operate together for maximal fitness. Specific favorable linkage relations will be established, and genes which interact synergistically for increased adaptation and reproduction will be selected. The gene pool, that is, the collection of all genes in the population,

adjusts itself; Dobzhansky (1949) has called this internal adjustment coadaptation.

Vetukhiv (1953, 1954, 1956, 1957, 1959) performed a series of experiments to determine the extent of coadaptation in populations of Drosophilia pseudoobscura. Crossing flies from different populations, he obtained F_1 and F_2 generations, and compared the performance of both hybrid generations and the parental strains for longevity, viability, and fecundity. The F_1 hybrids often outperformed their parents as judged by several criteria; the F_2 hybrids fell below the F_1's and below the parental strains. The F_1 heterosis, or hybrid vigor, reflects perhaps the increased heterozygosity of the F_1's; crosses between geographically disparate populations should give the maximal heterozygosity. Each F_1 has inherited a complete, integrated set of genes and chromosomes from each parent. These balanced complexes of genes will be disrupted and shuffled by recombination in the F_1 parents of the F_2's (see Wallace and Vetukhiv, 1955, and Wallace, 1959). The more important the mutual adjustment of the genes within each population, the greater will be the decline of the performance in F_2's. Wallace (1955) studied viability in crosses between geographically separated populations of Drosophila melanogaster, and found the F_2 breakdown expected for coadapted genetic systems. Brncic (1954) extended Vetukhiv's work with Drosophila pseudoobscura by studying viability under intense competition; he found the F_2 breakdown and was able to show its basis in recombination. King (1955a, 1955b) found that coadapted gene pools had evolved in experimental populations of Drosophila melanogaster selected for resistance to DDT. Recent work of McFarquhar and Robertson (1963) on Drosophila subobscura, a wide-ranging European species, has raised some interesting questions concerning coadaptation. They found no evidence of F_1 heterosis or F_2 breakdown in crosses of widely separated populations. Their chief criterion was body size, which is a polygenic character that should reflect coadaptation about as well as any other. Since their criterion of body size was not employed in any of the previous experiments, though, they might have been so unlucky as to pick an insensitive trait. On the other hand, the difference between their results with Drosophila subobscura and the previous work on Drosophila pseudoobscura and Drosophila melanogaster might be due to different genetic systems in the species. Populations of Drosophila subobscura may possess less flexible, more rigid genetic

structures than do populations of Drosophila pseudoobscura or Drosophila melanogaster. Differences in genetic structures between species would constitute a valuable addition to our knowledge of the various paths populations may take in response to selection. Experiments were therefore set up to determine whether body size in different geographical populations of Drosophila pseudoobscura is coadapted.

B. Materials and Methods

1. The sampling of natural populations. Drosophila psuedoobscura is an American species confined to the West. Populations exist from Canada through Guatemala, and from the Pacific coast to the western margin of the great plains. Eleven widely separated localities were selected to represent, as far as possible, the whole range of the species. These localities are shown in Figure 1. The flies for the experiments on morphological variation were collected from April through August, 1964. Buckets of fermenting bananas were set out in likely spots, usually under trees and near a stream. The flies attracted to the bait were recovered with a sweep net. They were tentatively classified in the field and shipped to New York.

2. The experimental populations established from the collections in nature Experimental populations as representative of the natural ones as possible were established in the laboratory. Each female inseminated in nature was placed in a separate culture bottle. Twenty females and twenty male offspring of each female were placed in plastic population cages, one cage per natural population, and maintained at 16°C until December, 1964. Genetic changes are known to be slight at this temperature (Wright and Dobzhansky, 1946). Hence each population reached a large size with a minimum of selection. The population cages are plastic boxes, 31 cm by 26 cm by 11 cm, with fifteen food cups inserted into the bottoms (Figure 2). Large and fairly stable populations (1000-4000 flies) are maintained in these cages. Late in December, 1964, after they were sampled to study differences in body size, the populations were transferred to a constant temperature room at 25°C; they remained there until the end of the experiments. The temperature in this room has accidentally fluctuated on several occasions as much as two or three degrees below and one degree above, 25°C. The relative humidity was not controlled and fluctuated with the seasons from a low of 25% in the winter to a high of 65% in the

Fig. 1. The localities at which collections were made. Table II gives the physiographic province of each locality.

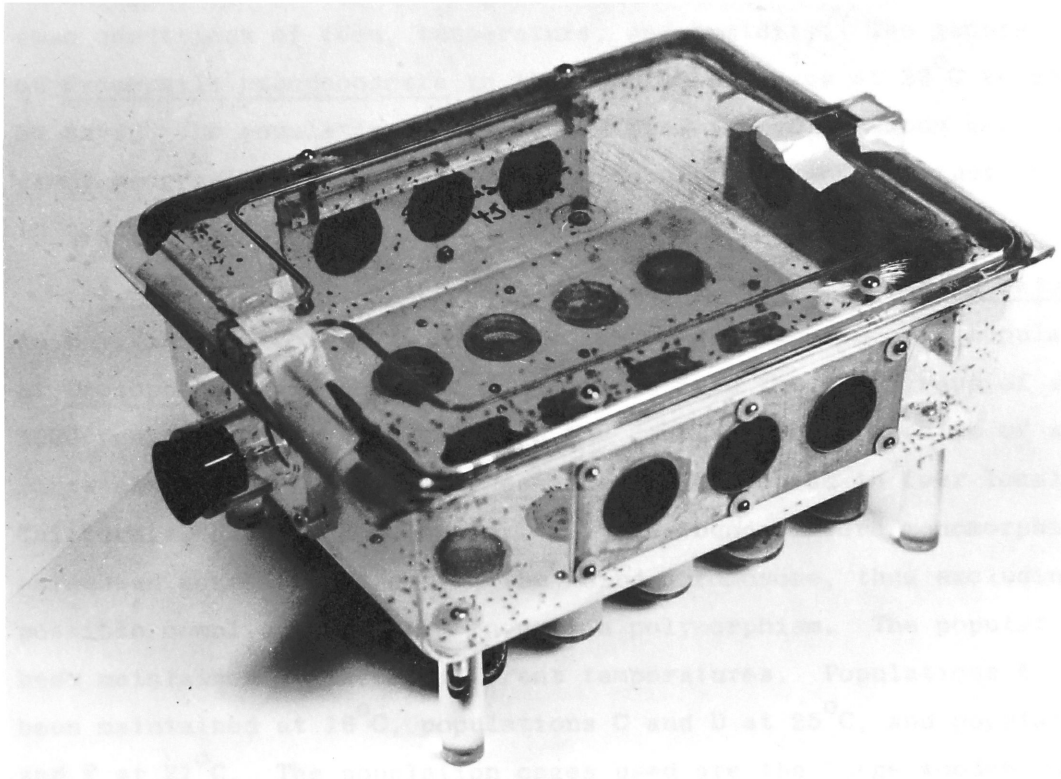

Fig. 2. The population cage for Drosophila.

summer. Spassky's (1943) cream-of-wheat medium was used in the food cups until September, 1965. Thereafter, a food enriched with Brewer's yeast, Ohba's (1961) 10% medium, was used for its convenience in the sampling of the populations. All the populations were exposed to the same conditions of food, temperature, and humidity. The generation time of Drosophila pseudoobscura in the population cages at 25°C is about 30 days. The populations established from the collections were used to study geographic variation among the populations and to study coadaptation in crosses among them.

3. The experimental populations kept at different temperatures In May, 1958, Dr. M. Vetukhiv established six experimental populations of Drosophila pseudoobscura, all derived from the same group of about 1000 founders. The founders were the double-cross progenies of about forty strains of Drosophila pseudoobscura collected in four localities in California, Utah, and Colorado. All the founders were monomorphic for the Arrowhead gene arrangement in the third chromosome, thus excluding any possible complications from inversion polymorphism. The populations have been maintained at three different temperatures. Populations A and B have been maintained at 16°C, populations C and D at 25°C, and populations E and F at 27°C. The population cages used are the large wooden boxes described by Wright and Dobzhansky (1946); these cages support large populations of several thousand flies. After his death, Vetukhiv's populations were maintained by Mr. Boris Spassky and Mrs. Olga Pavlovsky. Vetukhiv's populations were utilized to determine whether temperature directs selection for different body sizes.

4. The measurements The body weight of individual flies is difficult to measure and is sensitive to environmental conditions, humidity in particular. Following other workers, I chose wing length as the index of size. Wing length does not vary with the conditions at the time of measurement, and accurate measurement of each individual is possible. Sokoloff (1966) studied body size in Drosophila pseudoobscura and measured both wet weight and wing length to see how they were correlated. For flies from six localities he found the correlation to be 0.81. The sizes of males and females (measured as weight or wing length) was almost perfectly correlated. My own data show similar correlations. We may, then, confidently use the wing length of one sex as an index of the body size in a given population.

This procedure is standard in studies of size in Drosophila (Stalker and Carson, 1948; Prevosti, 1955; Teissier, 1957; Tantawy and Mallah, 1961; MacFarquhar and Robertson, 1963; and Misra and Reeve, 1964).

The length of the wing along the third longitudinal vein, from the outer margin of the anterior crossvein to the tip of the wing, has been used as the measure of size (see Figure 3). Left wings were removed and mounted in Canada balsam for later measurement. The measurements were made under a compound microscope at magnification X63, with an ocular micrometer of 100 divisions. At 19°C the average female wing measured about 90 scale divisions, and the average male wing, 80 divisions. Wing length was recorded to the nearest unit of the micrometer scale. A unit on the ocular micrometer scale corresponds to 20.8μ.

For the determination of wet body weight, small groups, containing nine flies on the average, were weighed on a chemical balance registering to 0.1 mg. Males and females were weighed separately when they were six to nine hours old. The average female weighed 1.25 mg at 25°C, and the average male, 0.98 mg. To measure development time, eggs were collected over an eight to twelve hour period, and samples of fifty eggs were placed in each of eleven replicate bottles for each population. The number of adults appearing was recorded once each day. Since almost all flies hatched early in the morning, counting was done late in the afternoon to insure fully expanded wings for the concomitant determinations of wing length.

5. Design of the experiments and statistical techniques The following procedure was adopted for all the experiments on morphological variation. Samples of approximately 1000 eggs were taken from each cage, and subdivided among six bottles. The adults coming from the initial egg sample were then placed in vials with spoons containing Kalmus' (1943) medium, blackened with charcoal, for the collection of eggs. Several hundred parents were used per population, distributed over five to ten vials. Counted samples of fifty (or, in a few cases, 100) eggs were then placed in yeasted half pint bottles with Spassky's (1943) cream-of-wheat medium. For each experiment these bottles were kept at the same temperature in which the initial egg sample was incubated. (The only exception to this routine was the study of wing length at 16°C; in this one case the initial sample

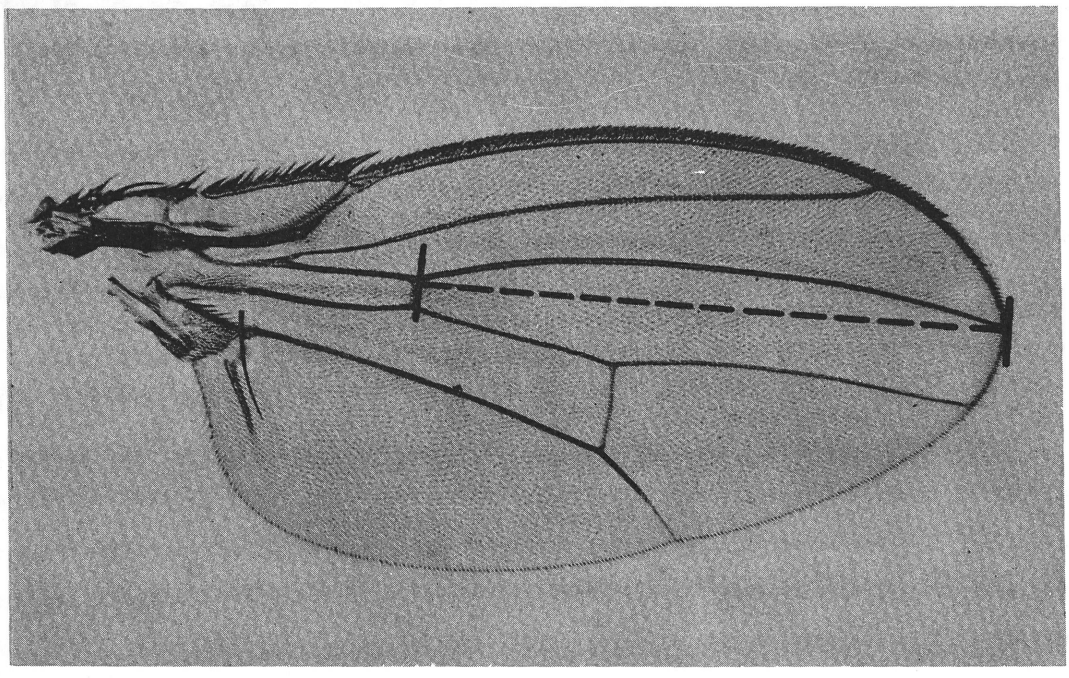

Fig. 3. The index of wing length used to measure body size.

was incubated at 25°C and the measurements made on flies raised at 16°C.) Thus, all of the flies actually measured were one generation removed from their cages and temperatures of origin and were raised under uncrowded, nearly optimal conditions. This procedure should have eliminated possible effects on the eggs of the different environmental temperatures at which the experimental populations were maintained. In all experiments, wings were removed from a random sample of all the flies hatching in a given culture. All experimental cultures were kept in circulating air incubators in which the temperatures only rarely varied as much as 0.5°C on either side of those desired. Bottles were randomized, and, wherever possible, all the bottles for a single experiment were kept on the same shelf within the incubator.

The experiments on wet body weight at 25°C and on wing length at 16°C had 100 eggs in each of four replicate bottles per population. All flies emerging were measured. On analyzing the data from these two experiments it became apparent that the variance between replicate bottles was large compared to the variance within bottles, undoubtedly a reflection of the unavoidable variations in food, humidity, and yeast among the culture bottles. Accordingly, all other experiments were set up with ten replicate bottles, each containing fifty eggs, for each population studied. Ten wings per sex from each of the ten bottles were measured per population. In many experiments only female wings were measured. A separate set of parental cultures was raised simultaneously with both F_1 and F_2 hybrid generations of crosses between populations. All reciprocal crosses were made.

Two experiments carried out at 19°C give an idea of the repeatability of body size measurements (Figure 4). The two sets of measurements, 19°C I and 19°C II in Figure 4, were obtained eight months apart. The agreement between them is apparent.

Individuals within a single bottle share a common, fairly uniform environment; the variance of body size within bottles is therefore largely genetic. The genotypes within different replicate bottles are, on the average, the same; hence the variance between bottles is largely environmental. The within-bottle variance is the index of genetic variability we shall adopt. Within each sex at each temperature there was no evidence of a dependence of within-bottle variance on the mean body size. The

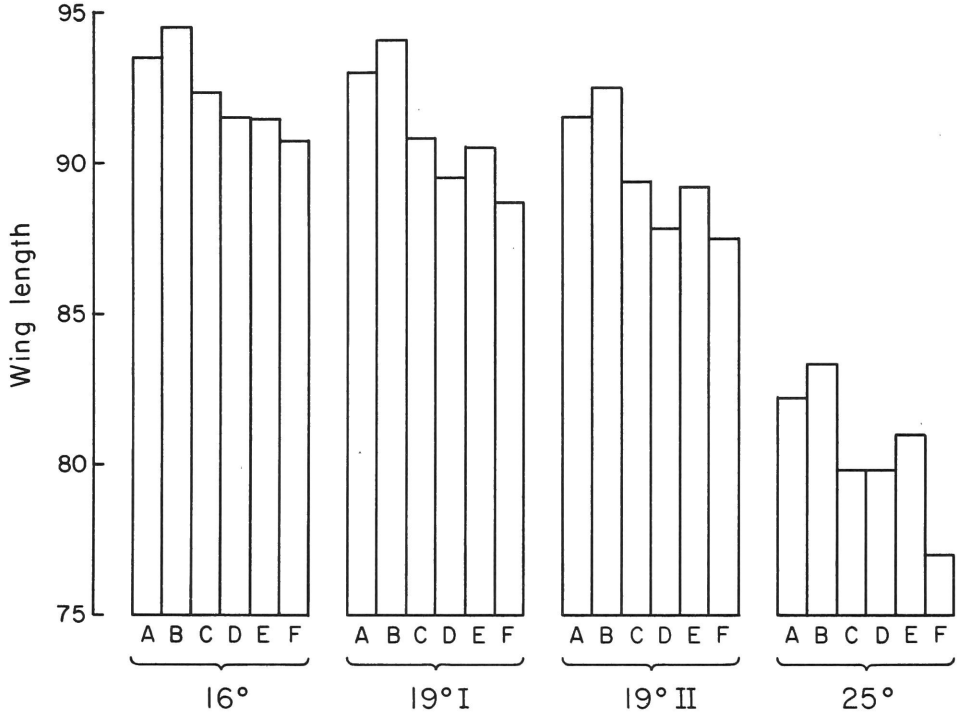

Fig. 4. Wing length of females from Vetukhiv's populations in tests at three temperatures. Note that the ordinate begins at 75 units. Length in units of the micrometer scale, 1 unit = 20.8 μ.

statistical analyses were therefore carried out on the untransformed data. Separate analyses were made for each sex at each temperature.

Error variances are based on variance within culture bottles and on variance between replicate bottles, The between-bottle mean square was significantly greater ($P<0.01$) than the within-bottle mean square for every experiment. This reflects greater environmental differences between bottles as compared to the relatively uniform environments within individual bottles. All but the earliest experiments were designed to minimize the error variance by increasing the number of replicates for each population studied.

Standard errors of the population means were obtained from pooled error variances. Steel and Torrie (1960) was used as a reference to the statistical calculations.

C. Results

1. The normal range of phenotypic variation In almost all experiments, counted numbers of fifty eggs were placed in the bottles. The viability varied from cross to cross, however, An experiment was performed at 25°C to see whether variation in the number of adults emerging in each bottle affected wing length over the range of densities encountered in the main experiments. The number of adults emerging is an accurate index of larval competition, since very few larvae fail to reach adulthood under these uncrowded conditions. The results are summarized in Table 1. The dependence of wing length on density is small and insignificant.

Since one of the aims of the present study was to explore the possible selective effects of temperature on body size, the normal phenotypic effects of temperature on body size may usefully be described first. No differences in the phenotypic response to temperature were found among various populations, so data from several were pooled. Ten or more replicate bottles, each containing fifty eggs, were placed at 16°C, 19°C, 25°C and 27°C. Ten female wings from each bottle were measured. The mean sizes and their standard errors were (1 unit = 20.8μ):

16°C	19°C	25°C	27°C
92.32 ± 0.57	87.58 ± 0.57	79.02 ± 0.35	74.81 ± 9.33

Table I. The relationship of wing length and larval density in *Drosophila pseudoobscura* raised under standard laboratory conditions.

No. adults emerging in bottle	Mean wing length ♀♀*	Mean wing length ♂♂*	No. adults emerging in bottle	Mean wing length ♀♀	Mean wing length ♂♂
7	81.67	74.50	35	80.80	73.70
8	79.67	72.00	36	81.20	72.00
8	80.00	72.25	37	81.30	74.20
9	81.17	74.68	37	80.00	73.50
9	80.00	71.00	40	80.30	73.60
14	79.50	75.20	40	79.70	72.80
16	80.71	72.56	41	79.90	73.30
19	81.00	73.30	42	80.40	73.70
22	79.90	73.13	50	80.60	73.10
25	79.60	71.78	51	81.00	74.90
26	81.40	75.00	51	80.30	73.30
28	79.30	72.50	53	80.70	73.80

Regression of ♀♀ wing length on density: $b = .003 \pm .009$

Regression of ♂♂ wing length on density: $b = .013 \pm .018$

*one unit = 20.8μ

The size difference between the flies raised at 16°C and 25°C is 17% of the wing length at 25°C, while the difference between flies raised at 25°C and 27°C is only about 5% of the wing length at 25°C. There are, then, large differences in size between flies raised at 16°C and at either 25°C or 27°C; there is little difference between flies raised at 25°C and 27°C.

2. The natural populations In Table II the body sizes in eleven widely separated geographic populations are given, along with the names of the physiographic provinces from which they came. The differences in size among the populations are highly significant ($P<0.005$), as shown in Table III. Figure 5 presents the geographic variation in size visually; to accentuate the differences between the populations, the scale and the heights of the bars are in units of (wing length-85) X10. The localities from the Pacific coast are smaller than those from the interior. Lumping the populations to form "interior" and "coastal" groups, Scheffe's (1953, 1959) test was applied to test the significance of the difference between the two groups. The average size in the four Pacific Coast localities was highly significantly different ($P<0.005$) from the average size in the interior populations. The collection from Sonora, Mexico was included among the interior populations since it came from a desert area typical of the interior.

3. The experimental populations exposed to different temperatures. The six experimental populations begun by Dr. M. Vetukhiv were split into three groups and placed, two each, at 16°C, 25°C and 27°C. When the populations were one and a half years old (after Dr. Vetukhiv's death), Mrs. M. Krimbas surveyed the populations for a variety of characters, wing length included. Adults taken from each population were allowed to oviposit and the eggs were placed in equal numbers in ten replicate bottles. Five bottles were raised at 15°C and the other five at 25°C. Mrs. Krimbas recorded wing length on a micrometer scale with units of 104μ. I have recalculated Mrs. Krimbas' data, adjusting her measurements to the scale used in my own measurements; the data are given in Tables IV and V. There were no significant differences among the populations at one and a half years.

Table II. Body size in geographic populations of Drosophila pseudoobscura.

Population	Province	♀♀ wing length at 19°C*
Austin, Texas	Texas	91.79
Raton, New Mexico	Rocky Mountains	90.48
Tucson, Arizona	Basin and Range	90.25
Black Canyon, N.M., Colorado	Rocky Mountains	89.80
Davis, Texas	Texas	89.02
Sonora, Mexico	Basin and Range	88.76
Hayden Creek, Colorado	Rocky Mountains	88.33
Methow, Washington	Pacific Coast	88.24
Okanagan, British Columbia	Pacific Coast	88.11
Berkeley, California	Pacific Coast	86.53
Riverside, California	Pacific Coast	86.37
Average standard error		0.618

* Mean of 100 measurements. One unit = 20.8μ.

Table III. Analysis of variance of body size in geographic populations of *Drosophila pseudoobscura*; females only, at 19°C.

	df	MS	F
Populations	10	269.02	7.04***
Error	99	38.23	

*** $P < .005$

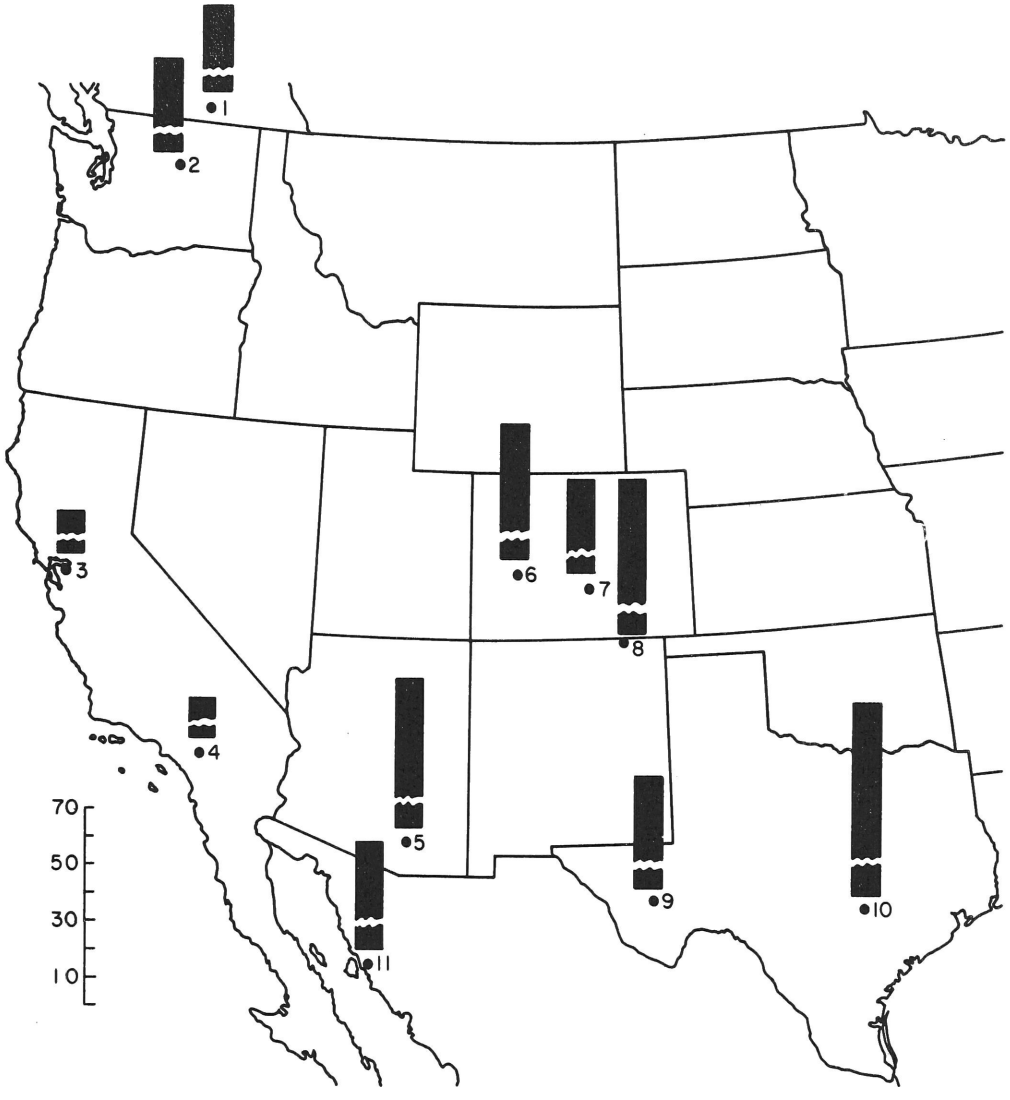

Fig. 5. Body size in natural populations of Drosophila pseudoobscura. The bars are proportional to the mean wing length of females in the populations. The scale is explained in the text. The localities are the same as in Fig. 1.

Table IV. Mean body weight, wing length, and development time of flies from the experimental populations maintained at different temperatures.

		Populations						
		A	B	C	D	E	F	S.E.*
A. Experiments at 1½ years								
WING LENGTH[1]	♀ at 15°	88.35	88.45	88.85	90.95	89.70	89.25	0.80
	♂ at 15°	81.90	81.95	82.05	83.00	82.20	82.20	0.65
	♀ at 25°	78.75	78.70	79.00	79.95	79.90	78.95	0.70
	♂ at 25°	73.05	72.85	73.35	74.30	74.00	74.00	0.60
B. Experiments at about 6 years								
BODY WT (MG)	♀ at 25°	1.26	1.33	1.18	1.18	1.24	1.16	0.06
	♂ at 25°	1.01	1.08	0.95	0.94	0.98	0.92	0.04
WING LENGTH[1]	♀ at 16°	93.47	94.55	92.44	91.60	91.47	90.72	0.57
	♀ at 19°	91.54	92.57	89.40	87.82	89.15	87.46	0.40
	♂ at 19°	84.22	84.52	81.80	80.10	82.11	80.34	0.34
	♀ at 25°	82.11	83.36	79.78	79.79	80.88	76.86	0.35
DEVELOPMENT TIME (DAYS)	♀ at 19°	19.83	19.86	20.12	20.05	19.72	20.03	0.09
	♂ at 19°	20.66	20.66	21.01	20.92	20.57	20.86	0.11

* Standard error for every mean in a given experiment, obtained from pooled error variance.

[1] one unit = 20.8 μ.

Table V. Analysis of variance for body size of flies from the populations maintained at different temperatures.

			Females			Males		
			df	MS	F	df	MS	F
A. Experiment at 1 ½ years								
WING LENGTH[1]	at 15°	Cages	5	94.25	1.47	5	16.25	
		Error	24	64.25		24	41.50	
	at 25°	Cages	5	32.25		5	33.75	
		Error	24	51.50		24	37.50	
B. Experiments at about 6 years								
WING LENGTH[1]	at 16°	Cages	5	408.94	6.22**			
		Error	17	65.75				
	at 19°	Cages	5	410.76	26.18**	5	349.64	29.76**
		Error	54	15.69		54	11.75	
	at 25°	Cages	5	478.06	39.32**			
		Error	55	12.16				
BODY WT (MG)	at 25°	Cages	5	.64	1.29	5	.51	2.10
		Error	18	.49		18	.25	
DEVELOPMENT TIME (DAYS)	at 19°	Cages	5	5.95	2.71*	5	8.12	2.41*
		Error	60	2.20		60	3.37	

* Significant at the .05 level.

** Significant at the .005 level.

[1] one unit = 20.8 μ.

When the populations were about six years old, measurements of wet body weight and of wing length were made at 16°C, 19°C, and 25°C. The mean size and its standard error for each population is given in Table IV, and the analyses of variance for these experiments are given in Table V. The differences in wing length among the populations are statistically highly significant ($P < 0.005$). The differences in wet body weight are consistent with the results of the wing measurements, but the variance between replicate bottles was large enough to obscure such differences as apparently did exist. The mean sizes show a pattern, illustrated in Figure 4. The two "cold" populations, maintained at 16°C, are consistently larger than the four "warm" populations, maintained at 25°C and 27°C, when compared at 16°C, 19°C and 25°C. Statistical comparison of the "cold" and "warm" groups of populations are given in Table VI. The comparisons are not significant at one and a half years, but are all highly significant at six years. Genetically larger size has developed at the lower temperature, and genetically smaller size, at the higher temperatures. The average of sizes in the two populations from 25°C is nearly identical with the average in the two populations from 27°C.

The genetic divergence in size among Vetukhiv's populations is striking. The difference between the largest mean size, always in population B from 16°C, and the smallest mean size, always in population F from 27°C, was 8% of the average of all body sizes in the experiment at 25°C. This is half the total phenotypic variation in size over the 16°C-25°C temperature range. An idea of how far the divergence has progressed can be gained from comparisons between all pairs of means. Since there are fifteen of these comparisons and they were not planned before the experiment was conducted, special precautions must be taken to avoid spurious statements of significance. Scheffe (1953, 1959) has designed a test for just these circumstances, a test which is necessarily conservative in judging a difference as significant. Comparisons of all pairs of means by Scheffe's test are given in Table VII. Notwithstanding the conservative nature of the test, many differences are highly significant.

The time of development from egg to adult and the wing length were both measured on the same flies in an experiment at 19°C. The time of development is different among the six populations (Tables IV and V),

Table VI. Comparison of mean body sizes in "cold" populations (A and B) and in "warm" populations (C, D, E, and F).

		$\frac{1}{2}$ (A + B) - $\frac{1}{4}$ (C + D + E + F)	
		♀	♂
A. Experiment at 1 1/2 years			
WING LENGTH[1]	at 15°	-1.30	-0.45
	at 25°	-0.70	-0.95
B. Experiments at about 6 years			
BODY WT(MG)	at 25°	0.10*	0.10*
WING LENGTH[1]	at 16°	2.45**	
	at 19°	3.60**	3.28**
	at 25°	3.41**	
DEVELOPMENT TIME (DAYS)	at 19°	-0.13*	-0.18*

* Significant at the .05 level.

** Significant at the .005 level.

[1] one unit = 20.8μ.

Table VII. Statistical significance of differences in mean wing length between the populations maintained at different temperatures at 6 years. (ns = not significant).

A. Experiment at 19°C. ♀'s above diagonal, ♂'s below

	A	B	C	D	E	F
A		ns	.05	.005	.01	.005
B	ns		.005	.005	.005	.005
C	.005	.005		ns	ns	.05
D	.005	.005	.05		ns	ns
E	.005	.005	ns	.01		ns
F	.005	.005	ns	ns	.05	

B. Experiment at 25°C. ♀'s only

	A	B	C	D	E	F
A		ns	.005	.005	ns	.005
B			.005	.005	.005	.005
C				ns	ns	.005
D					ns	.005
E						.005
F						

although at a marginal level of significance. The mean time of development is slightly shorter in the populations with the larger flies. The mean time of development in the progenies of the flies derived from the two "cold" populations was shorter than the mean for the four "warm" cages, again at a marginal level of significance. Development time at 16°C is twice as long as at 25°C. A test of development time at 25°C was not possible, since the differences among the populations become so small they are masked by sampling errors. At lower temperatures development time is extended, without a proportionate increase in variance. A shorter development time is a selective advantage; the genotype developing first leaves offspring first and will in time contribute more genes to the population than will the slower-developing genotypes. The slightly shorter development time in the populations kept at the lower temperature very likely resulted from such selection for shorter development time; the low temperature magnified the differences among the populations, making them more accessible to differential selection.

Two generations of hybrids between some of the populations were studied in order to understand something of the genetic systems responsible for the different sizes among the populations. In both F_1 and F_2 generations, samples from the original populations were simultaneously raised as standards. Those samples from the original populations which were raised with the F_2 hybrids were about 2% larger, on the average, than were those raised with the F_1's. The F_2 means were adjusted by this factor before comparison with the F_1 means.

Variation in body size in Drosophila is known to be polygenic. If the various genes for size act additively, then each hybrid generation should be the average of the sizes of its parents. This average is usually called the midparent. Heterosis occurs when the hybrids are larger than the midparent; breakdown occurs when they are smaller than the midparent. Overdominant loci often contribute to heterosis. The heterozygotes for an overdominant locus have a phenotype higher on the scale by which we judge performance than either homozygote. With body size as our criterion, overdominant loci would yield larger flies when the loci were heterozygous than when homozygous (see Robertson, 1954, for an example).

Heterosis in the F_1 may be due to the combination of two "internally balanced" sets of chromosomes, one complete set from each of the two populations. The favorable synergism within each balanced set of chromosomes is retained, and heterozygosity is increased. The increased heterozygosity has a particularly powerful effect at overdominant loci. Heterosis in the F_1 may also be due to partial dominance of the genes for larger size.

Breakdown in the F_2 could arise from the shuffling of synergistic combinations of genes by recombination. The comparisons F_1-midparent and F_1-F_2 are given in Table VIII. Six of the nine comparisons F_1-midparent are significant; only two of the nine comparisons F_1-F_2 are significant. The absence of widespread F_2 breakdown indicates that Vetukhiv's six populations have not evolved separate, integrated gene pools. The F_1 heterosis is probably the result of partially dominant genes for larger size. Another way to detect integrated genetic systems is to study variability. The F_1 hybrids between populations with coadapted, or internally adjusted, gene pools usually are less variable than their parental populations. The F_2's, by contrast, are usually more variable, an indication of the breakdown of highly selected synergistic systems. The pooled within-bottle variances are our best estimates of intrinsic, genetic, variability. The variability of the F_1 and F_2 generations are compared with those of the parental populations raised with each in Table IX. There is no evidence of a change in variability in either hybrid generation. We are justified in considering Vetukhiv's populations to have diverged genetically, probably by the selection of partially dominant genes for size, but not to have been separated long enough for the populations to have achieved new, different levels of internal genetic balance.

With two exceptions, the reciprocal crosses for each F_1 and F_2 were not significantly different. The exceptions involved population C from $25^{\circ}C$. The difference between the crosses C x A and A x C was highly significant ($P < 0.001$), CA being the larger. This effect persisted into the F_2 generation, the F_2 derived from the C x A F_1 being larger than the F_2 derived from the A x C F_1. The crosses C x F and F x C also suggested a possible maternal effect on size in population C. Unfortunately, all

Table VIII. Comparison of wing lengths in hybrids and parents from the populations maintained at different temperatures; females only, at 19°C.

Cross[1]	F_1 - midparent	F_1 - F_2
AB & BA	-0.26	-0.35
AC	-0.71	0.78
CA	1.65**	-0.49
AE & EA	1.26**	1.00*
AF & FA	-0.05	1.08*
BE & EB	1.15**	0.39
BF & FB	0.85*	0.23
CF	2.02**	-0.30
EF & FE	1.43***	0.47

Comparisons are in micrometer scale units; one unit = 20.8μ.

* Significant at the .05 level.

** Significant at the .01 level.

*** Significant at the .001 level.

[1] female parent given first; i.e., AB = A x B

Table IX. Comparison of pooled within-bottle variances of wing length in parents and hybrids from the populations maintained at different temperatures; females only, at 19°C.

		df	MS	F
a)	Parents	270	2.71	1.01
	F_1	621	2.69	
b)	Parents	270	3.61	1.15
	F_2	720	3.13	
c)	F_1	621	2.69	1.14
	F_2*	720	2.35	

* Adjusted for difference in average sizes of F_1's and F_2's.

but two bottles of crosses FC were lost; although the two remaining bottles contained sufficient flies to obtain an F_2 generation, no accurate data can be given for the F_1 generation. A highly significant ($P<0.01$) difference between the F_2's of crosses CF and FC was found, CF being the larger. A maternal effect on body size seems to exist in population C.

The means, numbers of flies examined, and standard errors are listed for reference in the appendix, Table XX.

4. Coadaptation in crosses among the populations from nature. The populations established from the collections in nature were kept at 25°C for one-and-a-half years. Sampling them in the usual way, body sizes were again determined; the data are given in Table X. The ranking by size is the same as in the determination one-and-a-half years previous, excepting only the Canadian population. The population from Okanagan, British Columbia, had a larger mean size in the later sample. On the whole, the sizes changed very little; the crosses between the populations will certainly reflect the genetic systems for body size in the natural populations. If anything, maintenance in the laboratory diminished the extent of coadaptation, and new integrated gene pools are very unlikely to have developed in so short a time. The results on Vetukhiv's populations bear out this last point. Our estimates of coadaptation, then, are conservative.

Vetukhiv and Beardmore (1959) found that F_1 heterosis and F_2 breakdown, as indices of coadaptation, depend on the environment of the experiment. Under stringent conditions, the effects are pronounced; under optimal conditions they may not be detected. My experiments were therefore conducted at 25°C, to accentuate the effects of coadaptation and to make their measurements more accurate.

The F_1 and F_2 generations of all combinations of the seven populations listed in Table X were studied, a set of the parental populations being raised simultaneously with each hybrid generation. All reciprocal crosses were made. Ten female wings from each of seven replicate bottles per reciprocal cross were measured. The data are presented in Tables XI and XII; maternal effects appeared, so the data have been divided into crosses in which the reciprocals were the same and into crosses in which the reciprocals were different. Splitting the data this way allows us to

Table X. Body size in geographic populations of Drosophila pseudoobscura after 1 1/2 years in the laboratory at 25°C. Females only.

Population	Wing length* at 25°C	Population	Wing length* at 25°C
Okanagan, British Columgia	82.057	Hayden Creek, Colorado	80.296
Austin, Texas	81.483	Berkeley, California	80.157
Tucson, Arizona	81.096	Riverside, California	79.133
Black Canyon N.M., Colorado	80.882	Average standard error	0.175

* Mean of 280 measurements. One unit = 20.8μ.

Table XI. Comparisons of wing length in hybrids between geographic populations and their parents.

A. Crosses not involving maternal effects; females only, at 25°C

Cross[(a)]	F_1 - MP	F_2 - MP	F_1 - F_2
SB & BS	0.45	0.04	0.37
SH & HS	-1.33***	-2.15***	0.94**
SR & RS	0.87**	-0.69*	1.53***
AB & BA	0.05	-0.28	-0.21
AR & RA	0.72*	-0.69*	0.90**
AC & CA	-0.24	1.45**	1.05**
BH & HB	0.25	0.11	0.00
BR & RB	0.22	0.55	-0.63
RC & CR	-0.72	-1.57***	0.91**

All comparisons are in micrometer scale units; one unit = 20.8 μ

* Significant at the .05 level.

** Significant at the .01 level.

*** Significant at the .001 level.

(a) Female parent given first.

Abbreviations:

A = Austin, Texas

B = Black Canyon N.M., Colorado

C = Okanagan, British Columbia

H = Hayden Creek, Colorado

R = Riverside, California

S = Berkeley, California

T = Tucson, Arizona

Table XII. Comparisons of wing length in hybrids between geographic populations and their parents. B. Crosses involving maternal effects; females only, at 25°C.

Cross [a]	F_1 - MP	F_2 - MP	F_1 - F_2	Cross [a]	F_1 - MP	F_2 - MP	F_1 - F_2
ST	1.38***	-0.06	1.59***	TR	0.34	0.85*	0.36
TS	-0.23		-0.02	RT		-0.86*	1.08*
SA	2.00***	1.68***	0.07	TC	-0.43	-0.27	0.07
AS	-2.23***	-2.04***	-0.44	CT		-1.27***	1.07*
SC	0.18	-1.35***	1.85***	AH	1.18***	-0.14	0.97*
CS		0.10	0.40	HA		-1.67***	2.50***
TA	1.45***	-0.75**	1.85***	BC	0.24	-1.68***	1.95***
AT	-0.58		-0.18	CB		0.31	-0.04
TB	-1.36***	1.34***	-2.83***	HR	-1.21*	-0.81**	-0.53
BT	0.74		-0.73	RH	-0.21		0.90
TH	0.35	0.35	0.05	HC	0.01	-0.23	0.46
HT	-0.86		-1.16*	CH		0.81*	-0.58

All comparisons are in micrometer scale units; one unit = 20.8 μ.

* Significant at the .05 level.

** Significant at the .01 level.

*** Significant at the .001 level.

[a] Female parent given first; abbreviations are as in Table XI.

see if the maternal effects bias our interpretation of the comparisons F_1-midparent, F_2-midparent, and F_1-F_2. There is no pattern to the comparisons F_1-midparent; in some cases the differences are positive and in others, negative. The comparisons F_2-midparent and F_1-F_2, by contrast, are more frequently significant and are consistently positive. There was no strong F_1 heterosis, but there was a pronounced F_2 breakdown. The findings are the same in the crosses which showed no maternal effects on size and in those which did. Body size in Drosophila pseudoobscura is evidently part of an integrated, internally adjusted genetic system. Recombination between different coadapted systems results in a loss of this integration and a consequent breakdown in the F_2 hybrids.

The reciprocal crosses are compared in Table XIII. Only in one case did a maternal effect persist for both hybrid generations. The maternal effects probably arose from interactions of the genotypes with the maternal cytoplasm. Each reciprocal line had an originally different maternal cytoplasm; the genotypes changed, however, by recombination in the F_1's. Similar maternal effects on body size in this (Prout, 1959) and other (McFarquhar and Robertson, 1963) species of Drosophila have been reported.

The variabilities of the F_1 and F_2 are compared with those of the sets of parental populations raised as standards with each in Table XIV. The variabilities of the F_1 and F_2 hybrids are also compared, after adjustment of the F_2 means for the size difference between the parents raised with the F_1's and the parents raised with the F_2's. The adjustment removes the average effect on size which different batches of food usually produce. The F_1's were highly significantly less variable than the parents, and the F_2's were highly significantly more variable than either the parents raised with them or than the F_1's. These findings corroborate the evidence for coadaptation from the comparisons of size among parents and hybrids.

The means of the populations, the numbers of flies measured, and the standard errors are given for reference in the appendix, Table XXI.

D. Discussion

Body size in Drosophila pseudoobscura is a continuously varying character with a known genetic basis; the heritability of size in this

Table XIII. Maternal effects on body size in crosses between geographic populations of Drosophila pseudoobscura; females only, at 25°C.

Cross [a]	Difference of F_1 reciprocals	Difference of F_2 reciprocals	Cross [a]	Difference of F_1 reciprocals	Difference of F_2 reciprocals
ST - TS	1.61*	0.27	AB - BA	-0.57	0.41
SA - AS	4.23***	3.71***	AH - HA	0.97	1.53***
SB - BS	-0.16	-0.10	AR - RA	-0.47	-0.85
SH - HS	0.56	0.69	AC - CA	0.79	0.06
SR - RS	0.37	-0.74	BH - HB	0.23	0.47
SC - CS	0.24	-1.46***	BR - RB	-0.58	-0.13
TA - AT	2.03***	-0.07	BC - CB	0.32	-1.99***
TB - BT	-2.10***	-0.01	HR - RH	-1.42**	0.07
TH - HT	1.21*	0.44	HC - CH	0.59	-1.04*
TR - RT	-0.45	1.72***	RC - CR	0.24	0.35
TC - CT	0.20	1.00*			

All comparisons are in micrometer scale units; one unit = 20.8 μ.

* Significant at the .05 level.

** Significant at the .01 level.

*** Significant at the .001 level.

[a] Female parent given first; abbreviations are as in Table XI.

Table XIV. Comparison of pooled within-bottle variances of wing length in parents and hybrids from the geographic populations; females only, at 25°C.

		df	MS	F
a)	Parents	837	3.23	1.22**
	F_1	2354	2.66	
b)	F_2	2296	3.50	1.24**
	Parents	882	2.83	
c)	F_2*	882	4.00	1.24**
	F_1	837	3.23	

* Adjusted for the difference in average sizes of F_1's and F_2's.

** Significant at the .01 level.

species is relatively high in comparison to many other quantitative characters (Falconer, 1960). Body size, is, thus, accessible to selection and is eminently suited to an evolutionary genetic study.

Body size in natural populations of Drosophila pseudoobscura does vary geographically. Not only are the sizes different in the eleven populations which were studied, but there is a pattern of sizes among the populations. Those from the Pacific Coast were consistently smaller than those more interior. This variation coincides with the physiographic division of the American West, and undoubtedly has an underlying environmental basis. The Pacific Coast region has a mediterranean climate, with moderate temperatures, winter rains, and summer drought. The intermontane plateau, the Rockies and the desert regions extending into Sonora are more arid, being shielded on the east and west by mountains. Summers are warm and winters are cold. Texas and adjoining regions of Mexico are influenced by the Gulf of Mexico. Summers are warm and humid, most of the rains coming during the warmest months; winters are relatively dry.

Like the chromosomal types studied by Dobzhansky (1944), body size is correlated with the major climatic regimes. Dobzhansky found that the frequencies of chromosomal types varied geographically; when transects within a physiographic province were taken, no gradients could be demonstrated; when transects across several provinces were taken, unmistakable graded variation appeared. We should expect such a pattern of physiographic variation in an organism which adapts to so varied a territory as does Drosophila pseudoobscura. Mountains and deserts, rainfall and vegetation, all vary rather irregularly. No simple North-South gradients, as are found for other species in more uniform environments, are expected. The parallel variation of body size and chromosomal types is double evidence of the different selections imposed in the different environmental provinces of the West. Both characters probably vary because the environment does; there is no evidence for a cause and effect relation between size and chromosomal type.

We have seen that Drosophila pseudoobscura, like other species in the genus, shows geographical variation in size. The complex nature of its territory, however, makes it difficult to isolate the effects of specific factors. Temperature is undoubtedly one of the most importnat features

of the environment. In other species, with more uniform environments, variation with latitude, and hence temperature, is clear (cf. Stalker and Carson, 1947, 1948; Prevosti, 1955; Misra and Reeve, 1964; Tantawy and Mallah, 1961). A study of experimental populations of Drosophila pseudoobscura exposed to different temperature allows us to isolate the effect of this factor and to learn something of the type of selection which generates geographical variation in nature.

Initially, Vetukhiv's populations were genetically identical, but each was genetically heterogeneous. The differences in body size among the populations kept at different temperatures were at first only phenotypic. Sokoloff (1966) has shown that the phenotypic effect of temperature on size persists, although smaller, even at larval densities approaching those in experimental populations. Flies in the two populations at 16°C were markedly larger, as measured by wing length, than flies in the two populations at 25°C. Flies in the two populations at 27°C were, however, only a little smaller than the flies at 25°C. Thus, the phenotypic differences among Vetukhiv's populations in their early stages were chiefly between the two populations at 16°C and the four populations at 25°C and 27°C.

After one-and-a-half years no indications were apparent that the populations were diverging genetically in body size. The changes in body size observed later clearly did not result from a rapid selection in the early generations. The experiments at six years disclosed a striking divergence among the populations. The size differences once induced by different environmental temperatures alone became, in part, genetically assimilated. For both wet body weight and for wing length, the flies in the populations kept at 16°C are genetically determined for larger size than those kept in the populations at the higher temperatures, 25°C and 27°C. The exact pattern varies somewhat; sometimes there are significant differences between replicate populations kept at the same temperature. But over and above these variations there is a clear distinction between those populations kept at the lower and those kept at the higher temperatures.

The divergence in body size among Vetukhiv's populations is impressive. Chance occurrence of such changes is extremely unlikely. The populations

have been too large for random genetic drift to have an appreciable effect, and the results of the study at one-and-a-half years rule out the possibility of a rapid reorganization of the gene pools during the initial adaptation of the flies to the different environments afforded by the six population cages. That the changes show as clear a pattern as they do, suggests that selection has favored larger body size at the lower temperature and smaller body size at the higher temperatures. This selection has acted slowly to produce a gradual genetic divergence of the populations. Selection has also acted to alter slightly the development time, the time being shortened in the populations at the lower temperature. A shorter development time would be maximally exposed to selection in the cooler environment. Development time and body size are often correlated traits. The postulated opposite selections for these characters in the populations at 16°C have probably balanced, neither character progressing as far as it might have if selected alone. The target character for the selection for size may not be size itself, but some other physiological character, which is genetically highly correlated with body size. The effect will be the same, however the selection acts, whether in the laboratory or in nature. The selection will produce genetic differences in body size. The changes observed in Vetukhiv's populations are examples of the selective process Waddington (1953, 1961) has called "genetic assimilation."

Ehrman (1964) and Mourad (1965) found that Vetukhiv's populations had diverged with respect to mating behavior and longevity. There was no pattern with temperature, however, for the differences in these characters.

The nature of the genetic systems of Vetukhiv's populations can to some extent be inferred from the hybrid studies. There is a partial dominance of larger size, the F_1's being significantly larger than the mean of their parents. There is no breakdown of size in the F_2's and no change in variability of the hybrid generations. The populations have not evolved distinct, coadapted systems; we do not expect this in populations only six years old.

It is of interest to compare these results with those of Druger (1962) who subjected Drosophila pseudoobscura to selection for body size, the

selection was practiced in lines kept at different temperatures, and then the selected lines were tested also at other temperatures. The results of selection for body size at low and at high temperatures were qualitatively similar when the selected lines were compared over a broad range of temperatures. The precise quantitative relationships, however, did depend on the temperatures at which the lines were selected and the temperatures at which the selected lines were compared. Vetukhiv's populations, which underwent natural selection for body size, show a behavior similar to Druger's artifically selected lines. Qualitatively, the distinction between the populations from 16°C and the populations from 25°C and 27°C is clearly revealed at all temperatures of comparison--16°C, 19°C, and 25°C. But the exact pattern of sizes varies according to the temperature at which the populations were compared.

The temperature-directed selection for body size found in Vetukhiv's populations may well be similar to that which has produced the temperature-oriented gradients of body size in some natural populations of Drosophila. It illustrates the selective role of the environment on organisms, and demonstrates how swiftly significant changes may be generated.

The geographic variation we have considered indicates selection on a grand scale, differentiating populations over a range of two thousand miles. The crosses among the experimental populations derived from the parents collected in nature, on the other hand, illustrate selection at a narrower level, within each population. The populations exhibited precisely the behavior we expected they would if their genes were mutually adjusted--coadapted--by selection. Heterosis in the F_1 generation is often found in crosses between geographically separated populations, but is not a necessarily expected phenomenon in crosses between coadapted populations. F_1 heterosis and F_2 breakdown are probably the results of different genetic mechanisms. F_2 breakdown occurs through the reassortment of genes by recombination and the consequent disruption of synergistic combinations of genes. F_1 heterosis, on the other hand, is most likely the result of increased heterozygosity for genes with overdominant effects. Many genes with overdominant effects may be fixed in the parental population, by chance or by selection. The fixation occurs at different loci in different populations, since the selections are different. Crossing two populations restores heterozygosity at the overdominant loci, with a

corresponding heterosis. Whether the populations have highly integrated, interacting genetic systems is another matter. Conversely, populations with coadapted genetic systems may not display F_1 heterosis when crossed.

In particular, we should not expect F_1 heterosis for a trait that is determined by genes with largely additive, and seldom overdominant, effects. The experiments of Kojima and his collaborators bear out this reasoning. Kojima and Kelleher (1963) compared the effectiveness of purebred and crossbred selection on fecundity in Drosophila pseudoobscura. In purebred selection, parents are chosen for their performance, mated and the process repeated again and again; it is the usual directional selection. In crossbred selection, parents are chosen for the performance of their hybrids with other, unrelated populations. Those parents whose hybrids with other lines are most successful by the particular criterion of selection being employed are remated, each within its own line. Thus there is no exchange of genes between lines; separate lines are developed which perform well when crossed. Komima and Kelleher found crossbred selection more successful in increasing fecundity than was purebred selection. After nineteen generations the crossbred lines were combined into one mixed population (Richardson and Kojima, 1965). The fecundity scarcely changed; there was no breakdown through recombination in six generations of the mixed population. The crossbred selection produced lines which yielded F_1 heterosis but which were not coadapted.

The crosses between the experimental populations begun from the collections in nature clearly indicate the coadaption within them. The irregular behavior of the F_1 suggests either a minor role for overdominant loci in determining body size, or that the populations are already heterozygous for a large fraction of the possible overdominant loci for size. The former possibility seems more likely in view of the increased heterozygosity expected in crosses between such geographically separated populations. The maternal effects on size complicate but in no way obscure our conclusions. Prout (1959) has found similar maternal effects in Drosophila pseudoobscura, and workers in other species have reported them for a variety of characters (Wallace, 1955; Poulson, 1934; Moriwaki and Tobari, 1963). Maternal effects illustrate the opportunism of evolution; an advantageous character may be determined in several different ways. What matters is that it is determined and increases the adjustment of the organisms to their environment.

E. Summary

1. Body size in Drosophila pseudoobscura is a continuously varying character with a high heritability; it is almost certainly related to fitness. Natural populations of Drosophila pseudoobscura from Canada to Mexico have been sampled and found to vary geographically in body size. The geographic variation for the genes determining size is correlated with the physiographic division of the West. The populations from the Pacific Coast have genetically smaller flies than do those from the interior provinces. Selection by the environment has probably brought about the differentiation.

2. Six experimental populations of Drosophila pseudoobscura, maintained at different temperatures for seven years, were studied to see if temparature could be an important factor in the differentiation of body size among populations. Two populations have been kept at 16°C, two at 25°C, and two at 27°C. One-and-a-half years after the start, there was no significant genetic divergence in body size among the populations. When the populations were about six years old, a striking genetic divergence in body size was found. The genetic difference between the populations showing the smallest and largest mean sizes is over half the total phenotypic variation in size between the two extreme temperatures at which the populations were kept. The populations kept at the lower temperature have genetically larger flies than the populations kept at the higher temperatures. The F_1 hybrids from crosses between the populations were significantly larger than the mean of their parents; the F_2's did not differ from their F_1 parents. There was no change in variability of body size in the F_1 or F_2 hybrids. The genes for larger size are partially dominant, and coadapted gene pools have not yet evolved in these populations. The temperature-directed selection for body size in these experimental populations may well be similar to that which has produced temperature-oriented gradients for body size in natural populations of several species of Drosophila.

3. Experimental populations derived from the samples of natural populations were crossed to yield F_1 and F_2 hybrid generations. The F_1's varied irregularly, while the F_2's showed a consistent "breakdown" of size, the F_2's being significantly smaller than their F_1 parents.

The natural populations possess coadapted, or internally balanced, genetic systems, with genes mutually adjusted by selection for favorable interactions. Recombination disrupted the balanced genic complexes to give the F_2 breakdown. Maternal effects on size were found and represent examples of the opportunism of evolution.

III. CHROMOSOMAL VARIATION IN DROSOPHILA PSEUDOOBSCURA

A. Introduction

1. Cytogenetic aspects The salivary chromosomes of Drosophila are particularly well-suited to cytogenetic analysis. They are giant, polytenic chromosomes, consisting of perhaps one thousand strands bound together as a cable. They pair somatically, so that a study of the mitotic cells of the salivary glands gives us a magnified model of the pairing which occurs in meiosis. Soon after the application of the aceto-carmine staining technique to salivary cells (Painter, 1933), the chromosomes of Drosophila pseudoobscura were described (Tan, 1935; Koller, 1936).

A remarkable array of gene sequences has been found in natural populations of Drosophila pseudoobscura. The different orderings of the genes, due to inversions in the chromosomes, have been an important tool in the genetic study of populations and in the understanding of evolutionary mechanisms. Let us first consider the mechanics of inversions, and then proceed to discuss their role in populations.

Imagine a chromosome with a gene sequence symbolized by the letters in alphabetical order. The chromosome will, with quite low frequency, spontaneously break. With far lower frequency, the chromosome may break in two places. The chromosome will in all probability be twisted upon itself in a complicated tangle. The broken ends seem to rejoin by chance, and sometimes the broken fragment will have rejoined so that its sequence of genes is reversed. This process of inversion is diagrammed in Figure 6. Let us suppose that the inverted chromosome pairs in meiosis with a homolog possessing the normal sequence of genes. The two homologs can effect the point-by-point pairing of synapsis only by throwing themselves into a loop. An inverted chromosome may itself suffer an additional inversion.

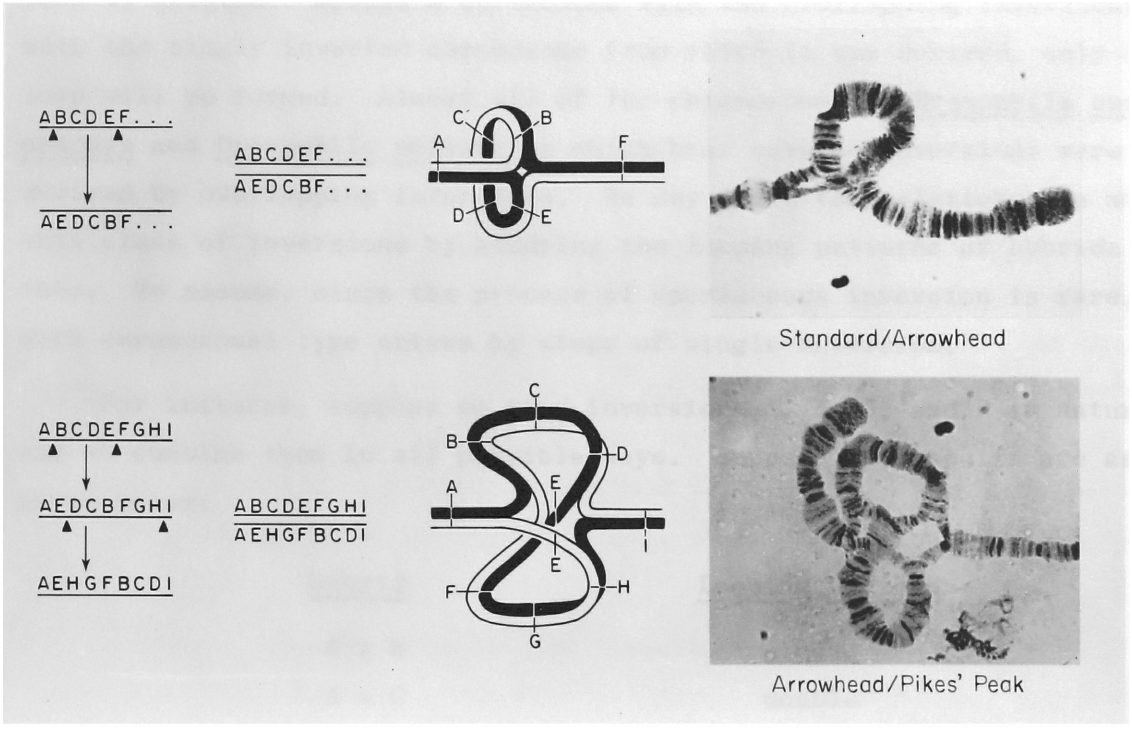

Fig. 6. Pairing in the salivary chromosomes of inversion heterozygotes in Drosophila pseudoobscura. Top: A single inversion; bottom: two overlapping inversions. (Redrawn in part from Sturtevant and Dobzhansky, 1938.)

The looping patterns which form when homologs pair will become progressively more complex as more inversions occur, one on top of another. An inversion which overlaps another is diagrammed in Figure 6; when the chromosome with the double, overlapping inversions pairs with a normal chromosome, a double loop is created. Should a chromosome with two overlapping inversions pair with the singly inverted chromosome from which it was derived, only one loop will be formed. Almost all of the chromosomes in Drosophila pseudo-obscura and Drosophila persimilis which bear several inversions were derived by overlapping inversions. We may infer the relationships among this class of inversions by studying the looping patterns of hybrids between them. We assume, since the process of spontaneous inversion is rare, that each chromosomal type arises by steps of single inversion.

For instance, suppose we find inversions A, B, C, and D in nature, and we combine them in all possible ways. Suppose our results are as given below:

Hybrid	Looping Pattern
A x B	single
A x C	double
A x D	complex
B x C	single
B x D	double
C x D	single

Then the relationship among the inversions is linear: A-B-C-D. We might, however, have obtained different results, as follows:

Hybrid	Looping Pattern
A x B	single
A x C	double
A x D	double
B x C	single
B x D	single
C x D	double

In this case, the relationship is branched: A-B<C/D.

Sturtevant and Dobzhansky (1936a) first showed how this method could be used to derive a "phylogenetic tree" for the inversions on the third chromosome of Drosophila psuedoobscura and Drosophila persimilis. Dobzhansky and Sturtevant (1938) presented the first phylogeny, supplemented by later discoveries in Dobzhansky (1944 and 1948), Spiess (1950 and 1965), and Epling and Lower (1957). I have used these data, as well as the unpublished work of Crumpacker and Kastritsis, and Strickberger and Wills to construct the phylogeny shown in Figure 7. By virtue of their central positions, the gene arrangements Standard, Hyopthetical, and Santa Cruz are most likely ancestral chromosomal types. Which is the original type cannot be decided with the present data. The Hypothetical chromosome is the leading contender because its sequence of genes is most like that in Drosophila miranda, a species closely related to Drosophila pseudoobscura and Drosophila persimilis. Of all the inversions shown in Figure 7, only the Hypothetical type has not been found in nature. It is surprising that the phylogeny is still so nearly intact, with only one intermediate step missing.

A single crossover inside the loop in an inversion heterozygote results in two normal and two abnormal products of meiosis. The abnormal products are a dicentric chromosome and an acentric fragment, each duplicated or deficient for part of the chromosome. The acentric fragment will be lost, since it has no means of movement at anaphase. The dicentric chromosome will be stretched between two centromeres moving in opposite directions, and may break to yield lethal fragments. Thus, two of the four meiotic products will be inviable. Normally, such a large inviability would be a potent selective force to reduce the frequency of paracentric inversions. The special biology of meiosis in Drosophila and many other Diptera removes most of the cytogenetic disadvantage of paracentric inversions. There is no crossing over in the males and consequently no disadvantage to their carrying inversions. Only one of the four products of meiosis in the females becomes a functional egg nucleus; the other three are discarded in the polar bodies. Always it is the innermost of the four meiotic nuclei in the egg which serves in fertilization. The separation of chromosomes to the poles in oogenesis occurs in essentially a straight line directed inward to the center of the egg. Because the dicentric chromosome is pulled in two directions, one of the two chromomsoes which did not

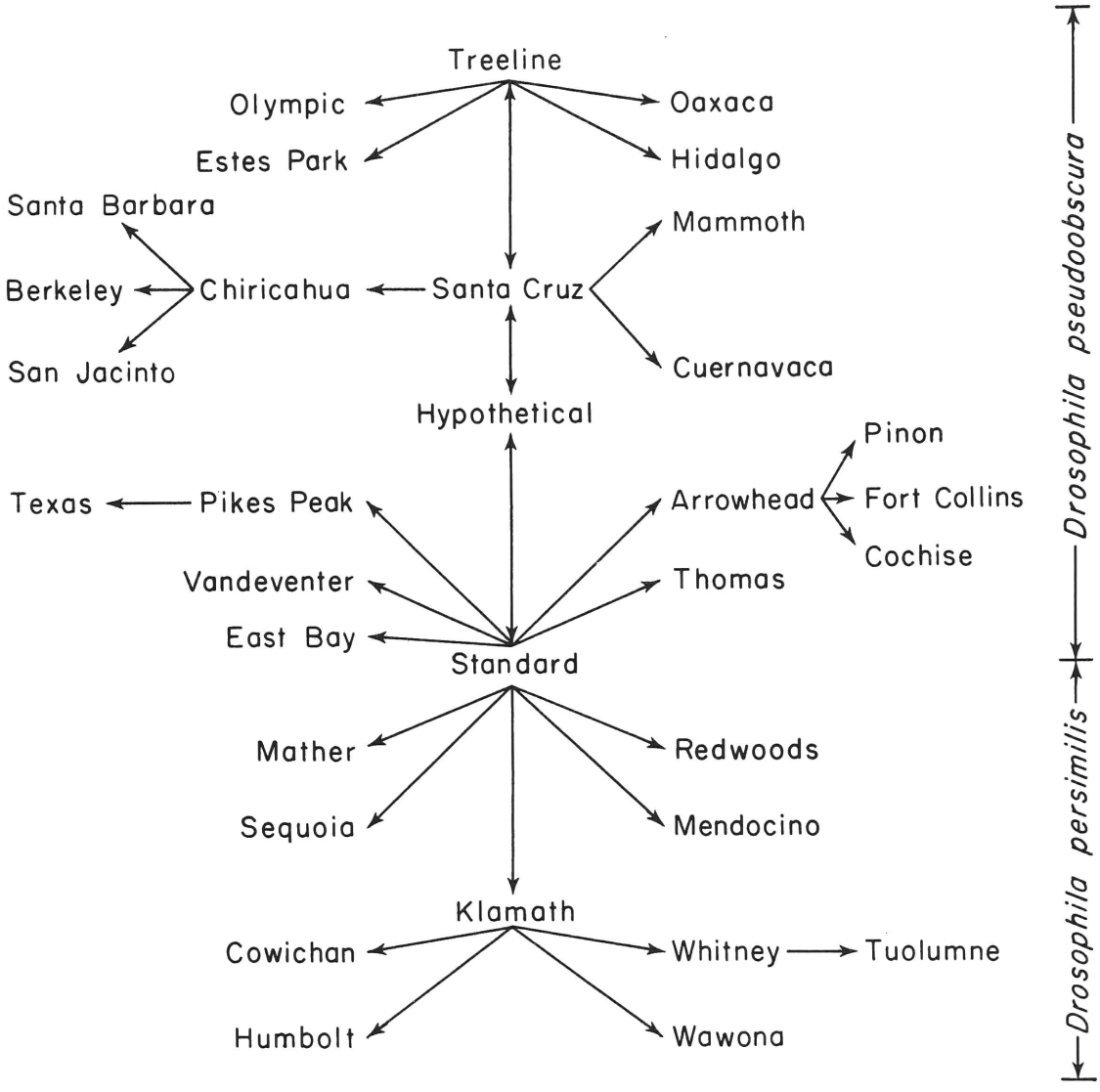

Fig. 7. The phylogeny of inversion types in the third chromosomes of Drosophila pseudoobscura and Drosophila persimilis.

participate in crossing over is nearest the center of the egg. This selective elimination of chromatids which participate in crossovers eliminates all but a small fraction of the cytogenetic disadvantage of heterozygous inversions. Multiple crossovers can lead to inviable eggs, but the frequency of such multiple events is too small for them to exert an appreciable selection. The mechanism of selective elimination was proposed by Sturtevant and Beadle (1936), and confirmed cytologically and genetically by Carson (1946) and Hinton and Lucchesi (1959).

The overall effect of heterozygous inversions is a suppression of crossing over; we shall see later that the importance of the inversions in nature stems from this property. The degree by which recombination is suppressed when the third chromosome of Drosophila pseudoobscura is structurally heterozygous was investigated by Dobzhansky and Epling (1948). About one-third of the third chromosome is inverted in the heterozygote of Arrowhead with Standard. Yet recombination is suppressed twenty-fold, declining from eighty map units to less than four. The Chiricahua and Tree Line inversions involve about sixty per cent of the chromosome, and they almost eliminate recombination in combination with Standard. Recombination is reduced from eighty map units to less than one.

2. Evidence for selection in nature The first surveys of chromosomal types in natural populations of Drosophila pseudoobscura and Drosophila persimilis were begun in the late nineteen-thirties. Differences between populations on different mountains in Death Valley (Dobzhansky and Queal, 1938) or in the different valleys of a single mountain (Koller, 1939) were observed and were attributed to the effects of sampling errors in small populations. The inversions were thought to be adaptively neutral, since they produced no outward manifestations in their carriers. Sewall Wright (1931) had only recently emphasized the importance of chance fluctuations in small populations. The hypothesis of adaptive neutrality was justified, and the data agreed satisfactorily. As further data were gathered, however, it became obvious that the inversions were being selected, and at unprecedented intensities.

The first indication that the inversions were changing in response to selection came in 1943, with the discovery of seasonal cycles in

inversion frequencies on Mount San Jacinto, California (Dobzhansky, 1943). The changes occur regularly each year and have continued to do so since 1939 (Epling, Mitchell, and Mattoni, 1957). Seasonal cycles have also been found at Aldrich, Texas (Dobzhansky, 1944) and in the Sierra Nevadas (Dobzhansky, 1948 and 1956).

Altitudinal gradients in inversion frequencies were found by Dobzhansky (1948) on the western slope of the Sierra Nevadas for both Drosophila pseudoobscura and Drosophila persimilis. Spiess (1950) extended Dobzhansky's analysis of the altitudinal gradient for Drosophila persimilis in the Sierra Nevadas; he related the variation to the different ecological life zones which the transect encompassed.

The American West is an area of complex topography. Any study of geographic variation must necessarily be related to the physiography. Dobzhansky (1944) summarized all collections of Drosophila pseudoobscura and Drosophila persimilis through the early nineteen-forties. He found that the distribution of chromosomal types was clearly associated with the physiographic provinces into which Fenneman (see Dobzhansky, 1944) divided the West. Only transects which cut across the provinces revealed the associations, the chromosomal frequencies changing in each province. Transects unrelated to the environmental subdivision, north-south lines, for instance, revealed no regular variation.

The seasonal, altitudinal, and geographic variations of the inversion types provide overwhelming evidence that natural selection regulates their frequencies according to the environment. Changes in the chromosomal system occur in time also, but are more difficult to analyze because we cannot specify the corresponding environmental changes with any accuracy.

The first long-term change was observed in Drosophila pseudoobscura at Keen Camp in the Sierras (Dobzhansky, 1947a). The frequency of the Standard arrangement increased while the frequencies of the Arrowhead and Chiricahua arrangements declined. Changes in the inversions of both Drosophila pseudoobscura and Drosophila persimilis near Mather, California were observed over a span of ten years (Dobzhansky, 1952, 1956). The changes could be correlated with the succession of wet and dry years.

The changes in inversions at Mather were later observed to continue through a reversal of wet and dry years, and the wet year-dry year hypothesis was discarded (Dobzhansky, 1963).

The changes in the inversion system of Drosophila pseudoobscura at Mather fit into a trend which has been observed in other localities in California (Dobzhansky, 1956, 1958, 1963; Dobzhansky, Anderson, Pavlovsky, Spassky and Wills, 1964). The frequencies of the Arrowhead and Chiricahua chromosomes have fallen, while that of the Standard chromosome has increased. The Pikes Peak chromosome appeared on the coast and rose dramatically, to a frequency of up to ten per cent. The Pikes Peak chromosome was predominant in Texas in the early samples (1939-1941) and common in Colorado and New Mexico on the eastern slope of the Rockies. There were only four Pikes Peak chromosomes out of the twenty thousand which were examined before 1946 in California and the adjoining section of Nevada. We do not yet know what factors have produced these long-term changes, although we shall discuss several possibilities later.

3. The experimental approach The nature of the selection which occurs in wild populations has been the subject of many laboratory investigations of Drosophila populations. The population cages described earlier can be set up under a variety of genetic and environmental conditions, and the response of the inversion frequencies accurately followed. These studies are evaluated in terms of simple mathematical models of selection. Wright and Dobzhansky (1946) followed the changes in the frequencies of inversion types in experimental populations of Drosophila psuedoobscura and found strong selection operating. In many cases the selection was heterotic, the heterokaryotype being fittest in the sense of leaving more offspring, and hence more chromosomes, than the other karyotypes. Heterotic selection results in stable equilibria, with several inversion types maintained in the populations. Dobzhansky and Pavlovsky (1960), Levine and Beardmore (1959), and Druger (1966) have shown that these stable equilibria may be maintained for many years in the laboratory.

Dobzhansky (1947) found that the genotypic frequencies among zygotes and young adults in experimental populations containing third chromosomal inversions conformed to the Hardy-Weinberg expectations, while those

among older adults did not. The differential mortality between young and old adults was sufficient to account for the selection which maintains the balanced polymorphism for the third chromosome, the heterokaryotype being fittest. Using a special statistical test, Dobzhansky and Levene (1948) found deviations from the Hardy-Weinberg frequencies among males captured in nature; the heterokaryotypes were more frequent than expected. Epling, Mitchell, and Mattoni (1955) repeated this analysis of genotypic frequencies among males taken in nature. They found in some cases an excess of heterokaryotypes, in some an excess of homokaryotypes, and in others no evidence of departure from the Hardy-Weinberg expectations. Heterozygote advantage is, thus, one way in which the balanced polymorphisms in Drosophila pseudoobscura are generated. The results of Epling and his coworkers show that it is not the only way, but must rather be one in a battery of mechanisms which together produce the "balancing selection" for polymorphism. We shall briefly consider some of the other possible components of balancing selection.

Selection dependent on population density was demonstrated by Birch (1955), by Beardmore, Dobzhansky, and Pavlovsky (1960), and by Dobzhansky and Pavlovsky (1961). Using the ST-CH and AR-CH combinations, these workers found entirely different selection when the larvae but not adults are crowded, than when the adults but not larvae are crowded. This type of selection may underly the seasonal cycling of the Standard and Chiricahua inversions on Mount San Jacinto, for the population densities change in nature with the seasons. Different microfloras in the populations create different selective pressures (da Cunha, 1951; Dobzhansky and Spassky, 1954). Likewise, selection for the inversions is different at different temperatures (Wright and Dobzhansky, 1946; Van Valen, Levine, and Beardmore, 1962). Preferential selection of the genotypes in different niches within the environment may sustain polymorphism with no heterozygote advantage (Levene, 1953; Li, 1955). The mating advantage of rare males described by Ehrman (1966) for Drosophila pseudoobscura will help maintain polymorphism. Spiess and Langer (1964) and Spiess, Langer, and Spiess (1966) found a mating advantage of heterokaryotypes which also contributed to polymorphism. Mutual facilitation of different genotypes, demonstrated by Lewontin (1955) and by Levene, Pavlovsky and Dobzhansky (1954) is yet another factor in balancing selection. The balancing

selection for polymorphism is a complex phenomenon and must be explained as a composite of these many types of selection. We have seen that the inversions effectively suppress recombination between structurally different chromosomes, that they are strongly regulated by selection in nature, and that at least parts of the selection in nature may be reproduced in the laboratory in experimental populations. Why are the inversions selected? Dobzhansky (1949) proposed that the different inversions contain different, coadapted blocks of genes. Since recombination cannot reassort the genes in each inversion type, favorable combinations of genes will persist. In particular, beneficially interacting constellations of genes may be maintained without disruption. Earlier, we considered experiments which confirmed the validity of the concept of coadaptation for the genes which control viability, longevity, fecundity, and body size in Drosophila pseudoobscura. Complexes of genes on different inversions within the same population will likewise be mutually adjusted by selection for maximal fitness. Dobzhansky and Levene (1951), Dobzhansky and Pavlovsky (1953), and Levine (1955) combined inversions from widely separated localities in experimental populations. The course of selection was extremely variable and led to many different equilibria or to elimination of one chromosomal type. By contrast, the course of selection in experimental populations containing chromosomes from the same population was both predictable and repeatable. The chromosomes from different localities formed combinations untested by selection; the many possible combinations on which selection could begin its work assured heterogeneous outcomes. Thus, the adaptive role of the inversions within populations lies in their ability to suppress recombination. The net effect is to allow different advantageous gene complexes to be formed on different types of third chromosomes within populations, just as such advantageous complexes are formed within different populations for characters such as body size.

4. "Sex ratio" The chromosomal polymorphism of greatest interest is that involving the third chromosome. The X-chromosome is also polymorphic, with only one variant of the standard gene arrangement known. A series of three inversions in the right arm of the X-chromosome is associated with the Sex Ratio character (Sturtevant and Dobzhasnky, 1936b; Dobzhansky, 1939). Ninety-five to one hundred per cent of the progeny of males carrying the Sex Ratio chromosome are daughters. The cytogenic

basis for the effect is problematical (Sturtevant and Dobzhansky, 1936b; Novitski, Peacock, and Engel, 1965). The unusual behavior of the Sex Ratio chromosome will "drive" it into the population; the Sex Ratio chromosomes will increase in frequency unless their ascent is counterbalanced by selection. The polymorphism for Sex Ratio in nature has not been duplicated in the laboratory in experimental populations, where it is quickly eliminated (Wallace, 1948).

Terzaghi and Knapp (1960) measured the hatchability of eggs produced by female Drosophila pseudoobscura which were heterozygous for inversions in 0, 1, 2, and 3 of the four large chromosomes of this species. The hatchability of eggs laid by females heterozygous for no inversions was not significantly different from that of females heterozygous for an inversion in one of the chromosomes; the average hatchability of this control group was 93.5 per cent. There was a 14.7 per cent decrease in the per cent of eggs hatching, compared to the control group, when the female parents carried inversions in two of their chromosomes. Females heterozygous for inversions on three chromosomes laid eggs of which only 58.7 per cent hatched, a 34.8 per cent decrease from the control group. A similar decrease of hatchability among eggs laid by females heterozygous for inversions on two different chromosomes has been found in Drosophila melanogaster and Drosophila paramelanica. Drosophila robusta, however, shows no pattern of increased egg lethality with an increasing number of maternal chromosomes structurally heterozygous for naturally-occuring inversions (Riles, 1965). This last species is normally polymorphic in nature for chromosomal arrangements on all three of its large chromosomes.

Terzaghi and Knapp used inversions on the X, second, third, and fourth chromosomes of Drosophila pseudoobscura in their study, but only the inversion on the third chromosome (Arrowhead) was one found commonly in natural populations. These authors suggested that structural heterozygosity for both the Sex Ratio inversion complex and for the third chromosome will result in a lowered egg hatchability. A fifteen per cent decrease in egg hatchability is a large selective disadvantage. We might, then, expect the frequency of females heterozygous for Sex Ratio to be negatively correlated with the frequency of heterozygotes for the third chromosome in natural populations.

Several aspects of the inversion system of Drosophila pseudoobscura were investigated in the work to be presented below. New collections were taken in all those localities in the United States for which adequate data from the ninteen-thirties and nineteen-forties were available. These new data permit us to assess the present geographic variation of the inversions and to compare the present distribution with that of thirty years ago. Experimental populations were begun with samples from widely scattered locations to see how selection operates on different genetic systems. The distribution of the Sex Ratio chromosomes in the natural populations was studied, along with laboratory crosses, in order to determine what relation there is between polymorphism on the third and X-chromosomes.

B. Materials and Methods

The method of collecting and the initiation of the experimental populations were described previously. It suffices here to emphasize that the experimental populations were started with exactly the same chromosomes and in exactly the same frequencies as in the samples from each locality. In this respect they differ from all the experimental populations set up in the past.

The populations were sampled twice after their initiation. Eggs for the samples were collected over six successive days and then cultured under near-optimal conditions of low temperature and abundant yeast. Squash preparations of the larval salivary glands were made according to the technique outlined in Strickberger (1962). The inversions were identified under 450X, following the description of Dobzhansky (1944), Kastritsis and Crumpacker (1966), and Crumpacker and Kastritsis (1966). We assume that little, if any, selection occurs between the formation of zygotes and the later larval stages whose chromosomes we study. We also assume that mating in the experimental populations occurs at random. Support for these assumptions comes from chi-square tests on the genotypic frequencies in the samples; the results of such tests on the final samples are given in Table XV. Only in the population from Davis, Texas, do the genotypic frequencies depart significantly from the Hardy-Weinberg expectations; such an isolated exception is expected when a large number of tests is performed.

Table XV. The goodness-of-fit of the genotypic frequencies in the egg samples from the geographic populations to those expected from the Hardy-Weinberg Principle.

Population	Chi-Square	Probability >	Probability <
Okanagan, British Columbia	0.001	0.95	0.975
Berkeley, California	0.005	0.90	0.95
Riverside, California	0.028	0.9	0.95
Tucson, Arizona	0.330	0.5	0.75
Sonora, Mexico	0.073	0.90	0.95
Black Canyon N.M., Colorado	0.257	0.75	0.90
Hayden Creek, Colorado	0.975	0.25	0.5
Raton, New Mexico	1.115	0.25	0.5
Davis, Texas	5.784	0.01	0.025
Austin, Texas	0.778	0.25	0.5

Stocks of Drosophila psuedoobscura from California and Utah were utilized for a test of the effect of simultaneous heterozygosity for the Sex Ratio X-chromosomes and for the third chromosomes. The heterozygotes for the third chromosome carried the Standard and Pikes Peak gene arrangements; Pikes Peak is a long single inversion of the Standard arrangement, involving more than half the chromosome. The egg-to-adult viability was measured. Hatchability is one component, perhaps the most important, of viability and of Darwinian fitness. The experiment was conducted at $25^{\circ} \pm \frac{1}{2}^{\circ}$C. Virgin females aged three days were placed with males in vials with spoons containing food medium darkened with charcoal. Eggs were collected over twenty-four hour periods, and fifty eggs were put into each of several bottles containing Cream-of-Wheat medium and a drop of yeast suspension. All adults emerging through the twenty-fourth day were counted. The chromosomal constitution of the stocks was verified by cytological examination of salivary gland preparations.

C. Results

1. Geographic variation The frequencies of the inversions in the samples from nature are given in Table XVI and presented visually in Figures 8-11; the collecting stations are numbered identically in the figures and the table. To facilitate comparison with previous collections, the data from previous samples are included in the figures and the table. Those localities without numbers in Figures 8-11 are included here to extend the geographic coverage of the maps; the data are taken from earlier work in which the author participated (Dobzhansky, Anderson, Pavlovsky, Spassky, and Wills, 1964).

The geographic variation of the inversion frequencies is clear. The Standard chromosome is predominant along the coast and the Arrowhead chromosome, in the intermontane region and the Rocky Mountains. The Chiricahua chromosome is common in the southwestern part of the United States and the Pikes Peak chromosome, in Texas and the adjoining parts of New Mexico and Colorado which lie on the eastern slopes of the Rockies.

Localities numbers 1-4 are in the northwestern United States and the adjacent part of Canada (British Columbia). Between 1940 and 1964 or 1965, the populations of all these localities changed quite appreciably

Table XVI. Numbers of the chromosomes studied (n), and percentage frequencies of chromosomes with different gene arrangements in samples of natural populations of Drosophila pseudoobscura.

Locality	Year	ST	AR	CH	PP	TL	EP	others	n
1. Okanagan, British Columbia	40	36.7	46.7	10.0	3.3	3.3			30
	64	55.0	30.0	1.2	8.8	5.0			80
2. Methow, Washington	40	52.0	47.0	1.0					100
	64	79.3	17.8			2.9			208
3. Service Creek, Spray, Oregon	40	25.0	56.8	11.4	4.5	2.3			88
	65	44.3	27.1	5.7	8.6	14.6			70
4. Kerby, Selma, Oregon	40	18.8	53.1	18.8		7.8		1.6*	64
	65	43.3	30.0	13.3	3.3	6.7		3.3*	30
5. San Gabriel Canyon, California	36-37	34.7	27.7	26.7		10.9			101
	63	70.5	14.1	5.1	1.3	9.0			78
	64	66.1	19.6	10.7		3.6			56
6. Ferron, Utah	50	6.4	87.3	4.5	1.8				110
	65	5.6	81.3	1.9	9.3	1.9			54
7. Bryce N. P., Utah	40	2.0	96.0	2.0					100
	50	4.8	92.9	2.4					84
	57	2.6	93.2	1.6	2.6				190
	65	2.5	92.0	4.0	1.5				200
8. Mesa Verde N. P., Colorado	40		100.0						100
	57		96.5		1.5		0.5	1.5†	200
	64	1.9	97.6		0.5				206
9. Black Canyon N. M., Colorado	50	3.3	81.6		8.6	1.3	5.3		152
	64	5.0	94.4		0.6				182
10. Hayden Creek, Colorado	50		41.7		20.8	29.2	4.2	4.2**	24
	64	17.2	47.2	2.2	25.0	1.7	6.7		180
11. Grand Canyon Arizona	40	1.0	98.0	1.0					100
	57	5.5	91.0	2.5	1.0				200
	65	2.5	96.5		0.5	0.5			200
12. Flagstaff, Arizona	40	1.0	97.0	1.0	1.0				100
	57	6.5	89.0	2.0	2.5				200
	65	0.5	96.0	1.5	1.5			0.5***	200
13. Sonoita, Arizona	41	7.1	59.5	33.3					42
	57	2.0	80.0	15.0	3.0				200
	65	6.5	77.5	11.5	4.0	0.5			200
14. Chiricahua, Arizona	40	0.5	88.5	6.3	4.2	0.5			192
	57	0.2	85.0	11.5	2.5	0.2		0.5†	400
	64	1.5	88.9	6.1	3.0	0.5			198
15. Raton, New Mexico	40		78.0	1.0	20.0	1.0			100
	64	0.5	78.0	1.5	19.0	1.0			200
16. Capitan, Hondo Ruidoso, Lincoln, N. M.	41		56.3	7.1	35.2	1.4			142
	64	2.4	69.5	1.2	25.6	1.2			82
	65	2.5	42.0	1.0	47.5	2.0	5.0		200
17. Marfa & Davis, Texas	39-41	1.3	33.8	3.4	56.1	4.7	0.7		148
	64		15.0	1.5	82.5		1.0		200
18. Austin, Texas	39-41		22.1		69.5	6.2	0.7	2.0**	1100
	53	2.0	38.5		54.5	4.0	0.5	0.5**	200
	64	2.6	16.4		72.0	3.5	1.3	4.3**	300

* Santa Cruz.

** Olympic.

*** New.

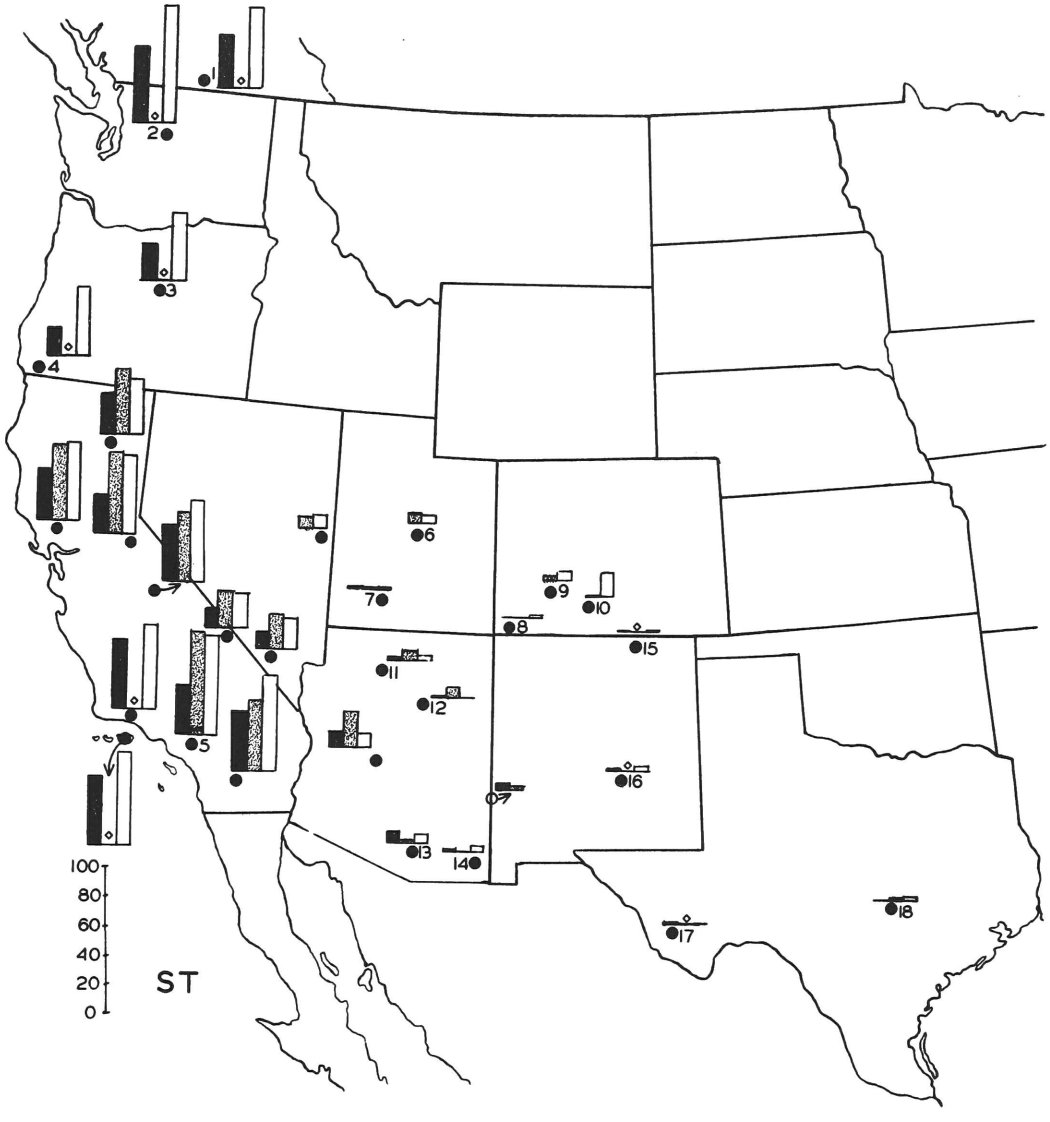

Fig. 8. The frequencies of Standard chromosomes in population samples of Drosophila pseudoobscura taken in 1940 or thereabouts (black columns), in 1957 (stippled columns), and in 1963-1965 (white columns); a diamond (◇) means that no sample was taken during a given period. The scale in the lower left corner indicates the frequencies in percentages. The localities are numbered as in Table XVI and in the text.

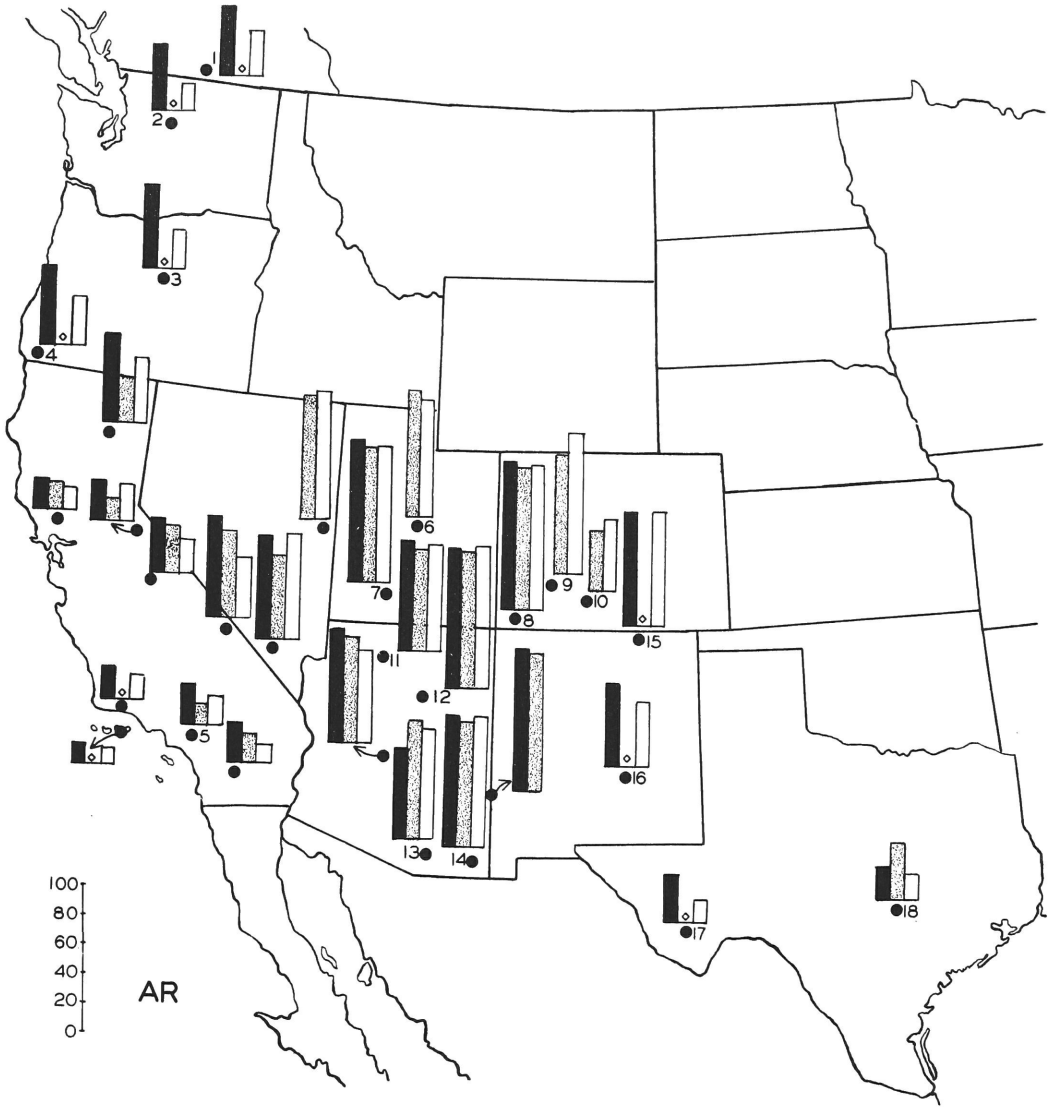

Fig. 9. The frequencies of Arrowhead chromosomes; the meaning of the symbols is as in Fig. 8.

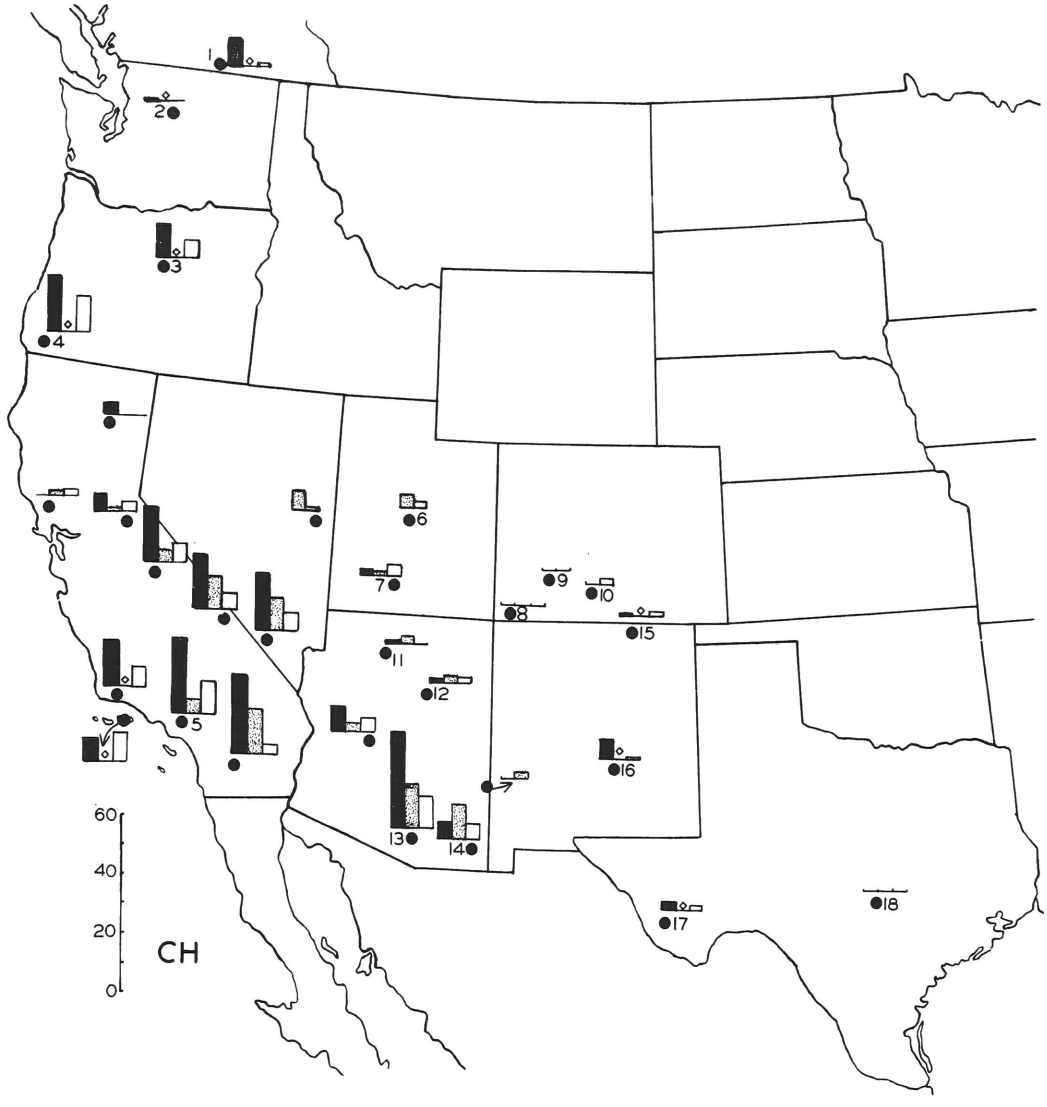

Fig. 10. The frequencies of the Chiricahua chromosomes; the symbols are as in Figs. 8 and 9, but note that the scale of the percentages is larger.

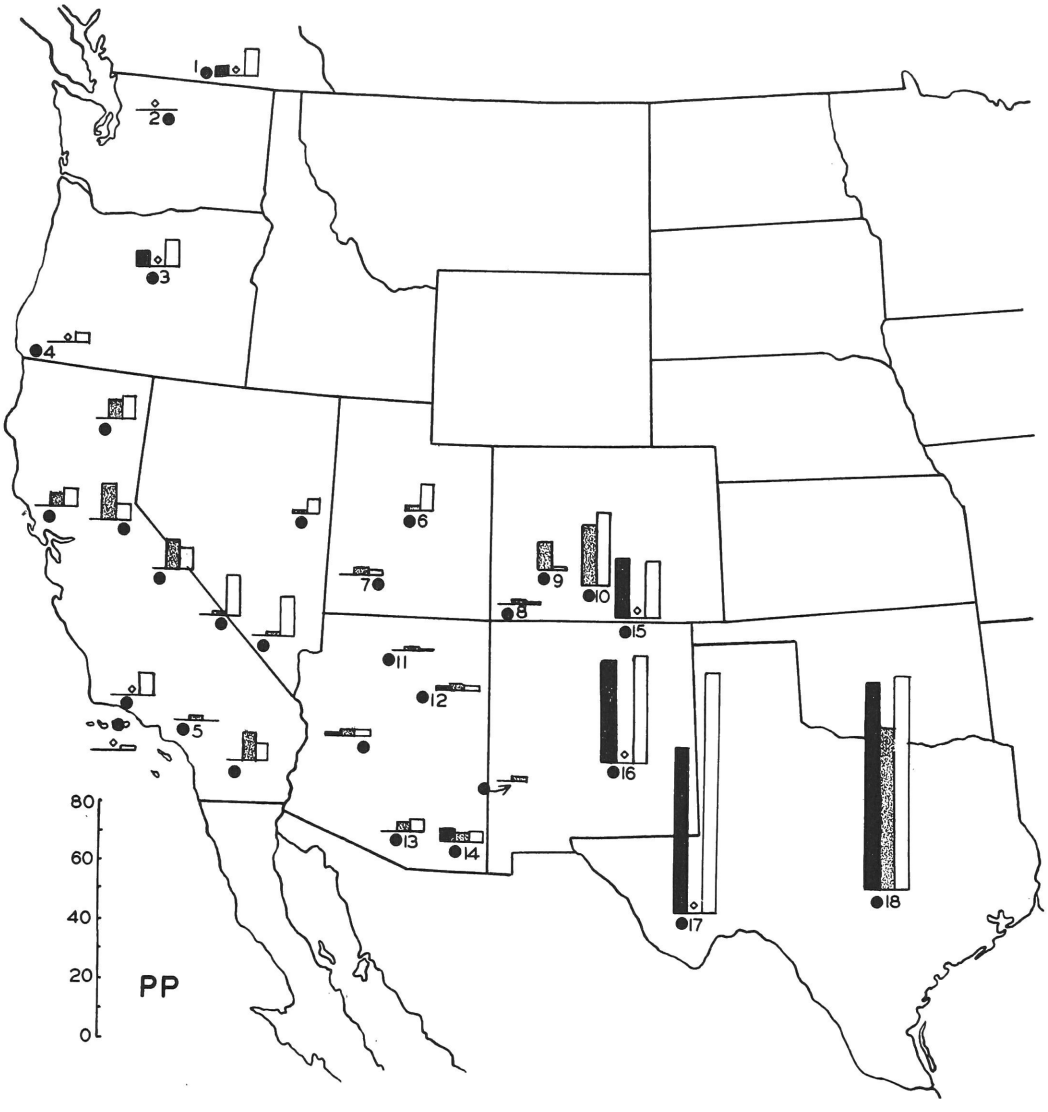

Fig. 11. The frequencies of Pikes Peak chromosomes; the symbols are as in Figs. 8-10, the scale as in Fig. 10.

and in similar ways. Chromosomes with the Standard gene arrangement increased, and those with Arrowhead decreased in frequency. The changes are significant even in the Kerby locality, from which only thirty chromosomes were scored in 1965. Chiricahua, Pikes Peak, and Tree Line gene arrangements are not frequent enough to make the frequency changes statistically assured, but ostensibly they behaved similarly in all four localities, Chiricahua decreasing and Pikes Peak and Tree Line increasing in frequency. Since these changes are all in the same directions as in most California localities, they are probably significant.

Localities numbers 6, 7, 8, 11, 12, and 14 are in Utah and Arizona. Their composition remained constant or nearly so during the period of observation, the Arrowhead chromosomes decidedly predominating. Only at Ferron (No. 6) was there an ostensible increase in Pikes Peak and a drop in Chiricahua, which agrees with the situation in the northwestern and the southwestern states. Sonoita (No. 13) is the only population in this group which seems to have changed considerably between 1941 and 1957, but not between 1957 and 1965. The change concerns chiefly a drop in the Chiricahua frequency and an increase in that for Arrowhead.

The Rocky Mountains of Colorado and New Mexico are represented by four localities (Nos. 9, 10, 15, 16). Here we meet a variety of situations. The population at the Black Canyon of the Gunnison National Monument (No.9) is the only one in which Arrowhead has increased, and Pikes Peak decreased, in frequency between 1950 and 1964. The changes in Hayden Creek (No. 10) were seemingly even more drastic, although the smallness of the 1950 sample makes the situation not clear; anyway, no Standard chromosomes were found in 1950, while in 1964 their frequency stood at 17 per cent; Tree Line chromosomes appeared to be the second most frequent in 1950, and were rare in 1964; Arrowhead and Pikes Peak showed no significant change. At Raton (No. 15) no changes are apparent. The locality Number 16 is problematical, because the samples were not taken in the same place. The 1941 samples were collected by the late Professor J.T. Patterson at Capitan and at Hondo, while the 1964 and 1965 samples came from the vicinities of Ruidoso and of Lincoln respectively. Although these localities are not far from each other, they are at different elevations and have different ecological conditions. The

1964 and 1965 samples are most different, while the 1941 sample is intermediate. A less serious uncertainty attaches to locality 17, in Texas. Prof. J.T. Patterson's samples in 1939-1941 were labelled "Marfa," while in 1964 we collected in Davis State Park. No vegetational or other serious difference is here apparent. The frequency of Arrowhead has decreased and of Pikes Peak increased between the two collections. Finally, at Austin, locality 18, the frequency of Arrowhead went up between 1939-1941 and 1953, and down between 1953 and 1964, while Pikes Peak changed downwards between 1939-1941 and 1953, and upwards between 1953 and 1964.

2. Changes in chromosomal frequencies in the experimental populations The frequencies of the chromosomal types in the experimental populations begun from the samples taken in nature are given in Table XVII. Most experimental populations of Drosophila pseudoobscura reach approximate equilibria for the gene arrangements on the third chromosome before one year at 25°C (Levine and Beardmore, 1959; Druger, 1966; Dobzhansky and Pavlovsky, 1960; and Pavlovsky and Dobzhansky, 1966). We are justified, then, in considering the chromosomal frequencies in the last sample as the values at, or very near, the equilibria. In Figure 12 the frequencies in the last smaple are drawn as histograms over the localities from which the populations were derived. The figure shows that the behavior of the experimental populations under the laboratory conditions reflects the chromosomal differentiation of the natural populations. In natural populations of the Pacific Coast, Standard is the most frequent chromosome, and in experimental populations of Pacific Coast origin the Standard chromosomes approach fixation. In the populations from the Great Basin and eastward, where the frequency of Standard is normally low, Standard increased, but generally to a frequency less than that of the normally more common Arrowhead. The different reactions of the inversion polymorphisms in the two groups of populations under the laboratory conditions is further evidence of the genetic differentiation of the chromosomal races. The frequency of Standard in nature seems to be an index of its success in the laboratory.

The nature of the polymorphisms which became established are of primary interest. In the populations from the Pacific Coast, the Standard gene arrangement was obviously the fittest; Arrowhead was,

Table XVII. Percentages of gene arrangements in experimental populations of Drosophila pseudoobscura begun with samples from natural populations.
N--the number of chromosomes examined.
F--the number of founder genotypes.

Population	F	Sample	Third Chromosomes ST	AR	CH	PP	TL	EP	SC	Other	n
Pacific Coast											
Okanagan, British Columbia	78	Sept. 1964	55.0	30.0	1.3	8.7	5.0				80
		May 1965	78.5	16.5	4.0	0.5	0.5				200
		July 1966	96.3	3.7							300
Methow, Washington	246	Sept. 1964	79.3	17.8			2.9				208
		May 1965	88.5	11.0			0.5				200
		July 1966									
Berkeley, California	96	May 1964	45.0	2.0	24.0	4.0	17.0	6.0	2.0		200
		May 1965	85.0	1.0	4.0	1.0	5.0	4.0			200
		July 1966	99.3		0.7						300
Riverside, California	50	June 1964	66.1	19.6	10.7		3.6				56
		May 1965	84.7	8.7	5.7	1.0					300
		July 1966	93.0		7.0						300
Basin and Range Province											
Tucson, Arizona	112	April 1964	3.6	94.5	0.9	0.9					110
		May 1965	5.0	92.0		3.0					200
		July 1966	43.0	57.0							300
Sonora, Mexico	48	March 1964	2.2	32.6	60.9						46
		May 1965	16.5	73.0	10.5						200
		July 1966	39.3	60.7							300
Rocky Mountains											
Black Canyon N. M., Colorado	178	Sept. 1964	5.0	94.4		0.6					180
		May 1965	23.7	76.0		0.3					300
		July 1966	44.3	55.7							300
Hayden Creek, Colorado	173	Sept. 1964	17.2	47.2	2.2	25.0	1.7	6.7			188
		May 1965	53.0	33.5		6.5	2.0	5.0			200
		July 1966	73.8	26.2							300
Raton, New Mexico	232	Sept. 1964	0.5	78.0	1.5	19.0	1.0				200
		May 1965	1.0	89.5		7.0				2.5*	200
		July 1966	16.3	81.7			2.0				300
Texas											
Davis, Texas	78	Sept. 1964		15.0	1.5	82.5		1.0			200
		May 1965		48.3		45.7	2.3	3.7			300
		July 1966		70.7		21.0			8.3		300
Austin, Texas	228	April 1964	2.6	16.4		72.0	3.5	1.3		4.3**	232
		May 1965	13.0	61.3		17.3	8.0	0.3			300
		July 1966	48.7	45.7		0.3	5.3				300

* New

** OL

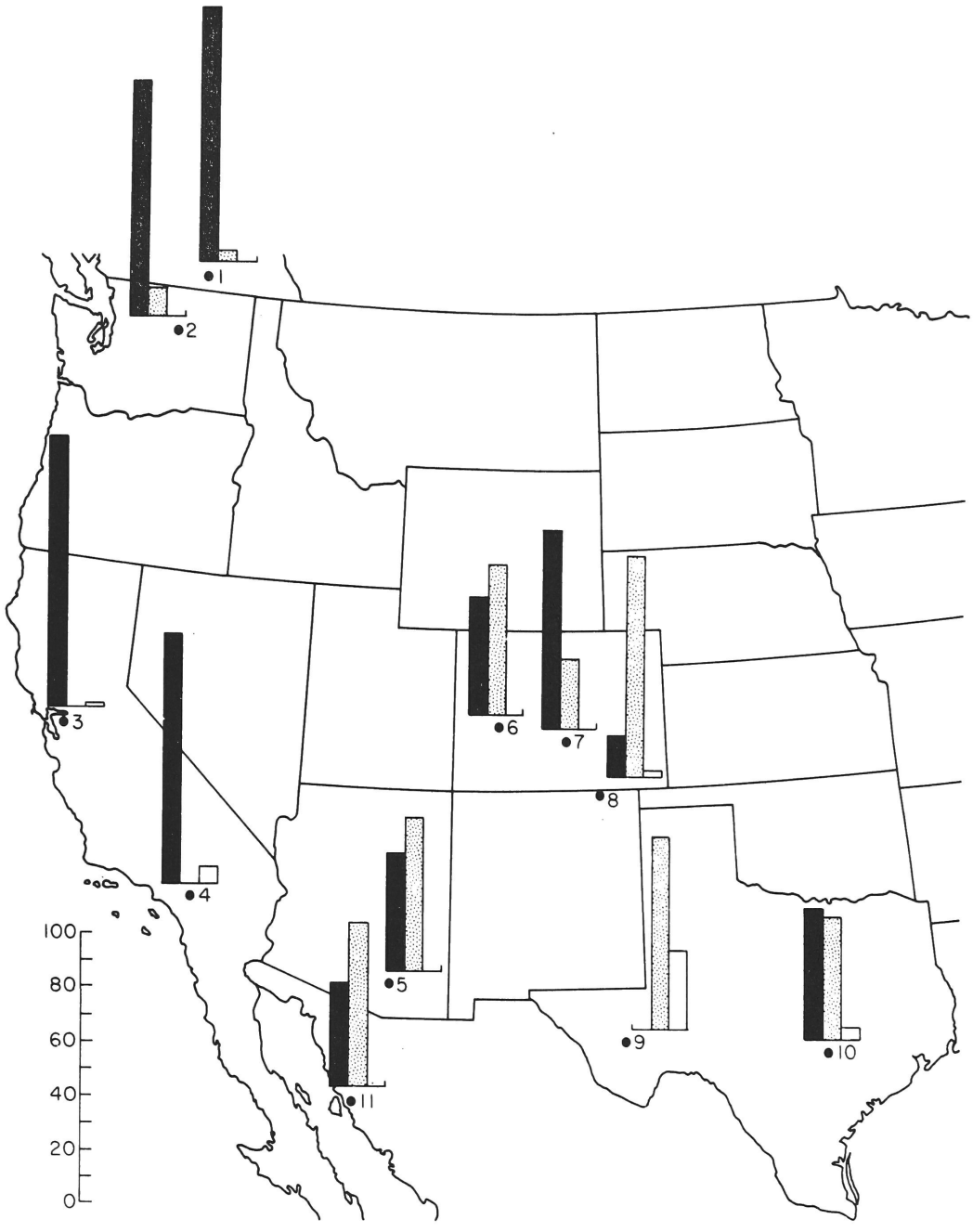

Fig. 12. Frequencies of the gene arrangements in the experimental populations begun from samples of natural populations, after 1½ years at 25°C. Black columns--Standard; stippled columns--Arrowhead; white columns--other gene arrangements (see Table XVII for details). The localities are as in Fig. 1.

by comparison, of low fitness and disappeared in all but the population from Canada. In the Riverside population, Chiricahua established a low-level polymorphism. The Arrowhead chromosome in the populations from the interior was much fitter under the laboratory conditions than its Pacific Coast counterpart; the Standard was less fit. Thus, many polymorphisms stabilized with Arrowhead and Standard at intermediate frequencies. The Davis, Texas, population had no Standard chromosomes, and a stable polymorphism between Arrowhead, Pikes Peak, and Santa Cruz was established. The behavior of this population, where Pikes Peak was initially most frequent, contrasts with that of populations begun with Arrowhead and Pikes Peak from California, where Pikes Peak was until recently quite rare (Pavlovsky and Dobzhansky, 1966). In the latter populations no equilibrium was reached, Pikes Peak tending to be eliminated; the heterokaryotype Arrowhead-Pikes Peak was less fit than the Arrowhead-Arrowhead homokaryotype. On a genetic background in which it is normally favored, however, the Pikes Peak from Davis formed a heterotic heterokaryotype with Arrowhead. The rise in frequency of the Santa Cruz gene arrangement was unexpected. Although not recorded in the earlier samples, Santa Cruz chromosomes were probably present at low frequency or were misclassified as Estes Park. In the Austin population, Standard rose and Pikes Peak fell, in frequency; the Tree Line arrangement was maintained, even increasing from its original frequency. The Tree Line gene arrangement was likewise maintained in the Raton population. In several of the experimental populations in this study, "rare" gene arrangements, which normally are eliminated in populations begun from laboratory strains, were retained. The balanced mixtures of chromosomes from the natural populations which were used to found the experimental populations may be the explanation for this different behavior.

3. Sex Ratio and polymorphism for X and third chromosomes. In Table XVIII are listed the frequencies of the Sex Ratio chromosomes and the frequencies of structural heterozygotes for the third chromosomes in the samples from natural populations. The latter are calculated by the binomial-square expansion of the gene arrangement frequencies. An examination of the data shows no correlation of the frequency of Sex Ratio with the extent of polymorphism in the third chromosome. Sonora, Ruidoso, and Fort Collins have the highest frequencies of Sex Ratio, but are all in

Table XVIII. The frequencies of the Sex Ratio chromosomes and of the third chromosome heterokaryotypes in samples from natural populations, 1963-1966. N--the number of chromosomes examined.

	Locality	Sex Ratio		3rd Chromosome	
		%	N	% Hetero-zygotes	N
1.	Okanagan, British Columbia	0.0	63	59.7	80
2.	Methow, Washington	0.0	165	33.9	208
3.	Mather, California	6.7	315	76.2	400
4.	Borrego, California	15.4	156	47.7	200
5.	San Jacinto, California				
	A. Pinon Flats	18.7	166	68.1	190
	B. Keen Camp	17.8	349	68.0	438
6.	Ferron, Utah	8.3	36	32.7	54
7.	Bryce N.P., Utah	13.2	144	15.1	200
8.	Fort Collins, Colorado	20.7	121	65.8	168
9.	Gunnison, Colorado	12.3	65	10.6	182
10.	Hayden Creek, Colorado	8.8	147	68.0	180
11.	Flagstaff, Arizona	16.0	150	7.8	200
12.	Grand Canyon, Arizona	16.6	163	6.8	200
13.	Tucson, Arizona	20.0	75	10.6	110
14.	Chiricahaua, Arizona	15.3	229	20.5	198
15.	Raton, New Mexico	16.0	156	35.5	200
16.	Ruidoso, New Mexico	22.4	76	59.4	200
17.	Austin, Texas	19.7	188	45.1	300
18.	Davis, Texas	14.9	134	29.7	200
19.	Sonora, Mexico	25.0	46	52.1	46

the upper forty per cent of the populations ranked according to the frequency of polymorphism in the third chromosome. The correlation between the frequency of Sex Ratio and the frequency of structural heterozygosity of the third chromosome is -0.08, which is not significant.

The north-south gradient in frequency of the Sex Ratio chromosome, first noticed by Sturtevant and Dobzhansky (1936b), is still evident. Sex Ratio is absent or rare in the northern populations and becomes more frequent in the south, reaching a maximum along the Mexican border. The reason for the gradient is still unknown, but evidently it does not depend on polymorphism in the third chromosome.

The results of the viability tests of crosses combining structural heterozygosity in the X and third chromosomes in different ways are given in Table XIX. The eggs of the doubly heterozygous females showed not only no increased embryonic lethality, but even a higher per cent of eggs giving rise to adults than in the single-inversion controls of Terzaghi and Knapp (1960).

D. Discussion

Like body size, the frequencies of the inversions vary with the physiographic divisions of the environment. The similar variation in the two characters is probably a reflection of the strong selection exerted in different areas; there is no basis for assuming that changes in one cause the other to vary.

The changes in time are of particular importance, for the pattern to them suggests a strong selection of some sort. The populations along the Pacific Coast, from Canada to Mexico, have undergone similar changes. The Standard chromosome increased, while the Arrowhead chromosome decreased, in frequency. Likewise, the Chiricahua and Pikes Peak chromosomes formed an associated pair, the rise in frequency of Pikes Peak being compensated by a drop in that of Chiricahua. The populations east of the Sierras show no pattern in the changes in the frequencies of the inversions. Two possible types of causes for the changes in the coastal populations were outlined by Dobzhansky (1958, 1963) and by Dobzhansky, Anderson, Pavlovsky, Spassky, and Wills (1964). New adaptive genotypes

Table XIX. Mean per cent of eggs giving rise to adults in crosses involving inversions on the X and third chromosomes of Drosophila pseudoobscura. N--the number of replicate bottles. X and SR are the standard and sex ratio X-chromosomes; AR, ST, and PP are the Arrowhead, Standard, and Pikes Peak gene arrangements on the third chromosome.

Cross			% Viability ± S.E.	N
$\frac{X}{X}$; $\frac{AR}{AR}$	x	$\frac{X}{Y}$; $\frac{AR}{AR}$	89.64 ± 1.15	22
$\frac{X}{X}$; $\frac{ST}{PP}$	x	$\frac{X}{Y}$; $\frac{PP}{PP}$	89.42 ± 1.25	38
$\frac{SR}{X}$; $\frac{ST}{PP}$	x	$\frac{X}{Y}$; $\frac{PP}{PP}$	94.39 ± 0.67	36

may have arisen, perhaps by recombination within preexisting structural types, and spread. Estimates of the rate of diffusion of genes in populations of Drosophila pseudoobscura do not support this hypothesis (Dobzhansky and Wright, 1947). Dispersal of flies over long distances by air currents could spread a new type, but there is no evidence that such air transport occurs in Drosophila. The second type of cause for the changes in the inversion system is selection by some agent of the environment. Correlations of the changes with many environmental variables have been attempted; rainfall, temperature, fallout from nuclear tests, and smog have all been eliminated as primary factors in the changes (Dobzhansky 1958, 1963; Dobzhansky, Anderson, Pavlovsky, Spassky, and Wills, 1964). The insecticides are currently being investigated in this regard. Insecticides have come into prominent use only after World War II, and hence after the early samples, and they have been intensively used particularly along the Pacific Coast. Oshima and Watanabe (1965) have published preliminary results of an experiment designed to test the effect of insecticides on inversion frequencies in experimental populations of Drosophila psuedoobscura. Exposure to low doses of DDT and Dieldrin each generation caused a rise in Standard, a drop in Arrowhead, and a longer retention of Pikes Peak than in control populations receiving no exposure to the insecticides. The insecticide hypothesis is thus at least tenable for present; its validation must await further analysis of these experiments.

The sudden rise in the frequency of Pikes Peak along the coast points to the importance of those chromosomes which are rare. They may remain at low frequencies for many years, to appear only when selection shifts. Their maintenance at low frequencies is very likely facilitated by heterozygote advantage and by frequency dependent fitnesses which increase as the chromosomes drop in frequency. The San Jacinto, Vandeventer, Mammoth, Cochise, Humbolt, and Texas arrangements, found in one or a few individuals in the early samples, have all been rediscovered in later collections.

The experimental populations begun from the natural collections underscore the flexibility of the inversion system in Drosophila pseudo-obscura. The equilibria established in the laboratory are very different from those prevailing in nature. Krimbas (1966) has run parallel

experiments with Drosophila subobscura, a European species which, like Drosophila pseudoobscura, is chromosomally polymorphic, wide-ranging, and successful. But Krimbas found little change in the karyotypic frequencies in his experimental populations. He also found little seasonal or altitudinal variation in chromosomal types in nature; Burla and Götz (1965) reported seasonal and altitudinal variation, but of very small magnitude compared to that in Drosophila pseudoobscura. Künze-Mühl, Müller, and Sperlich (1958), Dobzhansky (1962, 1965), and Krimbas (1964a and b, 1966) suggested that the chromosomal polymorphism in Drosophila subobscura is rigid while that in Drosophila pseudoobscura is flexible. Both my studies of body size and of inversion frequencies add fresh support to the theory that the two species possess different types of genetic systems. We recall from the studies of body size that the genes in populations of Drosophila pseudoobscura are coadpated, that is, selected for beneficial interactions; McFarquhar and Robertson (1963) found no such coadaptation in similar experiments with Drosophila subobscura. Drosophila pseudoobscura is genetically flexible and its inversion system changes rapidly as selection varies; Drosophila subobscura, on the other hand, is genetically rigid, and its genotypes withstand many alterations of selection without change. Here we see an example of the variability of evolution, of the different ways in which the same result may be achieved. Two species, similar in many respects, have evolved different methods of adjusting to similar environmental stresses.

The studies of the experimental populations reveal something about the association of inversion types. Wallace (1953, 1954, 1959) suggested that the distribution of inversion types in natural populations of Drosophila pseudoobscura is related to their ability to transfer genes serially from one to another. When groups of three gene arrangements--"triads"--from a linear sequence in the phylogenetic tree of inversions are present in the same population, gene transfer may break down the coadapted complexes of genes within each inversion. The Arrowhead-Standard-Tree Line and Arrowhead-Pikes Peak-Santa Cruz groups which became established in my experimental populations are not triads and thus may retain the coadapted blocks within the inverted sections of the third chromosome. The Arrowhead-Standard-Pikes Peak group which existed in the Austin population as Standard and Arrowhead rose, and Pikes Peak fell in frequency is a triad and thus

might suffer a selective disadvantage. Of course, the Standard-Arrowhead combination might simply be so fit under the laboratory conditions as to eliminate the weaker combinations with Pikes Peak. The Standard-Arrowhead-Chiricahua group, which is not a triad and thus not subject to the disadvantageous shuffling of the genes inside the rearranged segments, disappeared from the Californian and Mexican populations. The behavior of the gene arrangements in my experimental populations is, thus, consistent with Wallace's triad hypothesis. The inversions on the X and third chromosomes seem to be independent; there is no association of heterozygosity for these two chromosomes in nature. The crosses which combined heterozygosity for inversions on the X and third chromosomes showed that no association should be expected. The interchromosomal effects on viability found by Terzaghi and Knapp (1960) were not present in my tests of inversions from nature. The decreased viability found when two or more chromosomes simultaneously carry inversions has been demonstrated in Drosophila melanogaster and Drosophila pseudoobscura for induced inversions only. Riles (1965) found no inviability in combinations of inversions taken from natural populations of Drosophila robusta. The inversions found in nature have undergone selection for many years and are not a random sample of those which occur after x-irradiation in the laboratory. Those chromosomes which in combination lower fitness will be selected against, leaving in the populations those which do not produce deleterious effects.

E. Summary

1. Collections of Drosophila pseudoobscura were taken in many natural populations in the American West. As in samples taken up to thirty years ago, the frequencies of the inversions vary geographically, forming chromosomal races which are related to the different physiographic divisions of the environment. This geographic variation in inversion frequencies, similar to that for body size, is direct evidence of the important role of the inversions in nature. The recent collections are contrasted with earlier ones. A consistent pattern of change is found along the Pacific Coast but not eastward of the Sierras. Two "pairs" of chromosomal types, Standard-Arrowhead and Chiricahua-Pikes Peak show compensatory changes; the rise in the frequencies of Standard and Pikes Peak are associated with drops in the frequencies of Arrowhead and Chiricahua. The spread of the Pikes Peak arrangement is

particularly noteworthy. The causes of these changes over time are not yet decided; several hypotheses are discussed, the possible role of insecticides being stressed.

2. Eleven experimental populations of Drosophila pseudoobscura were begun from samples of natural populations ranging from Canada to Mexico. Each population was begun, as far as practicable, with the same chromosomal constitution as had the sample from the locality in nature. The frequencies of the inversions were followed for two years. The terminal frequencies of the gene arrangements in the experimental populations reflect the geographic areas from which the populations were derived. Thus the genetic differentiation of the chromosomal races can be demonstrated in the laboratory as well as in nature. The equilibrium frequencies established in the laboratory populations were quite different from those in the natural populations which were the ancestors of the laboratory ones. This result stands in interesting contrast with that obtained by Krimbas in Drosophila subobscura. The contrast between the two species extends both to the general coadaptive features of the gene pools, as revealed in the studies of body size, and to their chromosomal polymorphisms. These studies lend additional support to the hypothesis that Drosophila pseudoobscura is genetically flexible, while Drosophila subobscura is genetically rigid. The contrast between the mode of genetic adjustment in the two species illustrates the very different pathways evolution may take in adapting similar organisms to similar environmental stresses.

3. The nature of the polymorphisms in the experimental populations is consistent with the "triad" hypothesis, which states that groups of three chromosomes related one to the next by single inversions will not coexist in the same population because gene transfer between the inversions is then possible. Coadaptation would be lost as a consequence. The polymorphism on the third chromosome is independent of that on the X-chromosome. There is no correlation between the two polymorphisms in nature. Crosses which combined the natural inversions on the third and X-chromosomes showed that no correlation is expected from the effects of heterozygous inversions on viability, contrary to the results of other workers with induced inversions. The role of selection in weeding out polymorphisms which interact unfavorably is stressed.

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V. APPENDIX

Table XX. Body size in hybrids and parents from the experimental populations maintained at different temperatures; females only, at 19°C.

Cross[(a)]	F_1 *	F_2 *	Cross[(a)]	F_1 *	F_2 *
A	92.02	93.88	CA	92.62	94.90
B	93.64	94.42	AE & EA	92.00	92.75
C	89.92	91.74	AF & FA	89.48	90.10
D	89.14	89.78	BE & EB	92.70	94.08
E	89.46	91.54	BF & FB	91.19	92.71
F	87.04	90.26	CF	90.50	92.54
AB & BA	92.57	94.71	FC		90.62
AC	90.26	91.20	EF & FE	89.68	90.92
Average standard error	0.417	0.404			

(a) Female parent of each hybrid given first.

* Average of 50 measurements for each parental and reciprocal hybrid cross. One unit = 20.8 μ

Table XXI. Body size in hybrids and parents from the geographic populations; females only, at 25°C. N--the number of wings measured.

Cross(a)	F_1	N-F_1	F_2	N-F_2	Cross(a)	F_1	N-F_1	F_2	N-F_2
ST	82.08	36	80.63	70	TA	82.70	70	80.81	70
TS	80.47	70	80.36	70	AT	80.67	70	80.89	70
SA	82.83	36	82.76	70	TB	79.56	70	82.9	10
AS	78.60	70	79.04	70	BT	81.66	70	81.89	70
SB	80.87	60	80.53	30	TH	81.07	70	81.24	70
BS	81.03	70	80.63	60	HT	79.86	50	80.80	70
SH	79.33	39	78.37	70	TR	80.19	70	81.03	70
HS	78.77	70	77.69	70	RT	80.64	58	79.31	70
SR	80.77	26	78.6	10	TC	81.23	70	81.06	50
RS	80.40	68	79.34	70	CT	81.03	70	80.06	70
SC	81.50	20	79.46	70	AB	80.81	70	81.51	70
CS	81.26	70	80.91	70	BA	81.39	70	81.10	70
AR	80.67	70	79.59	70	AH	82.51	70	81.06	70
RA	81.14	70	80.43	30	HA	81.54	70	79.53	70
AC	81.84	70	80.43	30	RC	79.95	20	79.03	60
CA	81.06	70	80.37	70	CR	79.71	70	78.69	70
BH	80.89	70	81.00	30	Average S.E.	0.36		0.30	
HB	80.66	70	80.53	30	S	80.28	90	80.04	140
BR	79.77	60	80.64	50	T	81.12	140	81.07	140
RB	80.34	70	80.77	70	A	81.38	140	82.12	140
BC	81.75	67	79.64	70	B	80.72	140	81.04	140
CB	81.43	63	81.63	70	H	80.32	140	80.27	140
HR	78.44	70	79.00	70	R	78.99	140	79.28	140
RH	79.87	60	78.93	60	C	81.99	140	81.59	140
HC	81.46	70	80.70	70	Average S.E.	0.22		0.27	
CH	80.87	70	81.74	70					

All measurements are in micrometer scale units; one unit = 20.8 μ.

(a) Female parent given first; abbreviations are as in Table XI.

End