GENETICS OF NATURAL POPULATIONS. IV. MEXICAN AND GUATEMALAN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

NOTWITHSTANDING their external uniformity, free-living populations of *Drosophila pseudoobscura* are replete with genetic variability. The variations in the shape of the Y chromosome (DOBZHANSKY 1935, 1937), and in the gene arrangement in the third chromosome (DOBZHANSKY and STURTEVANT 1938) are especially striking. A genetic analysis of the third chromosomes has shown that a considerable proportion of them carry recessive lethals, semilethals, deleterious viability modifiers, and other morphological and physiological changes (STURTEVANT 1937, DOBZHANSKY and QUEAL 1938b).

The present study was undertaken in order to extend the above observations by using samples of the populations of *D. pseudoobscura* inhabiting the highlands of Mexico and Guatemala. A greater comprehensiveness was, however, not the only aim of the work. In the absence of suitable techniques of creating experimental populations of Drosophila, the general problems of population mechanics can be attacked only through comparative analysis of judiciously selected free-living populations. For reasons to be explained below, a comparison of Mexican and Guatemalan populations with those from the temperate part of North America seemed especially promising.

MATERIAL AND TECHNIQUE

The distribution area of *D. pseudoobscura* extends from the boreal forests of British Columbia to well below the Tropic of Cancer. In Mexico and Guatemala it inhabits the montane coniferous and broad leaved forests, within the altitudinal limits of approximately 5500–8500 feet. It does not occur in the tropical lowlands, even when pine groves are encountered therein (for example, south of Chilpancingo, Guerrero, Mexico, or near Quirigua, Guatemala). Its south-eastern limit is apparently reached in the highlands of western Guatemala; at least, it has not been found in the pine barrens around Guatemala City which appear, judging by analogies, not unfavorable for it. A list of the localities in which population samples were collected is given below (compare with figure 3). The names used to designate the strains are italicized.

Guatemala: Finca Santa Victoria, near Panajachel, Lake *Atitlan*, Feb-

ruary 9, 1938, mixed wood; *Totonicapan*, February 11, pine grove; slope of Santa Maria, at Zunil, near *Quezaltenango*, February 12, pine wood; between San Francisco and *Momostenango*, February 13, pine wood; Salegua valley, near *Huehuetenango*, February 14, mixed wood; Rancho Tejor, near *Sacapulas*, February 15, mixed forest; *Chichicastenango*, February 16, deciduous grove.

Mexico, *Oaxaca:* Cerro San Jose, near Ejutla, September 1936, mixed forest; Morelos: north of *Cuernavaca*, September 1936 and March 7, 1938, mixed forest. Mexico: foot of Iztaccihuatl, near *Amecameca*, March 9, pine wood. Michoacan: Lengua de la Vaca, near *Zitacuaro*, March 13, pine wood; Quiroga, Lake *Patzcuaro*, March 14, mixed wood; east of *Morelia*, March 15, oak grove. Vera Cruz: mountains above Tecamaluco, near *Orizaba*, March 20, mixed wood. Puebla: below Asseraderos de San Felipe, east of *Tehuacan*, March 22, pine grove; slope of Iztaccihuatl, northwest of *Puebla*, March 23, mixed forest. Hidalgo: Omitlan, near *Pachuca*, March 25, mixed grove. Durango: Rancho *Otinapa*, September 1936, pine forest.

The wild females trapped were isolated from the males, shipped to the Laboratory at Pasadena, and each of them became the progenitor of a separate strain. Chromosomes of the larval salivary glands were examined in temporary aceto-carmine mounts; the larvae to be used for this purpose were grown at 18°–20°C, with yeast added to the regular medium. The shape of the Y chromosome was studied in temporary aceto-carmine smears of the testicular tissue of freshly hatched adult males. The crosses made for the analysis of the gene contents in the third chromosomes were raised generally at room temperature, but the generations (F_3, F_4, and F_5) in which the counts were made were always kept in an incubator at 24.5°C.

GENE ARRANGEMENTS IN THE THIRD CHROMOSOME

Seventeen distinct gene arrangements, related to each other mostly as overlapping inversions, were identified in the third chromosome of *D. pseudoobscura* by DOBZHANSKY and STURTEVANT (1938). With the aid of a method described in detail in the paper just referred to, and which need not be dwelt upon here, the phylogenetic relationships of these seventeen structural types were established (compare with the phylogenetic chart in figure 3 of that paper). The arrangements denoted as Standard, Hypothetical (resembling that found in *D. miranda*) and perhaps Santa Cruz, are the ancestral ones. Standard has given rise to a family of related types which occur mostly in race B, but two of which, namely Arrowhead and Pike's Peak, are restricted to race A. Race B of *D. pseudoobscura* does not occur in Mexico and Guatemala. Santa Cruz has produced a family of four arrangements: Cuernavaca, Chiricahua I, Mammoth, and Tree Line. Tree Line has, in turn, given rise to a family of four derivatives: Estes Park,

Oaxaca, Olympic, and Hidalgo. Santa Cruz, Tree Line, and their derivatives are found exclusively in race A.

The identification of the gene arrangements is technically easiest if the individual to be tested is outcrossed to another whose third chromosomes are known beforehand. The chromosomes are examined in the salivary glands of the larvae in the F_1 of the cross. If the individual in question has the same gene arrangement in its chromosomes as that present in the tester strain, only simple chromosome strands are found in the hybrid. Any difference in the gene arrangement is reflected in formation of aberrant pairing configurations. If, finally, the tested individual is a structural heterozygote, two or more types of hybrid larvae are encountered. To

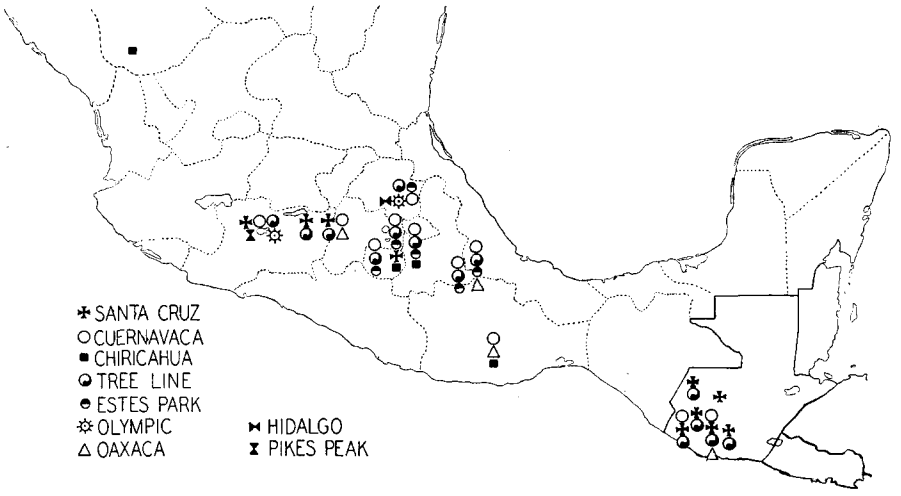

FIGURE 1.—The distribution of the structural types of the third chromosome in Mexico and Guatemala.

facilitate the observation, tester strains are so chosen that the gene arrangement found in them resembles as much as possible that expected in the materials tested.

Two strains, one homozygous for the Santa Cruz and the other for the Tree Line arrangements in the third chromosomes, were used as testers for Mexican and Guatemalan populations. Single sons of the females caught outdoors, and in some instances single grandsons of such females, were crossed to females from the tester strains. A summary of the data thus obtained is presented in table 1 and in figure 1.

Nine gene arrangements in the third chromosome are recorded in the material examined. In three localities from which adequate samples were available (Pachuca, Amecameca, and Patzcuaro), as many as five structural types were detected in the same population. This indicates that

Mexican and Guatemalan populations of *D. pseudoobscura* tend, on the whole, to be more heterogeneous with respect to the gene arrangement than is the rule in populations from more northern latitudes (DOBZHANSKY and STURTEVANT 1938). The total number of the arrangements recorded in race A from its entire distribution area, extending from British Columbia to Guatemala, is only thirteen. Among the nine found in Mexico, eight

TABLE 1

Frequencies of the gene arrangements in the third chromosome in populations from various localities in Mexico and Guatemala.

	SANTA CRUZ	CUER-NAVACA	CHIRI-CAHUA	TREE LINE	ESTES PARK	OLYM-PIC	OAXACA	HI-DALGO	PIKE'S PEAK	CHRO-MOSOMES TESTED
Orizaba	—	27	—	5	4	—	4	—	—	40
Tehuacan	—	11	—	2	3	—	—	—	—	16
Pachuca	—	45	—	27	2	14	—	2	—	90
Puebla	—	13	1	11	5	—	—	—	—	30
Amecameca	1	17	2	11	19	—	—	—	—	50
Cuernavaca	—	4	—	—	—	—	—	—	—	4
Zitacuaro	2	9	—	4	—	—	1	—	—	16
Morelia	2	—	—	4	—	—	—	—	—	6
Patzcuaro	18	8	—	2	—	2	—	—	2	32
Quezaltenango	35	6	—	15	—	—	—	—	—	56
Momostenango	1	—	—	1	—	—	—	—	—	2
Totonicapan	9	1	—	3	—	—	1	—	—	14
Huehuetenango	5	—	—	1	—	—	—	—	—	6
Sacapulas	4	—	—	—	—	—	—	—	—	4
Atitlan	2	1	—	1	—	—	—	—	—	4

were known previously, and one, Hidalgo, is new. Hidalgo/Tree Line heterozygotes have a single short inversion in the third chromosome, which includes sections 77B, 78, 79A, 68D, 74AB, 73, 72C, and a part of 72B (figure 2). The position of Hidalgo in the phylogenetic scheme is, accordingly, among the Tree Line derivatives.

The presumed ancestral arrangement, the Standard, is common on the Pacific Coast of the United States, from British Columbia to Lower California, and occurs sporadically further east, as far as the Rockies and possibly Texas. Yet, Standard does not penetrate Mexico and Guatemala. The same negative conclusion must be reached with respect to Arrowhead, which is the commonest type almost everywhere in the United States where the species is found. The sole derivative of Standard that is found in Mexico, and at that very rarely, is Pike's Peak, which is rather common in the Rockies and very common in Texas. Pike's Peak is not found on the

Pacific Coast of the United States, except in a small area north of San Francisco Bay, where it has been detected in two samples collected by DR. A. H. STURTEVANT (from Sebastopol and from Hopland, California). All other gene arrangements inhabiting Mexico and Guatemala belong to the Santa Cruz and the Tree Line "families"; furthermore, all the members of these "families" are represented in these countries, with the single exception of Mammoth which is endemic to the Sierra Nevada of California. On the other hand, only three of the arrangements found in Mexico and Guatemala, namely Cuernavaca, Oaxaca, and Hidalgo, are endemic, while the remainder are found also in the United States, especially in California and in the Rocky Mountains of northern Colorado, although none of them

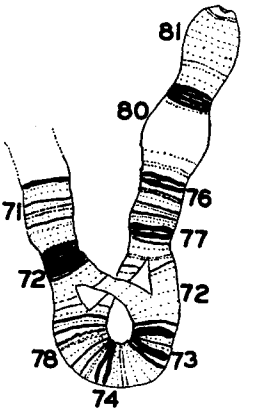

FIGURE 2.—The distal part of the third chromosome in the Tree Line / Hidalgo heterozygotes.

are known to go as far north as British Columbia. To summarize: populations inhabiting the extremes of the species area, British Columbia in the North and Mexico and Guatemala in the South, have no structural types in the third chromosome in common; populations from the intervening territories contain mixtures of the northern and the southern types in varying proportions.

Within Mexico and Guatemala the populations are also not uniform. The localities studied fall into two large groups. The first embraces east-central Mexico, from Orizaba to Cuernavaca (table 1), and the second includes west-central Mexico and Guatemala. The first group is characterized by high frequencies of Estes Park and Cuernavaca arrangements, and by the absence of Santa Cruz. A high frequency of Santa Cruz, a lower one of Cuernavaca, and absence of Estes Park are characteristic for the second group. The writer is insufficiently familiar with zoo- and phytogeography and geology to visualize the causal background of the division just described.

The geographical regularities discussed above represent what one could

perhaps call gross distributional features. Superimposed on them are much more local, microgeographic, variations, which manifest themselves primarily in unexpectedly large differences between populations inhabiting neighboring localities. Thus, 15.5 percent of the chromosomes at Pachuca have the Olympic arrangement, which is not found at all in other localities studied in east-central Mexico, and which reappears only in the relatively remote Patzcuaro. Although many samples are admittedly small, the statistical probability of the difference between Pachuca and adjacent localities being due to chance is negligible. A similar, though less pronounced difference exists between the Puebla and Amecameca samples, which come from places separated only by the northeastern spur of the volcano Iztaccihuatl, the crest of Rio Frio, which is completely covered with forest favorable for the habitation of *D. pseudoobscura*. Nevertheless, the Estes Park arrangement is more common at Amecameca than it is at Puebla; the χ^2 of the difference equals 4.062, which means that such or a greater difference may occur by chance less than once in twenty trials. The failure to find the Oaxaca arrangement anywhere in central Mexico except in Orizaba and Zitacuaro is also suggestive, although this may well be due to inadequacy of the samples. In general, although any one of such differences could conceivably be due to sampling errors, the combined data prove the existence of microgeographic variations rather convincingly. Whether such variations are permanant, in the sense that a population retains its characteristics from year to year, or are fleeting waves in the genetic composition of a local colony, can not be decided for the Mexican samples. DOBZHANSKY and QUEAL (1938a) and KOLLER (1939) found that populations inhabiting different mountain ranges in the Death Valley region of California, and even parts of the same range, are not alike. According to KOLLER, the composition of the population in a locality may differ in successive years.[1]

Somewhere between the macro- and the microgeographic variations stand the phenomena of discontinuity in the distribution of certain arrangements. The Chiricahua arrangement is rather common in the southwestern United States; the single strain known from northern Mexico (Durango) proved to be homozygous for it. Yet, in central Mexico this ar-

[1] The finding of DOBZHANSKY and QUEAL that populations inhabiting adjacent mountain ranges differ in composition, and that there are no clear geographical trends or "gradients" in these variations, has been misunderstood by some colleagues as implying a denial of the usual rule that such trends are present in intraspecific geographical variability. This is, of course, a misapprehension. The geographical trends certainly exist, and can be detected if samples from sufficiently remote localities are studied (compare with DOBZHANSKY and STURTEVANT 1938). But if samples are taken from close localities, the major trends are obscured by the microgeographic variations. The macro- and the microgeographic variations are not mutually exclusive, but, on the contrary, complementary, and the latter may prove to be the germs of the former.

rangement is rare (table 1). The two strains known from southern Mexico (Oaxaca) both contained it, and in Guatemala it has not been found. Estes Park is common in east-central Mexico, but not in the west-central part or in Guatemala. The only other area in which it is known to exist is the Rocky Mountains of northern Colorado. Olympic is known from the Olympic peninsula, Washington, from Mount Whitney, California, and from Pachuca and Patzcuaro, Mexico. The absence of Estes Park in west-central Mexico and in Guatemala appears to be a somewhat special case. A glance at table 1 or figure 1 shows that there is a kind of repulsion between the Estes Park and the Santa Cruz arrangements: they practically never occur together. Phylogenetically, Estes Park is descended from Tree Line, which is in turn a derivative of Santa Cruz. A comparison of Estes Park and Santa Cruz (compare with DOBZHANSKY and STURTEVANT 1938) reveals that these gene arrangements are very similar: the inversion transforming Tree Line into Estes Park has nearly undone the change produced by the inversion through which Tree Line arose from Santa Cruz. The Estes Park/Santa Cruz heterozygotes have in their salivary gland cells almost completely paired third chromosomes, showing only small deficiency-duplication buckles in the short sections where the homology is incomplete. STURTEVANT (1938) has shown that, on theoretical grounds, such inversions are not expected to coexist in the same population, since the crossing-over in the heterozygotes will give rise to gametes with unbalanced gene complements. According to STURTEVANT, this situation may serve as a starting point for the development of isolating mechanisms, and hence for species divergence. The "repulsion" between Estes Park and Santa Cruz observed in Mexican and Guatemalan populations may be regarded as evidence corroborating STURTEVANT'S theoretical deductions.

VARIATION IN THE Y CHROMOSOME

Seven distinct types of Y chromosome are known in *D. pseudoobscura* (DOBZHANSKY 1935, 1937), four of which, types IV, V, VI, and VII, occur exclusively in race A, two, types II and III, only in race B, and one, type I, in both races. The shape of the Y chromosome has been examined in 26 strains from Mexico and in 9 strains from Guatemala. The results obtained are as follows.

Type I. Mexico. Durango: Otinapa-7. Michoacan: Patzcuaro-1, Patzcuaro-2, Zitacuaro-3. Morelos: Cuernavaca-5. Puebla: Tehuacan-1. Oaxaca: Cerro San Jose-4, Cerro San Jose-5.

Type IV. Mexico. Durango: Otinapa-3. Michoacan: Patzcuaro-4, Morelia-1, Morelia-3, Zitacuaro-2. Hidalgo: Pachuca-2, Pachuca-5. Mexico: Amecameca-1, Amecameca-3. Morelos: Cuernavaca-2, -5, -6, -8, -11. Puebla: Puebla-1, Puebla-2, Tehuacan-2. Vera Cruz: Orizaba-2, Orizaba-3.

Type VII. Guatemala: Quezaltenango-1, 2-, -3, Momostenango-1, Huehuetenango-1, Sacapulas-1, Chichicastenango-1, Totonicapan-1, Atitlan-1.

A summary of the above data is presented in figure 3 in map form. Types I and IV occur throughout Mexico, the former apparently being commoner in the western and the latter in the eastern part of the country. Beyond the confines of Mexico, type I has been recorded in race A from a single locality only, namely from Santa Rita Mts., which is close to the Mexican border. Type IV is found along the Pacific Coast of the United States and Canada, and also in Arizona and New Mexico. Type V, which is the commonest in the Rocky Mountain area, does not occur in Mexico at all. This negative information is interesting since it parallels the results obtained with the gene arrangements related to Standard in the third chromosome (see above).

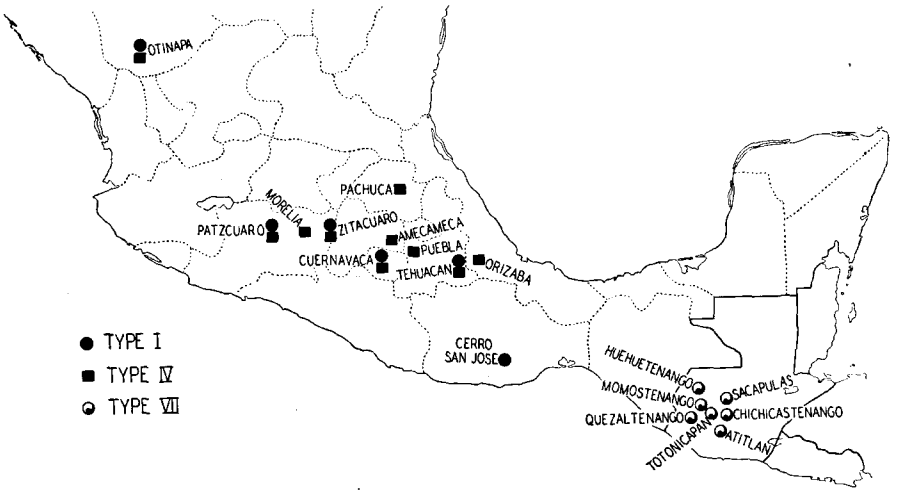

FIGURE 3.—The distribution of the types of Y chromosome in Mexico and Guatemala.

A totally unexpected finding is that the Guatemalan populations are uniform in having the type VII of the Y chromosome. This type has been recorded previously only from two localities in northern Colorado, and from nowhere else in the United States or in Mexico. The possibility that we are dealing with two distinct but mimic types, one endemic to Guatemala and the other to Colorado, should not be disregarded: small differences in the metaphase chromosomes are easily overlooked. But the great difference between the Mexican and the Guatemalan populations with respect to the Y chromosome is also unexpected, since no analogous difference exists with respect to the gene arrangements in the third chromosome. The strains from Cerro San Jose, Oaxaca, which were recorded in 1937 as having the type I of the Y were reinvestigated, in order to ascertain that these strains have actually type I, and not VII, of the Y. The original

identification proved to be correct. The boundary between the Mexican and the Guatemalan populations is probably the low Isthmus of Tehuantepec, which, being covered with tropical vegetation, is in all likelihood not inhabited by *D. pseudoobscura*.

GENE ARRANGEMENT IN CHROMOSOMES OTHER THAN THE THIRD

The "sex-ratio" condition in race A of *D. pseudoobscura* (STURTEVANT and DOBZHANSKY 1937) is now known to be associated with a triple inversion in the right limb of the X chromosome. These inversions were repeatedly observed in preparations of the Mexican material, but estimates of the frequency of "sex-ratio" in populations can be made more easily by observing the progeny from the crosses of single sons of wild mothers to unrelated females. "Sex-ratio" males produce progenies consisting of daughters, and few or no sons. Sometimes two sons of the same female were tested; since a female has two X chromosomes, her sons may be either alike or different. If a single son is tested, the presence or absence of the "sex-ratio" in a single X is determined; if a second son of the same female is tested, the probability that the second X of that female is recovered is 50 percent. Therefore, it was considered that 1½ chromosomes were tested wherever the progeny of two brothers was observed. In east-central Mexico (the first six localities in table 1) 24½ "sex-ratios" were found among 126½ tested chromosomes, so that the frequency is 19.4 percent. In west-central Mexico (Zitacuaro, Morelia, and Patzcuaro) the figures are 5½ among 32, or 17.2 percent, and in Guatemala 1 in 65½, or 1.6 percent. The figure for Guatemala is astonishingly low.

In the chromosomes other than the third and the right limb of the X no inversions or other changes were detected, although in the older material from Cuernavaca a fourth chromosome inversion has been seen (DOBZHANSKY and STURTEVANT 1938).

GENIC VARIABILITY IN THE THIRD CHROMOSOME

Analysis of the population samples from the mountains of the Death Valley region has shown that about 15 percent of the third chromosomes carry recessive lethal or semilethal genes. Among the remaining 85 percent, a very large fraction carry viability modifiers, a decided majority of them unfavorable to the organism. Other types of hereditary variability, such as mutations producing visible external effects and modifiers of the rate of development, are also encountered (DOBZHANSKY and QUEAL 1938b). To obtain comparable data for Mexican and Guatemalan populations, the technique used previously in the work with those from Death Valley was followed.

Each female caught outdoors was allowed to produce offspring. A single

male was taken from every culture, and crossed to females homozygous for the third-chromosome recessives orange and purple (*or pr*). In the F_1, males were taken, one per culture, and crossed to females having *or*, Blade, Scute, and *pr* in one chromosome and the Cuernavaca inversion in the other. The inversion is used to prevent crossing over in the *or Bl Sc pr / Cuernavaca* females. In the next generation, denoted F_2 although it is not that strictly speaking, females and males showing *Bl* and *Sc* are selected from each culture and intercrossed; their constitution is evidently *or Bl Sc pr / wild*. In the F_3 a segregation in the ratio 66.7 percent *Bl Sc* : 33.3 percent wild type is expected, provided the wild chromosome in question carries no viability modifiers. A more detailed description of this technique, including a discussion of its limitations, may be found in the paper by DOBZHANSKY and QUEAL (1938b). Suffice it to add here that, since the *or Bl Sc pr* chromosome has the Standard gene arrangement and the wild chromosomes from Mexico and Guatemala always have arrangements other than the Standard, few or no crossovers appear in the offspring of *or Bl Sc pr* females.

Altogether 120 wild chromosomes, 38 from Guatemala and 82 from Mexico were analyzed. The proportion of the wild-type individuals in different F_3 cultures varied from zero to a high value of 57.05 percent (table 2). Complete absence of wild-types in a culture indicates that the wild third chromosome involved carries a lethal gene, or genes. The number of such cultures in the Guatemalan samples amounts to 18.4 percent, in the Mexican, to 22.0 percent and in the combined data to 20.83 ± 2.50 percent of the total. This may be compared with the figure for the Death Valley populations, which is 11.90 ± 0.75 percent. The infestation with lethals is greater in the Mexican and Guatemalan populations, the difference being significant.

In some cultures a few wild-types do appear, but their frequency is far below the theoretically expected 33.3 percent. Such cultures contain semilethals. The distinction between the lethals and the semilethals, and between the semilethals and the "normal" viability range, is not sharp. Arbitrarily we may consider any culture producing less than one-half of the expected number, that is, less than 16 percent, of wild-types as having a semilethal. Although this limit is an arbitrary one, shifting it a little upward or downward fails to alter the results appreciably, since cultures giving frequencies of wild-types in the neighborhood of 16 percent are not numerous. On this basis, the proportion of the chromosomes carrying lethals or semilethals in Guatemalan populations turns out to be 34.21 ± 5.20 percent, in the Mexican 28.05 ± 3.35 percent, and in the combined data 30.00 ± 2.82 percent. The comparable figure for the Death Valley populations is 14.96 ± 0.82; the difference is 15.04 ± 2.94 per cent, or five

times greater than the probable error. STURTEVANT (1937) recorded 19.25 ± 1.95 percent of lethals, and no semilethals, in samples from various parts of the distribution area of the species, including some Mexican chromosomes. Comparing this figure with ours for lethals and semilethals combined, which is entirely fair since STURTEVANT'S failure to discover semilethals is due clearly to chance, we find that Mexican and Guatemalan populations have 10.75 ± 3.43 percent more lethal-bearing chromosomes than the species at large, and hence the germ plasm of the Mexican and Guatemalan populations is infested with these genetic variants to a greater extent than is the case in other territories. With 30 percent of the third

TABLE 2

Frequency of wild-types (in percent) in the F_3 cultures; further explanation in text.

LOCALITY	0	0+–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	...	57.05	TOTAL
Quezaltenango	3	3	—	—	—	—	1	—	1	—	—	—	1	—	4	3	4	2	1	1	...	—	24
Totonicapan	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	2	1	1	—		—	6
Momostenango	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Sacapulas	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—		—	2
Huehuetenango	1	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—		—	3
Chichicastenango	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Atitlan	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Total Guatemala	7	3	—	—	1	—	1	—	1	—	—	—	1	1	5	5	7	3	2	1		—	38
Patzcuaro	1	1	—	—	1	—	—	—	—	—	—	—	—	—	3	—	4	1	1	—		—	12
Morelia	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Zitacuaro	2	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—		—	4
Cuernavaca	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1		—	2
Amecameca	4	—	—	—	—	—	1	—	—	—	—	—	1	2	2	4	2	1	2	3		—	22
Puebla	3	—	—	—	—	—	—	—	—	1	—	—	—	—	—	2	—	2	—	2		1	11
Tehuacan	1	—	—	—	—	—	—	—	1	—	—	—	—	—	1	1	—	—	1	—		—	5
Orizaba	3	—	—	—	—	—	—	—	—	—	—	—	2	—	1	1	—	—	1	1		—	9
Pachuca	2	1	—	—	—	—	—	—	—	1	1	—	1	3	1	—	—	3	3	—		—	16
Total Mexico	18	2	—	—	1	—	1	—	1	2	1	—	5	5	8	9	6	7	8	7		1	82
Grand Total	25	5	—	—	2	—	2	—	2	2	1	—	6	6	13	14	13	10	10	8		1	120

chromosomes carrying lethals and semilethals, only half of the flies are free of them, 42 percent have one, and 9 percent have two lethal-bearing third chromosomes (this, of course, does not take into account the infestation which is probably present in the second, fourth, fifth, and X chromosomes).

Disregarding the lethals and the semilethals, a residue is obtained in which the frequency of wild-types varies from 16 percent to 38 percent, with one culture giving as much as 57 percent (table 2). To test whether this variation is genetic or is due to a combination of environmental influences with sampling errors, a F_4 generation was raised. For this purpose, *Bl Sc* females and males were selected in F_3 cultures and inbred; counts in the

offspring were made to determine the frequencies of wild-type and *Bl Sc* individuals. The results are shown in figure 4 in form of a correlation table, comparing the frequencies of the wild types in the F_3 and F_4 cultures of the same strains. The strain that gave 57.05 percent of wild types in F_3 is omitted; three F_4 cultures were raised from this line, giving respectively 29.3 percent, 33.1 percent, and 34.9 percent wild-type. The unreasonably

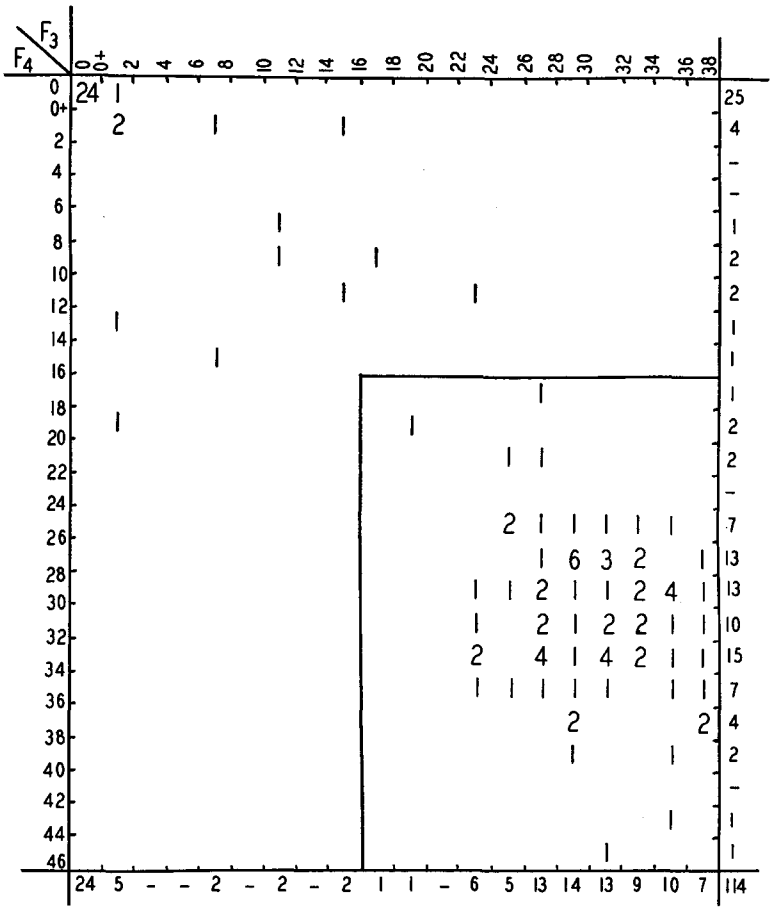

FIGURE 4.—Correlation between the frequencies of wild-type individuals produced in the F_3 and F_4 generations of the same lines. The frequencies are expressed in percent.

high frequency observed in F_3 is probably due to contamination; the F_3 culture contained 149 flies.

The correlation table conveys, first of all, an idea about the validity of the classification of lethals and semilethals. The 24 chromosomes, which behaved in F_3 as carrying lethals, showed a similar behavior in F_4. One of the F_3 semilethals behaved in F_4 as a complete lethal, and another of the F_3 semilethals moved in F_4 into the subnormal range. Two lines which on

the basis of the F_4 data must be classified as semilethals were in the subnormal range in F_5. If the frequencies of the lethals and semilethals were determined on the basis of the F_4, instead of the F_5, data, the figure arrived at would have been 31.3 percent, instead of 30.0 percent. This is a very good agreement.

The strains which gave more than 16 percent wild type in both F_3 and F_4 generations are found in the lower right part of figure 4. If this part is treated as an independent correlation table, a correlation coefficient $r = \pm 0.253 \pm 0.072$ is obtained. This is a weak, but still perceptible, positive correlation. The existence of the genetic viability modifiers is hereby established.

Wild type individuals obtained in F_3 and F_4 of the above experiments are made homozygous for wild third chromosomes. In natural populations, with the possible exception of local colonies *in extremis* reduced to very few individuals, there is no such rigidly enforced homozygosis. The "normal" viability of *D. pseudoobscura* must be looked for in individuals that receive from their parents unlike chromosomes of each kind, or, to put it more exactly, a random sample of the chromosomes present in the population in question. To obtain such a standard of comparison, *Bl Sc* / wild females and males from *different* F_4 cultures were intercrossed. The frequency of the wild type individuals in the resulting offspring is shown in table 3. The

TABLE 3

Viability of types heterozygous for two different third chromosomes extracted from wild populations.

PERCENT OF WILD TYPES	0	...	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	Total
No lethals	—	...	1	3	4	12	18	26	34	37	22	14	6	6	1	1	185
Two lethals	4	...	—	3	3	8	13	22	29	19	10	10	1	3	3	—	124
Total	4	...	1	6	7	20	31	48	63	56	32	24	7	9	4	1	309

distribution curves for the homozygotes and the heterozygotes are shown superimposed on each other in figure 5. It is quite evident that, even disregarding lethals and semilethals, the curve for the heterozygotes is displaced toward the right compared to that for the homozygotes. This is tantamount to saying that the viability of the former is greater than that of the latter.

The task of determining the frequency of the chromosomes containing minor deleterious modifiers is a difficult one, because the intensity of such modifiers ranges from semilethality up to the norm. In working with the Death Valley populations, an attempt was made to estimate this frequency by counting the number of strains which gave less than 34 percent wild

type in both F_3 and F_4. The figure arrived at was 39 per cent of all chromosomes studied, or 43.6 percent of the chromosomes having no lethals or semilethals. An obvious objection against this procedure is that a strain may deviate twice in the minus direction from the expectation without containing a viability modifier, but due to chance alone. On the other hand, strains containing mild deleterious modifiers may, also by chance, give more than 34 percent wild types in one or the other, or in both, generations. This should mitigate the above objection, but nevertheless the figure obtained is at best a rough approximation. In Mexican and Guatemalan populations, strains giving less than 32 percent wild type in both

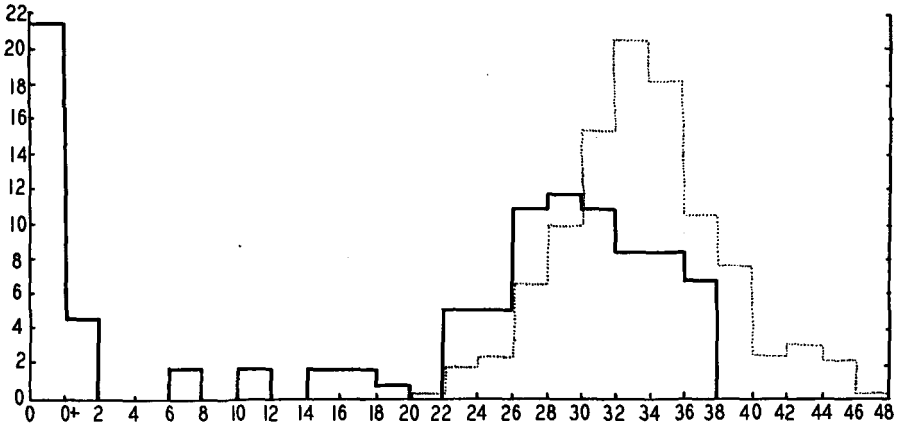

FIGURE 5.—Distributions of the frequencies (in percent) of wild-type individuals produced in cultures where the wild types are homozygous (solid line) and heterozygous (dotted line) for wild third chromosomes.

F_3 and F_4 may be taken to contain deleterious viability genes (the lowering of the standard from 34 percent to 32 percent is due to the average viability of the Mexican and Guatemalan heterozygotes being lower than that of the Death Valley ones, see below). The data presented in figure 4 show that 31 strains gave less than 32 percent, but more than 16 percent, of wild type in F_3 and F_4. This constitutes 27.2 percent of all chromosomes tested and 40.3 percent of those not having lethals or semilethals. The latter figure agrees with that obtained for the Death Valley populations.

More accurate, though less interesting biologically, is the determination of the average reduction of viability produced by homozygosis for a wild third chromosome. Again disregarding the lethals and semilethals, the F_3 cultures have produced on the average 29.84 $\pm$ 0.34 percent (computed from the data in table 2), and the F_4 cultures 30.31 $\pm$ 0.38 percent wild type (computed from figure 4). A comparable figure for the heterozygotes (computed from the data in table 3) is 33.40 $\pm$ 0.18 percent. The difference

is clearly significant. The survival value of homozygotes is distinctly below that of the heterozygotes, so that natural populations resemble to a certain extent the balanced lethal systems used in some laboratory experiments. This conclusion agrees with that arrived at on the basis of the study of Death Valley populations, although the exact figures obtained for the latter are somewhat different, namely 35.52 ± 0.20 percent for heterozygotes and 32.58 ± 0.12 percent for homozygotes. Taken at their face value, the data suggest, therefore, that the genotype of the Mexican and Guatemalan populations is on the whole less favorable than that of the Death Valley ones under the environmental conditions at which the experiments were done. This difference must not be stressed unduly, since the experiments done at different times, actually more than a year apart, are not strictly comparable. Moreover, the mixture of the modifiers contained in the Mexican third chromosomes with those in other chromosomes of the *or pr* and *or Bl Sc pr* strains may be in part responsible.

Lethals and viability modifiers are not the only genetic variants detected in the wild third chromosomes. Although no sharp mutant types, such as are commonly used in linkage studies, were found in the Mexican and Guatemalan materials, at least nine of the homozygotes were phenotypically abnormal in varying degrees. Of these, five were also semilethal, and four possessed normal or subnormal viability. Four were wing characters, two were dwarfish, one had a pale body color, one had short legs and antennae, and one showed black spots on the trochanters of the legs.

Genes modifying the development rate are also common. Table 4 presents the data for eight cultures in which such modifiers are manifestly at work. In Huehuetenango-2, Patzcuaro-2, and Amecameca-19 the early counts give a greater proportion of wild type than do the later ones. Here, consequently, the wild third chromosomes contain genes which speed up the development of the homozygotes (wild-type) relative to that of the heterozygotes (*Bl Sc*). The reverse is observed in the remaining five cultures, in which the early counts show a preponderance of *Bl Sc*, and the late ones an excess of wild-type flies. In these cultures the wild third chromosomes carry modifiers that slow down the development. Following the advice of DR. K. MATHER, the reality of these phenomena was tested by calculating the χ^2 values for heterogeneity, using the BRANDT and SNEDECOR method. The χ^2 values are shown in table 4 together with estimates of P, that is of the probability that such or greater deviations from homogeneity may arise by chance. Except for Orizaba-1, the P values are very small, and hence the heterogeneity is a real one. As expected, in the F_4 generation the strains shown in table 4 have repeated their performance shown by F_3, with the sole exception of Amecameca-19, which in F_4 showed mere traces of an excess of wild-type in the early counts. Since

out of 120 chromosomes tested in F_3, 25 chromosomes contained lethals or extreme semilethals, an opportunity to detect modifiers of the development rate was present in only 95 cultures. Among these, 8 cultures manifested such modifiers, thus giving the frequency 8.4 percent. There is no doubt that this is a gross underestimate compared to the real frequency, because the experiments were so arranged that the detection of the development rate modifiers was made difficult. The parents were allowed to oviposite until their offspring started to hatch, thus obscuring the action not only of the slight but even of the medium strong modifiers.

TABLE 4

Proportions of wild-type and Blade-Scute individuals on successive counts in certain F_3 cultures.

DATE	HUEHUE-TENANGO-2		PATZ-CUARO-2		PATZ-CUARO-13		PACHUCA-13		PACHUCA-24		DATE	ORIZABA-1		AMECA-MECA-19		QUEZAL-TENANGO-21	
	WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*		WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*
June 21	22	44	20	76	—	10	—	6	—	3	June 19			19	26		
June 24	7	13	1	20	3	38	3	14	—	15	June 21	2	23	5	9	—	15
June 27	3	16	—	45	17	38	7	21	1	51	June 23	1	21	21	36	—	51
June 30	4	8	—	33	18	34	19	37	14	29	June 26	14	45	6	24	2	15
July 4	—	10	—	40	17	18	11	23	15	29	June 29	19	40	4	45	9	13
July 9	—	22	—	39	11	34	2	25	6	30	July 2	11	27	6	36	7	13
July 11					3	11					July 4	12	47	22	68		
											July 9	14	43	5	19		
χ^2	15.7838		42.8639		22.1360		17.4181		27.3419		χ^2	11.3578		22.8096		30.6264	
P	0.01–0.02		<0.01		<0.01		<0.01		<0.01		P	0.10–0.20		<0.01		<0.01	

RECESSIVITY OF THE LETHALS

For an understanding of the population dynamics it is very important to know whether the lethals and other genetic variants present in the wild flies are completely or only partly recessive. The equilibrium frequency of a lethal in a population is determined by the mutation frequency producing that lethal from the normal allele, by the frequency of the reverse mutation, and by selection eliminating the lethal-bearing chromosomes. A completely recessive lethal is sheltered by its normal allele as long as only individuals heterozygous for it are present in the population; the elimination occurs only when homozygotes are produced. If the frequency of a lethal is q, that of the homozygotes is evidently q^2. If, on the other hand, the adaptive value of the heterozygotes is lower than that of the nonlethal homozygotes, the elimination can go on at a much faster rate.

To approach this problem experimentally, intercrosses of *Bl Sc* / wild females and males from *different* F_4 cultures were arranged. Two series of such intercrosses were made: in one of them the flies were taken from cultures known to contain no lethals in the third chromosomes, and in the other both parents carried lethals. The wild-type individuals appearing in

the F_5 of the first series are free of lethals (except where the lethals might have arisen by mutation, which is negligible), and the *Bl Sc* sibs contain only one lethal, namely Blade. In the F_5 of the second series the wild-type flies have two lethals, one in each of their third chromosomes, and the *Bl Sc* sibs have also two lethals, namely Blade and one or the other of the two "natural" ones. It is evident that if the lethals are incompletely recessive, the average frequency of the wild-type individuals in the first series must be greater than that in the second series.

Experiments of the type just outlined were made also with lethal-bearing and with non-lethal bearing chromosomes from the Death Valley populations (DOBZHANSKY and QUEAL 1938b). The results obtained there suggested that at least some of the lethals have slight dominant effects. Since however the two series of the experiments were not run simultaneously, these results were not conclusive, in view of the possible environmental differences between the series. The problem being a rather important one, it was decided to repeat the experiments on a larger scale and with all possible precautions, using Mexican and Guatemalan materials. The new results are shown in table 3. Some of the intercrosses containing lethals gave no wild-type flies at all; cultures of this kind may be disregarded since they obviously represent instances in which the pairs of lethals found in wild populations happened to be alleles. Otherwise, the average frequencies of wild types in the cultures with and without lethals are respectively 33.11 $\pm$ 0.27 percent and 33.59 $\pm$ 0.22 percent. The difference, 0.48 $\pm$ 0.35 percent is less than one and a half time greater than its probable error, and therefore not significant. As an additional check, the average numbers of offspring produced in the cultures of each series were computed, on the assumption that the possible dominant effects of the lethals may cause a drop in the productivity of the flies. The figures obtained were 160.25 $\pm$ 4.12 and 163.11 $\pm$ 3.50 per culture containing and not containing lethals respectively. The result is again negative.

The problem of the recessivity of the naturally occurring lethals is nevertheless not closed. In statistics, negative results are incapable of demonstrating the absence of a supposed effect, although they can show, and in the present case do show, that this effect, if any, is slight. Yet, even a very slight effect of this type is not negligible in population mechanics. An indirect method of attacking the same problem is to compare the elimination rate of the lethals calculated on the assumption that they are completely recessive, with the mutation rate producing lethals. It is hoped to present results obtained with the aid of this method in a separate publication.

DISCUSSION

The genetic structure of Mexican and Guatemalan populations of *D. pseudoobscura* proved to be, in its broad outline, the same as observed pre-

viously in the populations of the Death Valley region. It encompasses an extensive variation in the gene arrangement in the third, contrasting with a relative stability in other chromosomes, some variation in the shape of the Y chromosome, and the presence of an amazing store of recessive lethals, semilethals, deleterious viability modifiers, visible mutants, and modifiers of the development rate. Although it is premature to generalize these results as universal, even for all populations of *D. pseudoobsura*, it is reasonable to assume, as a working hypothesis, that similar conditions may prevail in many sexually reproducing and cross-fertilizing organisms. Pragmatically, differences are however at least as interesting as are the similarities, since the former may permit an insight into the operation of the forces of population mechanics.

The environment in which *D. pseudoobscura* lives below the Tropic in Mexico and Guatemala is obviously distinct from that of the high mountain ranges of the Death Valley region. In Mexican and Guatemalan populations about 30 percent of the third chromosomes carry lethals and semilethals, contrasting with only 15 percent in the Death Valley region. Is there any functional relation between the environmental differences and those in the genetic structure of the fly populations? Three hypotheses, not necessarily alternative to each other, may be advanced in this connection. First, the mutation rate producing lethals may be higher in the Mexican and Guatemalan populations than in the Death Valley ones. Indeed, DEMEREC (1938) and DUBOVSKIJ (1935) found that certain strains of *D. melanogaster* have a greater inherent mutability than others. Since the equilibrium point for a recessive lethal is the square root of the mutation rate, the mutation rate in the Death Valley race must be merely one-fourth of what it is in the Mexican-Guatemalan race. Experiments to test this possibility are in progress. Second, if the lethals are not completely recessive, their frequency may be influenced by selection in the heterozygous state. The stringency, as well as the sign, of the selection may differ in the respective geographic regions. Even if we were able to ascertain that lethals have dominant effects, it would still remain next to impossible to test this hypothesis experimentally. A third possibility, which seems most plausible, is that the greater concentration of lethals in Mexico and Guatemala is due to the effective size of the breeding population being greater in these countries than it is in the Death Valley region.

If there is no reverse mutation from lethal to normal, the equilibrium concentration of a lethal in a population is, as stated above, equal to the square root of the mutation rate from normal to lethal. This is however a limiting case, fully realized only in very large populations, while in smaller ones the equilibrium may be, as shown by WRIGHT (1931, 1937) much lower. In the theoretical example discussed by WRIGHT, the equilibrium

value for a lethal with a mutation rate of 1 : 100,000 is 0.0032 in an effective population of one million or more, and only 0.00008 in a population of ten individuals. It is not possible to tell how great must be the difference between the population sizes to account for a 100 percent increase or a 50 percent reduction in the concentration of lethals, since the relation between these variables in not a linear one. In WRIGHT'S example, a factor of ten can more than double the concentration of lethals in populations of the order from one to ten thousands, and smaller factors suffice for smaller populations. The accumulation of lethals in all parts of the distribution area of *D. pseudoobscura* would have been equal if there were no racial variation in the mutation rate, and if the effective population sizes were large enough throughout to be treated as practically infinite ones. Our data show however that this is not the case.

Although no exact information on the effective population size is available, the following considerations are germane. In exceptionally favorable localities and in favorable season, a hundred or more individuals can assemble in each trap bottle exposed for several hours. Even where the productivity of a trap is very much lower, there is no apparent decrease in the catch if the traps are exposed in the same place day after day. In localities where several species of Drosophila occur together, the compositions of the samples caught in different traps, placed about one or two hundred feet apart, may be very different, proving that the flies entering the traps come from their immediate neighborhoods, and certainly do not travel for miles to reach them. The absolute numbers of the flies present at the peak of the season in mildly favorable forested areas are, it appears, enormous. Yet, to infer from this that the effective sizes of the breeding population must always be practically infinite would be fallacious. Two things must not be lost sight of. First, in a species subject to seasonal fluctuations in numbers, the effective population size is close to that reached at the minimum phase (WIGHT 1938). Second, as shown by DOBZHANSKY and QUEAL (1938a) and by KOLLER (1939), populations inhabiting not only adjacent mountain ranges but even parts of the same mountain range are frequently distinct genetically. The microgeographic variations described in the present article also indicate that the elementary breeding unit may be a population inhabiting a very small territory, although how small that territory may be remains to be determined by future studies.

Curiously enough, the apparent abundance of the flies in Mexico and Guatemala is lower than it is on the mountain ranges of the Death Valley region, although these latter are also by no means the most densely populated places known. The writer collected in Mexico in September, which is the end of the rainy season, and again in March, which is in the second half of the dry period; at both times it proved to be not an easy matter to

assemble adequate samples in many localities. It may also be noted that in the Death Valley region, and in the California mountains in general, *D. pseudoobscura* is by far the commonest species attracted to the traps, while in Mexico and Guatemala it shares the available habitats with a variety of others, among which *D. azteca* and species of *D. hydei* and *D. saltans* groups are equally or more common.

There is however an important difference between the Mexican and Guatemalan populations on one hand and those from the Death Valley region on the other, a difference which has, in fact, provided the stimulus for the present investigation. Beginning with the region lying several degrees of latitude below the Tropic of Cancer, the yearly climatic cycle consists chiefly in an alternation of rainy and dry, rather than of cold and warm, seasons. Despite the fact that *D. pseudoobscura* occurs in the highlands of Mexico and Guatemala and avoids the strictly tropical zone, it can breed throughout the year without major interruptions, and without apparent shrinkages and expansions in numbers. The situation is quite different in the Death Valley region, where the forest belt is so high above sea level that it is covered with snow, or at least subjected to freezing temperatures, for about five months in a year, and in addition is exposed to extreme aridity in late summer. While in Mexico no difference in the apparent density of the flies was observed between September and March, which are climatically very distinct, sharp seasonal fluctuations occur in the California mountains. Although more information is admittedly needed on the seasonal populaton changes than is now available, it is safe to say that the amplitude of the fluctuations in numbers is smaller in *D. pseudoobscura* living below the Tropic than it is in the Temperate Zone. Although the apparent abundance of the flies at the peak of the season is greater in the Death Valley region than it is in Mexico or Guatemala, the abundance at the eclipse stage, and therewith the genetically effective population size, are the opposite. The greater genetic heterogeneity observed in the Mexican and Guatemalan populations is in accord with the expectation arrived at on theoretical grounds.

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SUMMARY

1. Samples of natural populations of *Drosophila pseudoobscura* were collected in eighteen localities in Mexico and Guatemala (figure 3), and a cytogenetic study of the material so obtained was made.

2. Nine distinct gene arrangements in the third chromosome, one of them first described in the present article, occur in Mexico and Guatemala (table 1, figure 1). All but one are phylogenetically interrelated, and belong to the Santa Cruz "family," which is indigenous also in the southwestern United States, but is rare or absent in the Northwest and in British Columbia.

3. The relative frequencies of the gene arrangements vary from locality to locality, showing both the major geographical trends and microgeographic fluctuations.

4. Santa Cruz and Estes Park arrangements, which are sufficiently similar presumably to allow crossing over throughout the length of the chromosome, practically never occur together.

5. Two types of the Y chromosome are found in Mexico, and a third occurs in Guatemala (figure 3).

6. The "sex-ratio" condition is moderately common in Mexico and rare in Guatemala.

7. In Mexico and Guatemala, about 30 percent of the wild third chromosomes contain lethals and semilethals. This contrasts with the 15 percent obtained for the Death Valley region.

8. Among the chromosomes free of lethals and semilethals, a large fraction, perhaps as much as 40 percent, have modifiers deleterious for the viability. Visible mutants and modifiers of the development rate are also common.

9. The adaptive value of individuals homozygous for wild third chromosomes is markedly below that of individuals heterozygous for different chromosomes found in the same population (figure 5). The lethals and other deleterious viability changes carried in wild third chromosomes seem to be completely recessive, although this is not regarded as established beyond doubt.

10. The greater genetic heterogeneity observed in Mexican and Guatemalan populations as compared with those from the Death Valley region is probably due to the effective size of the breeding population being greater in the tropical than in the more northern localities.

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