EXPERIMENTS ON CHROMOSOMAL VARIABILITY IN DROSOPHILA ROBUSTA [1]

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MOST species of Drosophila so far studied in this respect are polymorphic for gene arrangements in their chromosomes. In *D. pseudoobscura, D. persimilis,* and *D. funebris,* this polymorphism is adaptive (DOBZHANSKY 1943, 1947, 1948a, 1948b, 1948c; DOBZHANSKY and EPLING 1944; DUBININ and TINIAKOV 1945, 1946a, 1946b; SPIESS 1950). The adaptive nature of the chromosomal polymorphism is shown by three types of evidence. (1) The frequencies of the gene arrangements often show clear geographic and altitudinal gradients. (2) Cyclic seasonal changes in relative frequencies of gene arrangements are observed in some localities. (3) Experiments on the behavior of chromosomes with different gene arrangements in artificial populations in the laboratory show that inversion heterozygotes possess, as a rule, a much higher fitness than do the corresponding inversion homozygotes. CARSON and STALKER (1947, 1949) STALKER and CARSON (1948) have discovered chromosomal polymorphism also in *D. robusta* Sturtevant, a species common in forested regions of the eastern United States. In addition to the usual paracentric inversions, *D. robusta* populations contain also at least two pericentric and two cytologically apparently terminal ones. Geographic and altitudinal clines have been demonstrated for certain gene arrangements, but seasonal cyclic variations are small or absent in the populations living near St. Louis, Missouri.

The work reported in the present paper is a study of natural and experimental populations of *D. robusta* in the New York City area. The evidence shows that the chromosomal polymorphism in this species is also adaptive, inversion heterozygotes possessing higher adaptive values than do the homozygotes. The work was started during the summer of 1947. Preliminary collections showed that the gene arrangements 2L, 2L–1 and 2L–3 in the left arm of the second chromosome would be especially suitable for experimental study. Experimental populations with different combinations of the gene arrangements were created, and the ensuing changes in the relative frequencies of the chromosomal types in these populations were recorded by means of periodic sampling. In the spring and fall of 1948 and in the spring of 1949 systematic collecting was made in a single locality in New Jersey to detect possible seasonal variations in the incidence of the gene arrangements and to obtain a larger number of strains for further experimental populations.

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MATERIALS AND METHODS

Collections of Drosophila for this study were made in 1947 in deciduous woods on the outskirts of Bronx County, New York (3); in Tibbetts Brook Park, Westchester County, New York (10); near Fort Lee, Bergen County, New Jersey (6); in the Mariners' Harbor section of Richmond County, New York (11); at Cold Spring Harbor, Suffolk County, New York (2); and at Tenafly, Bergen County, New Jersey (1). In the parentheses are the number of strains developed from each place. Mass cultures were developed from each collected female, and the salivary glands of twenty to thirty larvae from each culture were smeared in aceto-orcein for a qualitative analysis.

The quantitative collections were made at Englewood Cliffs, Bergen County, New Jersey, in an area about 30×120 feet. The traps (Mason jars) were maintained throughout the study by fastening them to branches with wire. Collected females were placed individually in culture bottles; after five days they were transferred to fresh food. If larvae appeared in either bottle a salivary smear was made from a single female larva, and the recorded arrangements contributed to an "egg sample" (following the terminology of DOBZHANSKY and LEVENE 1948). If no larvae appeared, the female, presumably uninseminated, was mated to males homozygous for all chromosomal arms. Examination of seven to nine larvae from this cross determined the chromosomal constitution of the collected fly; the sum of these results may be called an "adult female sample." The corresponding "adult male sample" was obtained from matings of collected males and virgin stock females, care being taken in each instance to examine the salivary glands of at least one *female* larva. The homozygous stock used for most of the determinations, derived from a Westchester County strain, was homozygous for arrangements XL, XR, 2L, 2R, 3L and 3R.

The population cages used for *D. pseudoobscura* by WRIGHT and DOBZHANSKY (1946) are applicable also for *D. robusta*. The approximate duration of the egg to egg cycle in the cages was 35 days at 25°C, 50 days at 21°C, and 68 days at 15½°C.

The earliest populations were taken from pair matings out of the mass cultures as described by WRIGHT and DOBZHANSKY (1946). The offspring of similar homozygotes from the collections, as well as from these matings, were retained as homozygous stocks for use in subsequent cages. In addition, the F_1 or F_2 from collected flies were used in the makeup of later populations studying 2L vs. 2L–1 or 2L vs. 2L–3 when this would introduce no other arrangements of this arm.

In New York experiments at 15½°C were kept in a constant temperature room. The relative humidity varied considerably throughout the year, the range of monthly means being from 62.7 ± 0.8 percent (March, 1949) to 83.5 ± 0.9 percent (July, 1948). Some experiments at 25°C were also run in a constant temperature room. The monthly means of relative humidity were between 65.8 ± 0.4 percent (January, 1949) and 76.5 ± 0.7 percent (July, 1949). For the other experiments incubators were used. In one set at 25°C

the monthly means of relative humidity ranged from 47.2 ± 0.3 percent (April, 1949) to 59.2 ± 0.7 percent (June, 1949). In another 25° incubator the monthly means varied from 89.9 ± 0.5 percent in March, 1949, to 92.0 ± 0.2 percent in August, 1949. One experiment was kept in a 21° incubator at generally close to 70 percent humidity. On September 8, 1949, a number of the experiments were moved to Virginia Polytechnic Institute, Blacksburg, Virginia, and continued in incubators there.

Because of the long life cycle of *D. robusta* a considerable problem in these experiments was the prevention of mite infections and contaminations with foreign Drosophila species. Benzyl benzoate described by WALLACE (1948) and a 5 percent dust of the miticide "DMC" and pyrophyllite (DAHM and BAUER 1949) in the cups with growing larvae were used to control the mites. (Technical compound "DMC" obtained through the courtesy of the Sherwin-Williams Company, Cleveland, Ohio, and pyrophyllite from the R. T. Vanderbilt Company, New York, New York.)

CHROMOSOMAL POLYMORPHISM IN NATURAL POPULATIONS

Data concerning the frequencies of the gene arrangements at Englewood Cliffs, New Jersey, are summarized in table 1. The fifteen gene arrangements found in more than one locality by CARSON and STALKER (1947) are all represented. The relative frequencies of the gene arrangements in this population are generally similar to their northern populations. Arrangements that show most obvious increases from south to north, XL–1, 2R, and 3R, show at Englewood Cliffs the highest frequencies yet recorded for these arrangements; correspondingly, XL, 2R–1, and 3R–1 show their lowest recorded frequencies. Another inversion more common in northern than in southern populations, the pericentric inversion, 2L–3, has here almost as high a frequency as in Iowa. Arrangements common in southern United States, such as XL–2 and 2L–2, are comparatively rare in Englewood Cliffs.

Comparison of the arrangement frequencies in spring and fall samples discloses no significant differences. The differences within the 1948 collections are probably attributable to sampling errors; even were they real, the spring, 1949, data show that the changes are not cyclic.

An autumnal virginal diapause similar to that described by CARSON and STALKER (1948) was found at Englewood Cliffs; all except the first two out of 84 mature-appearing females collected in the fall of 1948 were uninseminated in nature.

Zygotic frequencies in the egg sample, adult male sample, adult female sample, and combined adult sample (data kept on file to minimize publication costs) were studied by means of LEVENE's t-test (DOBZHANSKY and LEVENE 1948). In no instance is there a significant difference, at the 5 percent level using both tails of the curve, between the number of heterozygotes and homozygotes observed and the number expected in each sample by HARDY's formula. The adult female sample does show a large excess of heterozygotes for the X-left arrangements but this is probably not significant ($P = 0.088$). In

TABLE 1

Gene arrangements and their frequencies (in percent) in D. robusta collected at Englewood Cliffs, New Jersey, May 6, 1948–June 8, 1949.

	n_X	XL	XL-1	XL-2	XR	XR-1	XR-2	n_A	2L	2L-1	2L-2	2L-3	2R	2R-1	3R	3R-1
May 6–June 14, 1948	32	34.4	65.6	0.0	90.6	0.0	9.4	44	31.8	27.3	2.3	38.6	100.0	0.0	100.0	0.0
March 28–June 8, 1949	131	42.0	56.5	1.5	87.0	0.0	13.0	178	46.1	28.6	3.4	21.9	96.3	3.4	98.3	1.7
Both springs	163	40.5	58.3	1.2	87.7	0.0	12.3	222	43.2	28.4	3.2	25.2	97.3	2.7	98.6	1.4
August 24–October 3, 1948	236	39.1	60.2	0.4	88.1	0.4	11.4	323	41.2	28.2	5.9	24.8	98.8	1.2	96.9	3.1
Total	399	39.8	59.4	0.8	88.0	0.3	11.8	545	42.0	28.3	4.8	25.0	98.2	1.8	97.6	2.4

most other cases " t " is positive, indicating an excess of homozygotes; the deviation of this type is greatest for the combined adult sample of second-left arrangements (P = 0.102). In unpublished data CARSON and STALKER found more significant excesses of homozygotes. Natural populations of *D. pseudoobscura* have an excess of inversion heterozygotes in adult male samples (DOBZHANSKY and LEVENE 1948).

Table 2 shows gametic frequencies in adult samples, by season. Surprising differences are disclosed between the gene arrangements carried by males and females in the fall, 1948, collection. The females seem to have a significantly higher frequency of XL and 2L and a lower frequency of XL–1 and 2L–3 than do the males; the excess of 2L is highly significant (P less than 0.01). The data suggest that selection for the carriers of these arrangements is not equal in the sexes. The data suggest also that these sex differences show seasonal variation; for example, males seem to carry less 2L than females in the fall but more 2L in the spring. The data were tested for seasonal and sexual differences simultaneously by comparing the frequencies of the ar-

TABLE 2

Seasonal comparison of gametic frequencies (in percent) of XL and 2L arrangements in adult samples.

	n_X	XL	XL-1	XL-2	n_A	2L	2L-1	2L-2	2L-3
Fall males	87	31.0	69.0	0.0	175	34.3	29.7	6.9	29.1
Fall females	144	44.4	54.9	0.7	144	50.0	25.7	4.9	19.4
Spring males	61	41.0	57.4	1.6	118	44.9	28.0	3.4	23.7
Spring females	20	45.0	55.0	0.0	20	25.0	30.0	10.0	35.0
Total males	148	35.1	64.2	0.7	293	38.6	29.0	5.5	27.0
Total females	164	44.5	54.9	0.6	164	47.0	26.2	5.5	21.3

rangements in spring males, spring females, fall males and fall females, against the frequencies expected if neither season nor sex had any effect. No significant differences appeared between the carriers of X-left arrangements, 2L–1, and 2L–3, but males and females apparently do show a significant seasonal difference in their frequencies of the arrangement 2L (P slightly more than 0.01 for 3 degrees of freedom). A serious limit to the validity of this test is the rarity of virgin females in the spring collections.

EXPERIMENTAL POPULATIONS

Table 3 contains data describing the initial compositions of the population cages in this study. Experiments of DOBZHANSKY, HOLZ, and SPASSKY (1942) demonstrated the presence of many recessive deleterious mutants in chromosomes of *D. pseudoobscura* derived from natural populations. Presumably this is no less true for *D. robusta;* hence, an attempt was made to ensure heterogeneity of gene contents of the chromosomes used by including a minimum of five different strains of each gene arrangement. The number of strains used, listed in the table as " number of kinds of chromosomes," repre-

sents the minimal number of kinds of chromosomes, since the parental flies of a strain may contribute more than a single chromosome with the same gene arrangements. Populations 1 and 2 were derived from the same pair matings, hence from identical strains; with two exceptions the same was true of 3 and 4. In cages 1–16, initial populations contained equal numbers of males and females.

The experimental results are given as gametic frequencies, the bulkier zygotic data being kept on file. Unless otherwise noted, the chromosome number per sample is 300. The sample of the parental population, taken about two weeks after the experiment began, was called "control" sample. The

TABLE 3

Composition of the initial populations in 21 population cages.

Experiment number	Date begun	Temp. °C.	Gametic frequencies			Number of kinds of chromosomes			Number of flies
			2L	2L-1	2L-3	2L	2L-1	2L-3	
1	March 8, 1948	25	25	75	....	16	16	....	1000
2	March 18, 1948	15½	25	75	....	16	16	....	1000
3	August 30, 1948	25	78	22	....	14	11	....	518
5	November 12, 1948	15½	90	10	....	18	9	....	1000
6	November 12, 1948	15½	76	....	24	22	....	20	1000
7	November 17, 1948	15½	....	80	20	....	12	5	638
8	November 19, 1948	25	82	....	18	26	....	17	1110
9	December 1, 1948	25	....	85	15	....	13	6	1000
10	December 22, 1948	15½	....	20	80	....	13	7	1000
11	January 5, 1949	15½	20	....	80	14	....	19	1000
13	January 18, 1949	25	20	....	80	20	....	21	1000
14	January 25, 1949	25	....	20	80	....	17	9	1000
4	September 23, 1948	25	81	19	...	12	12	...	878
12	January 10, 1949	25	10	90	....	23	11	..	2000
15	May 13, 1949	25	....	60	40	....	11	7	1000
1A	January 8, 1949	25	22	78	....	16	16	..	?
2A	April 1, 1949	21	30	70	....	16	16	...	?
9A	May 27, 1949	25	....	80	20	....	13	6	?
12A	June 1, 1949	25	11	89	....	23	11	....	4562
3A	November 14, 1949	25	33	67	...	14	11	....	152
16	November 30, 1949	25	50	...	50	11	....	6	1000

sample taken after the first generation hatched was called "F_1." Since the intervals between subsequent samples were equal to the generation time, they were analogously labelled; no implication that the sampled population consisted wholly of that generation is intended.

A. *Populations with 2L and 2L–1*

Table 4 shows the results of two experiments with the arrangements 2L and 2L–1 maintained at about 15½°C. The first of these, cage 2, apparently reached an equilibrium at about 30 percent 2L and 70 percent 2L–1. For calculation of the adaptive values of the gene arrangements in this environment, cage 5, where changes continued for several generations, is more suitable.

TABLE 4

Samples from experimental populations of 2L vs. 2L-1 at 15½° and 21° C. (2 and 5 at 15½°, 2A at 21°).

Sample	Cage 2		Cage 5		Cage 2A	
	2L	2L-1	2L	2L-1	2L	2L-1
Initial	25.0	75.0	90.0	10.0	30.0	70.0
Control	22.0	78.0	90.7	9.3	....	....
F_1	25.3	74.7	62.7	37.3	....	....
F_2	31.0	69.0	54.0	46.0	19.7	80.3
F_3	32.3	67.7	42.5	57.5*	18.7	81.3
F_4	30.0	70.0	55.3	44.7†	....	...
F_5	....	..	49.7	50.3	....	...
F_6	....	....	48.3	51.7	...	..
F_7	....	...	51.0	49.0	. .	..
F_8	....	...	46.3	53.7	...	..
F_9	....	...	50.3	49.7	..	..

*n = 200.
†first sample in Virginia.

Using the method of iteration and the underlying assumptions described in WRIGHT and DOBZHANSKY (1946) gives the following results:

Genotype	W		
2L/2L	0.060	s = 0.940	
2L/2L-1	1.000		$\hat{q} = 0.501$
2L-1/2L-1	0.055	t = 0.945	

W represents the relative adaptive value, taking that of the heterozygote to be 1.00, s, the selective disadvantage of the 2L homozygotes, and t, the selective disadvantage of the 2L–1 homozygotes; " $\hat{q}$ " is the equilibrium frequency of 2L expected from these selection coefficients. Here both homozygotes appear to be nearly lethal relative to the heterozygotes. The indicated equilibrium point is apparently the one obtained. With the exception of the small F_3 sample, the data fit closely the curve determined by these coefficients.

When cage 2 had apparently ceased changing, it was transferred to 21°C and 70 percent humidity, and renamed cage 2A. Table 4 shows that a change occurred at the new temperature in the reverse direction from the change in cage 2. Unfortunately, the experiment had to be discontinued before the point could be determined. The small change observed in the third generation makes it likely that an equilibrium was being approached, if not already reached.

At 25° these gene arrangements were studied in three groups of experiments (table 5). Cages 1 and 12A were kept in a constant temperature room where the average humidity varied from 65 to 76 percent. In No. 1 the fall of 2L frequency from the control of 28 percent during the first three generations certainly seemed to be produced by selection, but beyond this the experiment gives no clear results. Humidity in the room decreased at this time, and the erratic changes observed may be due to selection, the sign of which

TABLE 5

Samples from 2L vs. 2L-1 experimental populations at 25° C.

Sample	65–76% Humidity				45–60% Humidity				89–92% Humidity					
	Cage 1		Cage 12A		Cage 3		Cage 1A		Cage 4		Cage 12		Cage 3A	
	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1
Initial	25.0	75.0	11.0	89.0	77.5	22.5	22.0	78.0	81.3	18.7	10.0	90.0	33.0	67.0
Control	27.7	72.3	...	....	77.7	22.3	20.0	80.0	77.3	22.7	12.3	87.7	42.0	58.0
F_1	23.0	77.0	13.3	86.7	64.7	35.3	16.0	84.0	72.7	27.3	13.0	87.0	41.7	58.3
F_2	...	....	13.7	86.3	53.7	46.3	8.3	91.7	54.3	45.7	10.7	89.3	33.7	66.3
F_3	13.3	86.7	13.7	86.3	50.0	50.0	9.0	91.0	50.7	49.3	11.0	89.0	40.7	59.3
F_4	14.7	85.3			36.3	63.7	8.3	91.7	45.3	54.7			31.0	69.0
F_5	17.3	82.7			32.7	67.3	7.0	93.0	44.3	55.7			33.7	66.3
F_6	22.7	77.3			41.0	59.0	7.7	92.3	42.3	57.7				
F_7	22.0	78.0			24.7	75.3			35.7	64.3				
F_8	20.0	80.0*			34.7	65.3			33.3	66.7				
F_9					34.3	65.7			...	...				
F_{10}					32.3	67.7†			42.3	57.7†				
F_{11}					33.0	67.0			31.7	68.3				
F_{12}									29.0	71.0				
F_{13}									38.7	61.3				
F_{14}									34.0	66.0				
F_{15}									40.7	59.3				
F_{16}									36.7	63.3				
F_{17}									35.7	64.3				

* Part of this generation as No. 1A.
† First sample in Virginia.

changed with the changes in humidity. Evidence for this comes from the equilibrium of about 20–22 percent which 2L apparently attained when the environment became stabilized. An alternative hypothesis would assume an equilibrium between 15 and 20 percent 2L which was obscured by sampling errors and genetic drift. Responsibility of sampling errors is contradicted by the zygotic data for F_3–F_8 which show greater differences than might be expected from sampling variation (P about 0.01 for twelve degrees of freedom). In cage No. 12A no significant changes were detected during two and two-thirds generations. The seeming inability of the 2L frequency to increase beyond 13.7 percent may, considering sample errors, support the hypothesis of an equilibrium frequency of 15–20 percent 2L in this environment.

Cages 3 and 1A were kept at 25° but at a lower humidity. Cage 1A was a continuation of experiment 1. Once in the new conditions (see table 5) the frequency of 2L fell again, apparently reaching an equilibrium around 8 percent 2L. This result seems very unlikely on a basis of genetic drift alone. Even better evidence for natural selection in an experimental population comes from cage 3. Starting with a high frequency of 2L and a low one of 2L–1, this population showed clearly directional changes for five generations, the fall in 2L and rise in 2L–1 apparently ceasing after a frequency of 33 percent 2L and 67 percent 2L–1 had been reached. After that, a few irregular changes occurred, and the cage was discontinued after the eleventh generation. The population was always small; thus, it contained 152 flies in F_{11} generation. Calculation of adaptive values using all the data for No. 3 gives the following results:

Genotype	W		
2L/2L	0.26	s = 0.74	
2L/2L-1	1.00		$\hat{q}_{2L} = 0.327$
2L-1/2L-1	0.64	t = 0.36	

The expected equilibrium proportion of 2L agrees with the one apparently attained. However, the inclusion of several generations of no apparent change has a considerable effect on the calculations. Thus, if the data used are restricted to the period until equilibrium seemed to be attained (F_5) the following picture of the selection coefficients emerges:

Genotype	W		
2L/2L	0.39	s = 0.61	
2L/2L-1	1.00		$\hat{q}_{2L} = 0.116$
2L-1/2L-1	0.92	t = 0.08	

The adaptive values of the heterozygotes and the 2L–1 homozygotes seem to have been more nearly similar during the period of rapid change than was indicated by the previous calculation. An equilibrium is again predicted. However, this equilibrium is very different from one apparently attained in this cage; in fact, it closely resembles the one attained in cage 1A as well as several results at this temperature at higher humidities (cages 12, 12A, and the

first part of cage 1). Below are the calculated expected frequencies of 2L on the basis of each set of adaptive values for comparison with the observed results:

Sample	Actual	Expected (First set)	Expected (Second set)
Control	.777	....	...
F_1	.647	.617	.651
F_2	.537	.504	.536
F_3	.500	.437	.447
F_4	.363	.397	.378
F_5	.327	.373	.330
F_6	.410	.357	.294
F_7	.247	.347	.266
F_8	.347	.341	.244
F_9	.343	.337	.226
F_{10}	.323	.334	.212
F_{11}	.330	.332	.200
Limit	..	.327	.116

As expected, the adaptive values indicating an equilibrium of 11.6 percent 2L (second set) fit the gametic data of the first five generations more closely than do the ones in the first set. If, then, this is the correct set for these generations, the discrepancies between observed and expected in the later data would mean that the adaptive values of the genotypes are different when the frequencies are about 33 percent 2L and 67 percent 2L–1 in this environment than they are at higher frequencies of 2L or lower ones of 2L–1. However, the calculations based on all the data (first set) also generally agree, within the commonly accepted limits of sampling error, with the observed frequencies during the period of rapid change. The only exception is the F_3 sample, which has $t = 2.20$, and this also is not highly significant ($P = 0.028$). Since it is also simpler, because no variation of the adaptive values need be postulated, the hypothesis of adaptive values leading to an equilibrium of 33 percent 2L is probably the preferred one.

Experiments 4, 12, and 3A were kept in the 25° incubator at about 90 percent humidity. These cages consistently contained large populations; for example, F_3 of cage 12 numbered 4562 individuals. Table 5 shows that the changes in No. 4 were of the same general nature as those in its sister cage, No. 3, but they were slower arriving at the equilibrium point. The calculated selection coefficients are:

Genotype	W		
2L/2L	0.35	$s = 0.65$	
2L/2L-1	1.00		$\hat{q}_{2L} = 0.350$
2L-1/2L-1	0.65	$t = 0.35$	

Cage 12, intended as a check on population 4, showed no significant changes. Experiment 3A determined whether the equilibrium attained in No. 3, of which it was a continuation, would change in higher humidity. The available data indicate no change, thus possibly duplicating the result in cage 4.

Because of the relative rarity of 2L–3 in the 1947 collections, experimental

populations involving this pericentric inversion were not begun until November, 1948. It was, therefore, not possible to carry out as many tests in different environments as was possible for 2L and 2L–1. It is hoped to study the adaptive properties of 2L–3 more fully later.

B. *Experiments with 2L and 2L–3*

The studies with 2L and 2L–3 are shown in table 6. At the lower temperature these consist of cages 6 and 11. Cage 11, which could be carried on for only two generations, showed no significant changes. No. 6 did show significant changes for a number of generations. These changes make it likely that these arrangements are responsive to natural selection in this environment and indicate that the data of cage 11 resulted from beginning the experiment at or near the equilibrium point. The relative adaptive values of the genotypes in experiment 6, based on the available data, are:

Genotype	W		
2L/2L	0.53	s = 0.47	
2L/2L-3	1.00		$\hat{q}_{2L} = 0.434$
2L-3/2L-3	0.64	t = 0.36	

The gametic frequencies expected from these adaptive values agree closely with those found in the samples. This applies to the period of rapid change as well as the later data, indicating that natural selection was governed by the same adaptive values throughout the range of frequencies covered by this experiment. Not explained, however, is the discrepancy between the equilibria for experiments No. 11 and No. 6.

At the higher temperature also, the cage begun with high frequency of 2L–3, No. 13, showed no significant directional change. An increase in frequency of 2L and decrease in 2L–3 did occur during the second and third generations, but the population reverted to its initial frequencies thereafter. This suggests these are equilibrium frequencies for 2L or 2L–3 at this temperature. The population begun with low frequency of 2L–3, in this case No. 8, showed steady, albeit slow, decreases in the frequency of 2L and increases in 2L–3, apparently due to natural selection. This experiment had to be discontinued before the final outcome could be determined. To see if the results in No. 13 could be duplicated, No. 16 was begun with 50: 50 proportions of 2L and 2L–3. The control sample showed a few 2L–1 chromosomes had also been introduced. The main change observed is the rise in 2L–1, obscuring the relations of 2L and 2L–3.

These results indicate that the carriers of 2L and 2L–3 have different adaptive values, probably resulting in equilibria, at both temperatures, but the data do not show conclusively whether the equilibria differ at the different temperatures.

C. *Experiments involving 2L–1 and 2L–3*

The several experiments involving these overlapping inversions are shown in table 7. Cages 7 and 10 show no significant changes and apparently dem-

TABLE 6

Samples from experimental populations of 2L vs. 2L-3. Cages 6 and 11 at 15½° C., 13 and 16 at 25° C. and 45-60% humidity, 8 at 25° C. and 65-76% humidity.

Sample	Cage 6		Cage 11		Cage 8		Cage 13		Cage 16			
	2L	2L-3	2L	2L-3	2L	2L-3	2L	2L-3	2L	2L-3	2L-1	n†
Initial	76.2	23.8	20.0	80.0	82.0	18.0	20.0	80.0	50.0	50.0	....	....
Control	80.3	19.7	30.3	69.7	78.0	22.0	21.0	79.0	45.4	53.2	1.3	308
F_1	77.3	22.7	24.3	75.7	80.7	19.3	21.0	79.0	44.7	54.6	0.7	304
F_2	69.3	30.7	26.7	73.3*	78.3	21.7	26.0	74.0	44.6	53.5	1.9	312
F_3	63.3	36.7			74.3	25.7	32.0	68.0	40.7	52.3	7.0	300
F_4	52.7	47.3 $			73.7	26.3	22.0	78.0	....	....	....	....
F_5	54.0	46.0			70.7	29.3	21.7	78.3	40.0	51.7	8.3	300
F_6	50.7	49.3			70.7	29.3	....	....	43.3	44.7	12.0	300
F_7	51.0	49.0			60.3	39.7	....	....	41.0	41.3	17.7	300
F_8	47.3	52.7										
F_9	47.3	52.7										

* n = 270.
† applies to cage 16 only.
$ First sample in Virgina.

TABLE 7

Samples from experimental populations of 2L-1 vs. 2L-3. Cages 7 and 10 at 15½° C., 14 and 9A at 25° C., 45-60% humidity, 9 and 15 at 25° C. and 65-76% humidity.

Sample	Cage 7		Cage 10		Cage 14		Cage 9A		Cage 9		Cage 15	
	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3
Initial	80.2	19.8	19.8	80.2	20.0	80.0	80.0	20.0	85.0	15.0	60.0	40.0
Control	72.7	27.3	24.3	75.7	30.3	69.7	82.0	18.0	83.3	16.7	67.3	32.7
F_1	78.7	21.3	30.3	69.7	48.0	52.0	84.3	15.7	76.0	24.0	63.7	36.3
F_2	75.1	24.9	22.0	78.0	63.0	37.0	....	....	79.0	21.0	69.7	30.3
F_3	79.3	30.7	24.4	75.6*	63.7	36.3	88.7	11.3 $	80.0	20.0		
F_4					74.7	25.3	88.7	11.3	80.0	20.0		
F_5					75.0	25.0 $	85.7	14.3	82.0	18.0**		
F_6					73.0	27.0	70.3	29.7				
F_7					72.7	27.3	70.0	30.0				
F_8					69.3	30.7	76.0	24.0 †				
F_9					67.0	33.0	74.0	26.0				
F_{10}					61.0	39.0						
F_{11}					52.0	48.0						
F_{12}					....	....						
F_{13}					44.0	56.0						
F_{14}					49.7	50.3						
F_{15}					48.0	52.0 †						
F_{16}					51.0	49.0						

* n = 250.
† n = 50.
$ First sample in Virginia; development and hatching of these generations occurred in New York.
** Part of this generation spent as Cage 9A.

onstrate adaptive equivalence of the two gene arrangements at the low temperature. The behavior is different at 25°. Here a significant change was found in the population, No. 14, begun with a high frequency of 2L–3 and a low frequency of 2L–1. Calculation of adaptive values for the genotypes in this population, based on the samples of generations that developed in New York (Control–F_5), gives the following results:

Genotype	W		
2L-3/2L-3	0.17	s = 0.83	
2L-1/2L-3	1.00		
2L-1/2L-1	0.84	t = 0.16	$\hat{q}_{2L-3} = 0.153$

Had no change of environment taken place, it is not inconceivable that this equilibrium might have been reached. However, it is apparent that in Virginia there was a new change leading to equilibrium around 50 percent of each arrangement. The environmental factors responsible for the new change are presently under investigation.

Population No. 9, started with a high frequency of 2L–1 and low one of 2L–3, showed no significant changes in five generations. Upon transfer to a lower humidity, however, as No. 9A, this population changed in a manner reminiscent of the transfer of population 1 to a lower humidity (cage 1A, table 7). The decrease in 2L–3 and increase in 2L–1 in 9A is similar to what is expected from the New York equilibrium indicated for this environment in cage 14. An opposite change seemed to occur in Virginia in the same direction, but not to the same extent, as the new change noted in cage 14.

Since significant changes were noted only at the low humidity, the possibility exists that at higher humidity at this temperature (as in cage 9), the carriers of these arrangements are adaptively neutral. Cage 15, run at the

TABLE 8

Population counts of experimental populations.

Cage	Approximate generation	Temp. °C.	Humidity	Cage Style	Total flies	Females %	Males %
5	F_2	15½	63	New*	524	75.4	24.6
7	F_3	15½	79	Old*	624	78.7	21.3
10	F_3	15½	79	Old	591	82.5	17.5
2A	F_3	21	68	New	440	70.0	30.0
14	F_1	25	53	Old	137	64.3	35.7
13	F_5	25	57	Old	217	56.7	43.3
3	F_{11}	25	55	New	152	63.2	36.8
9A	F_5	25	53	Old	403	74.4	25.6
16	P_1	25	50	Old	361	71.7	28.3
16	F_1	25	52	Old	393	74.6	25.4
8	F_7	25	73	Old	1563	64.4	35.6
15	F_2	25	73	New	1157	76.2	23.8
12A	F_3	25	73	New	807	74.8	25.2
12	F_3	25	92	New	4562	61.7	38.3

* explanation in text.

same humidity as No. 9, is an attempted test of this hypothesis. Its results are inconclusive.

Size of the populations

Table 8 shows the counts made of various experimental populations along with the approximate mean humidity during the previous generation period. Females are consistently more common than males. At the high temperature the order of magnitude of the total count seems directly related to the relative humidity. At similar humidities, it is greater at high than at low temperature. At similar temperatures and humidities, the newer cages, those with netting instead of glass on two windows, and, therefore, with more evaporation surface, tend to have the smaller populations.

DISCUSSION

The *Drosophila robusta* population of Englewood Cliffs, New Jersey, repeatedly sampled for the purposes of the present investigation (see table 1), shows no cyclic seasonal changes in chromosomal composition, being in this respect like the St. Louis population studied by CARSON and STALKER (1949). It may be noted that no seasonal changes are observed for the two gene arrangements, XL–1 and 2L–3, which show clear geographic and altitudinal gradients (CARSON and STALKER 1947; STALKER and CARSON 1948) but whose seasonal variation could not be determined in Missouri (CARSON and STALKER 1949). The available data confirm the conclusion of CARSON and STALKER that the adaptive function of the chromosomal polymorphism in *D. robusta* is not related to seasonal changes in the environment. In this respect the species resembles *D. persimilis* rather than *D. pseudoobscura* or *D. funebris* (DOBZHANSKY 1943, 1948a; DUBININ and TINIAKOV 1945).

Experiments on artificial populations nevertheless show that the gene arrangements in *D. robusta* are not adaptively neutral. The relative frequencies of the gene arrangements in experimental populations often show rapid and directional changes, clearly caused by operation of natural selection. In no case, however, do these changes lead to elimination of one and establishment of the competing gene arrangement. Instead, the relative frequencies of the gene arrangements reach equilibria, and the composition of the populations becomes more or less constant.

Equilibria may be produced by natural selection if heterozygotes are at all frequencies adaptively superior to the homozygotes. In the text the relative adaptive values of the genotypes were calculated on the basis of this hypothesis, and the results expected from the calculated adaptive values generally corresponded well with the results actually obtained. Implicit in these calculations were two further assumptions: that the adaptive values were constant throughout the range of arrangement frequencies and that they were identical in the sexes. There is some evidence, however, that these assumptions may represent an oversimplification. Thus, in several experiments (see especially No. 3, table 5) the equilibrium apparently attained may not be the

one expected from the adaptive values responsible for earlier changes. This may occur if at certain frequencies the genotypes take on adaptive values leading to a different equilibrium. Or, the homozygotes, adaptively inferior at most frequencies, may be adaptively equal to the heterozygotes (and to one another) in the vicinity of the frequencies where observed changes stopped, for the effect of adaptive equality of the genotypes is there indistinguishable from a stable equilibrium under natural selection. In either case, the postulated stability of the adaptive values would be contradicted. Also, it is probable that variations in adaptive values, possibly related to small environmental variations, were responsible for temporary changes noted in several experiments (especially in No. 1, table 5 and No. 13, table 6). The observation made in the text that, in nature, carriers of several arrangements are apparently subject to different selection pressures depending on their sex makes the assumption that a given genotype has the same adaptive value in both sexes in the laboratory populations also questionable.

An alternative hypothesis for production of adaptive equilibria is that heterozygotes be at almost all frequencies intermediate in adaptive value to the homozygotes but that the adaptive value of each homozygote fall as the frequency of the arrangement increases. There would then be a range of arrangement frequencies where adaptive values of the homozygotes were similar or equal, and here no selective changes would occur. The margins of the range would be points of equilibrium. This possibility is more difficult to attack and was not considered in the text. However, instances described in the text where different equilibria seemed to be attained by the same arrangements in the same environment may well depend on some such explanation. Although the issue is complicated by strain differences and other variables, in some of these cases there was indeed a range of frequencies not attained from either extreme (compare, for example, cages 2 and 5, table 4; cages 4 and 12, table 5; cages 6 and 11, table 6). This possibility is supported also by the excess of homozygotes observed in several adult samples in nature (discussed in the text). WRIGHT and DOBZHANSKY (1946) point out, however, that such an explanation may well apply in nature, where a genotype may be adaptively superior until so numerous that it must attempt to use ecological niches for which it is not well adapted, but it is more difficult to conceive of such a phenomenon in an experimental population cage, where the number of niches is limited. Determination of zygotic frequencies among adults in the cages, currently being investigated, should help clarify this problem. In one completed study, F_{16} adults of cage 4 (table 5) a significant excess of *heterozygotes* was found in females, the adult males fitting the HARDY ratio. (Details of this and further studies of adults will appear in a later paper.)

The observations on experimental populations lend support to the view that the relative frequencies of different gene arrangements in natural populations are not accidental, but are determined by natural selection. Of course, this applies to populations in apparent equilibrium as well as to populations with changing gene or inversion frequencies. However, the time of action of natu-

ral selection in the life cycle of this fly in nature is not clear. The egg sample, albeit small, indicates that at least the spring sample is panmictic and lacks differential viability at the egg stage. The various adult samples indicate no differential survival of heterozygotes over homozygotes, in either males or females, beyond the egg stage; the overall adult sample for left arm second chromosome arrangements even suggest superior survival value of the homozygotes. The remaining possibility is greater fecundity or sexual activity of the heterozygotes. This hypothesis calls for no deviations from HARDY-WEINBERG ratios, provided the adaptive differences between genotypes are similar in the sexes. Unfortunately, the hypothesis is exceedingly difficult to test by observations on natural populations; laboratory experiments are planned. DA CUNHA (1949) found an even more significant excess of adult homozygotes for the color types of *D. polymorpha* in natural populations, although there is evidence the heterozygotes are adaptively superior. WALLACE (1948) showed, in laboratory experiments, that superior fecundity of females heterozygous for the sex-ratio factor in *D. pseudoobscura* apparently maintains this factor in populations despite the adaptive weakness of females homozygous for it.

Females heterozygous for a pericentric inversion may possess inferior fecundity, because crossing-over within the inversion would generally lead to inviable duplication-deficiency gametes. This explains the rarity of pericentric inversions in natural populations (DOBZHANSKY 1941). MILLER (1939) found a pericentric inversion in natural populations of *D. algonquin*. Since most individuals with the pericentric inversion also carry an arrangement differing from it by two overlapping inversions, MILLER suggests this reduces the probability of crossing-over and hence erases the theoretical selective disadvantage. CARSON and STALKER (1947) point out also that single crossovers within the overlap lead to a bridge and fragment as in paracentrics. In *D. robusta* the pericentric inversion 2L–3 is sometimes found associated with arrangements differing from it by two overlapping inversions, 2L–1 or 2L–2, and these may "protect" it somewhat, but most commonly it is found with 2L, which differs from it by only a single inversion. The extent of crossing-over within the inversion is unknown, but the fact that about half the euchromatic area of the left arm of the second chromosome is involved indicates that it probably does occur in 2L/2L–3 females. Hence, the high frequency of 2L–3 here (table 1) and in Mt. Vernon, Iowa (CARSON and STALKER 1947), and its even higher frequency at elevations more than 1400 feet near Gatlinburg, Tennessee (STALKER and CARSON 1948), cannot be explained on the same basis as the *D. algonquin* case. The equilibria obtained or indicated in the experiments with 2L–3, especially those involving 2L, show that a more likely explanation for the persistence of this inversion in populations is the selective advantage conferred on individuals heterozygous for it by heterotic effects of genes on it with those on 2L and 2L–1. The data indicate that these effects probably do not depend on differential mortality in nature.

Under natural selection the carriers of genotypes best adapted to the en-

vironment leave more surviving progeny than do carriers of genotypes with lesser fitness. The fitness or "adaptive value" is, however, a composite of relative superiorities and inferiorities in a number of factors operating at all stages of the life cycle. One of the most obvious and most easily measured of these is differential viability or mortality, but it is apparent that fitness depends also on relative fecundity, sexual activity, longevity and other factors. Moreover, each of these general classes is further compounded of particular variables; thus, a genotype which produces a higher ratio of inviable gametes may yet possess superior fecundity by virtue of a large total of gametes produced.

Several experiments with gene arrangements 2L and 2L–1 attained similar equilibria although maintained at different temperatures; and at all temperatures used, 15½°, 21°, and 25°C, the equilibria were at 50 percent 2L or less. Similar results were obtained in the experiments with 2L and 2L–3, though these are not as extensive and the results not as definite. This indicates that heterosis involving these gene complexes is largely independent of temperature. Only 2L–1 with 2L–3 shows clear-cut temperature differences in equilibria, these gene arrangements being adaptively neutral or nearly so at 15½° but not at 25°. However, temperature is but one of many environmental variables to which the arrangements might be adapted. Several experiments attempted to study another variable, namely humidity. These showed that populations of *D. robusta* are indeed sensitive to this factor, for the size of the population was consistently greater at higher humidities than at lower humidities at the same temperature. Whether any of the gene arrangements studied are involved in adaptations to this factor is not so clear, however. When a population containing 2L and 2L–1 was kept at a lower humidity (see experiment 1A, table 7), the frequency of 2L fell from 20–22 percent to about 8 percent, 2L–1 showing a corresponding increase. However, several experiments with these arrangements, notably 1A and 12, attained similar results at very different humidities, while still other experiments, notably 4 and 12, attained very different equilibria at the same humidity. Repetition of these experiments with better control of humidity and strain differences should yield interesting results. Controlling humidity in desiccators, HEUTS (1947 and 1948) was able to demonstrate differential survival of pupae and adults of *D. pseudoobscura* carrying various gene arrangements. DOBZHANSKY and ZIMMERING have shown that selective responses by carriers of certain gene arrangements in *D. pseudoobscura* also depended on the nature of the yeast used (DOBZHANSKY 1948d and unpublished data). The recent discovery that the natural food of *D. robusta* consists of microorganisms that occur in slime fluxes (CARSON and STALKER 1950) may lead to experimental study of the food preference of carriers of different gene arrangements also in *D. robusta.*

In some of the experiments described in this paper the same arrangements were involved under similar conditions of temperature and humidity. In none of these duplicate experiments were quite the same equilibrium proportions

of the arrangements attained; in some, the differences were quite large. Contrastingly, replicate experiments with gene arrangements of *Drosophila pseudoobscura* usually result in attainment of uniform equilibria (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY 1947 and 1948c). This difference between *D. pseudoobscura* and *D. robusta* may be related to the sensitivity of *D. robusta* to small environmental variations noted on numerous occasions during the course of the experiments. The genetic basis for this sensitivity, apparently intimately bound up with the chromosomal polymorphism, should prove an interesting subject for further study.

SUMMARY

A study is made of chromosomal polymorphism in natural and experimental populations of *Drosophila robusta*. The natural population of one locality in the New York City area proved to contain the fifteen widespread gene arrangements known in the species. The observed high frequencies of inversions XL–1, 2L–3, 2R, and 3R are characteristic for northern populations of the species. Cyclic seasonal variation in the frequencies of inversions has not been observed, but there is evidence of differential selection in the two sexes for several arrangements at different seasons. The adult population consists of heterozygotes and homozygotes in proportions consistent with the HARDY-WEINBERG equilibrium.

Twenty-one experimental populations containing mixtures in various proportions of two paracentric inversions, 2L and 2L–1, a pericentric inversion, 2L–3, in pairs, were placed in population cages and were kept at three temperatures, 15½°, 21°, and 25°C. At the highest temperature they were also kept at three different humidities. Most of these populations showed significant and directional changes in arrangement frequencies resulting from natural selection. These populations usually attained equilibria between the arrangements at which the competing gene arrangements were present with constant frequencies. This probably indicates that inversion heterozygotes are superior in adaptive value to homozygotes. In the experiments with 2L and 2L–1, and those with 2L and 2L–3, similar selective responses occurred at all temperatures used; experiments with 2L–1 and 2L–3 showed significant selective differences at 25° but not at 15½°C.

Results of the experimental populations indicate that the frequencies of the gene arrangements in nature are determined by natural selection, probably acting through adaptive superiority of inversion heterozygotes. Heterosis of this sort also accounts for the persistence of a pericentric inversion in these populations.

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