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DARWINIAN FITNESS AND ADAPTEDNESS IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA WILLISTONI*

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Fifteen second chromosomes were extracted from *Drosophila willistoni* flies collected in four natural populations. The adaptedness of populations homozygous for each chromosome was measured by average population size and productivity. Six 'control' populations were established with mixtures of the wild second chromosomes. The Darwinian fitness of flies homozygous for each wild second chromosome, and of flies carrying random combinations of these chromosomes, was measured relative to the fitness of flies heterozygous for a wild and a marker chromosome. The Darwinian fitness of homozygotes for each second chromosome relative to the fitness of flies carrying random combinations of the natural chromosomes was then inferred. The estimated loss of fitness on making the natural second chromosomes homozygous was substantial, ranging from 39 to 83 percent, with an average reduction in fitness of 66 percent. These results with *D. willistoni* are consistent with those from similar experiments with other *Drosophila* species, and they are compatible with a significant role for heterosis in the maintenance of genetic variability.

Populations homozygous for wild chromosomes differ in their adaptedness to the experimental environment. Population size and productivity are correlated, although the correlation is far from complete. Some populations have high productivity and low population size, or vice versa. The control populations, with greater genetic variability, were superior in adaptedness to the average of the single-chromosome populations. The Darwinian fitness and the adaptedness of the genotypes in this experiment were not significantly correlated. It follows that certain measures used by population geneticists, such as genetic load and average Darwinian fitness, cannot be taken as general indices of how well adapted a population is to its environment.

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Introduction

Darwianian fitness and adaptedness are related but distinct concepts. Darwinian fitness is a relative measure; the carriers of a genotype or of an array of genotypes can be more, equally, or less fit than the carriers of other genotypes coexisting in the same environment. The Darwinian fitness is measured as 'the average contribution which the carriers of a genotype, or an array of genotypes, make to the gene pool of the following generation, relative to the contributions of other genotypes' (DOBZHANSKY, 1968). Adaptedness refers to the ability of the carriers of a genotype or a group of genotypes to survive and reproduce in a given environment. In principle at least, adaptedness can be measured in absolute rather than in relative units. The ability of a population to transform the available food and energy into living matter of its own kind can be a measure of the adaptedness to the environment in which the population lives (AYALA, 1969). Two estimates of adaptedness can be obtained, productivity and population size. The productivity is the number or the biomass of the individuals born in the population per unit of time; the population size is the number or the biomass of the individuals composing the population. Darwinian fitness and adaptedness must often be correlated. If not, evolution could hardly have achieved what it did. It is important, however, that occasionally they do not coincide. Genotypes with high Darwinian fitness do not necessarily give high adaptedness.

The experiments reported in this article were undertaken to provide an experimental comparison of Darwinian fitness and adaptedness. Population size and productivity are used to measure the adaptedness of 15 different populations of *D. willistoni*, each homozygous for a second chromosome extracted from a natural population. The Darwinian fitness of the homozygotes for these chromosomes is measured in relation to a standard, which is the average fitness of individuals which carry random combinations of the second chromosomes from the same natural population. This study complements a previous analysis (MOURÃO & AYALA, 1971) of the fitness of four of these *D. willisoni* populations in competition with *D. pseudoobscura*.

Materials and Methods

D. willistoni is a widely distributed neotropical species. Flies were collected in four localities of the state of São Paulo, Brazil during September, 1968. A strain homozygous for the second chromosome was obtained from each female collected, using the technique described by PAVAN et al. (1951) and diagrammed in Figure 1. The second chromosome is a large metacentric one, with a known map length of 78

	♀		♂
P	*abb px bw*/*abb px bw*	×	+1/+2
		↘	
F_1	S/lethal	×	+1/*abb px bw*
		↓	
F_2	S/+1	×	S/+1
		↓	
		♀ and ♂	
F_3	S/S	S/+1	+1/+1
	1 (dies) :	2 :	1

Fig. 1. The crosses used to obtain strains homozygous for the second chromosomes. See text for explanation of symbols.

units. A description of mutant genes and their linkage relationships are given by SPASSKY & DOBZHANSKY (1950). A stock containing the recessives abbreviated (*abb*), plexus (*px*), and brown (*bw*) was used in the initial cross. In the F_1 a balanced lethal stock containing the marker chromosome Star (S) was employed. This chromosome carries the dominant markers *Star* (*S*) and *Hook* (*Hk*). and the recessives *abb* and *bw*, as well as a large pericentric inversion (No. 207). The gene *Star* is lethal when homozygous. PAVAN et al. (1951) detected no recombination in the 78 map units separating *S* and *bw* in heterozygotes for this inverted marker chromosome. The natural chromosomes are symbolized as '+'. The F_3 of the crosses should in theory yield heterozygous Star and homozygous wild-type flies in a ratio of 2 to 1. In

these flies more than 90 percent of the chromosomes other than the second come from the laboratory stocks.

Twenty-five second chromosomes were extracted from randomly chosen wild flies. Of these, six caused lethality, and four others caused sterility in homozygous condition. Of the other 15 chromosomes, 11 came from Mirassol ('M') and São José do Rio Prêto ('SJ'), two localities separated by only 15 km. Three additional chromosomes came from Ribeirão Prêto ('RP'), about 200 km east of Mirassol, and one more from São Paulo ('SP'), some 500 km southeast of Mirassol. The capitals in parentheses will be used to refer to these localities.

Thirty-six experimental populations were started as follows. For each of the 15 chromosomes, two populations were started, one with 80 percent flies homozygous for the wild chromosome and 20 percent heterozygous for the wild and the Star chromosomes, the other with 20 percent homozygotes and 80 percent heterozygotes. Six control ('C') populations were founded with F_1 flies from crosses involving 11 'M' and 'SJ' chromosomes. We shall call these flies the 'random heterozygotes' for the natural chromosomes. Three of the control populations were started with 80 percent wild flies and 20 percent heterozygotes for the wild and Star chromosomes; the other three were started with 20 percent wild and 80 percent heterozygous Star flies. In all 35 populations the second chromosomes segregated as single units, since the inversion in the Star chromosome effectively suppressed all recombination between the second chromosomes carried by the heterozygotes.

Each population was started with 300 pairs of flies. In order to maintain crowded conditions at the beginning of the experiment, 75 pairs of flies with the genetic composition of the founders were added to each population at the end of weeks 1 and 2. The populations were maintained at 25°C by the 'serial transfer' technique as described by AYALA (1965). Briefly, adult flies are introduced in a half-pint milk bottle with standard cream-of-wheat and molasses medium. Three drops of very diluted baker's yeast are added to the medium; a piece of toweling paper (4.5 × 18 cm) partially pressed into the medium provides additional surface for the flies. Twice a week, on Mondays and Thursdays, the flies are transferred to new bottles with fresh food. When adult flies begin to emerge in the bottles where the eggs were laid, they are collected and added to the bottle with the adult population, on the same days in which the adult population is trans-

ferred to a new bottle. Each bottle is discarded after four weeks. A population consists of eight bottles: one bottle contains the adult, egg-laying flies while seven others contains eggs, larvae, pupae, and newly emerged adults.

Once in a two-week period, on Monday, the adult population is etherized, a sample of at least 100 (usually about 150) flies is counted and weighed, the rest of the population is weighed, and the total number estimated by a simple proportion. The newly emerged flies are weighed twice a week on the same days when the adult population is transferred to a fresh bottle. Once every two weeks, a sample of newly emerged flies is counted and weighed, and the number of flies emerged during the week is estimated from the weighing by proportion. Etherization was performed by shaking the flies into an empty bottle and applying either for 80 seconds. Under the conditions of the experiment, the generation time of *D. willistoni* is approximately four weeks. All populations were started between March and May, 1969.

Results

DARWINIAN FITNESS

The effects of the second chromosomes from the natural populations on viability may be assessed by examining the ratio of Star to wild-type flies in the F_3 of the crosses used to establish the homozygous strains (Fig. 1). The data are given in Table 1. For twelve of the fifteen chromosomes the percent of wild-type was not significantly different from the expected 33.3 percent. Of the three which differed significantly, two showed a lowered frequency, indicating some disadvantage of the homozygotes. The other chromosome caused a homozygote advantage, as reflected by a significantly higher frequency of wild-type flies than expected. Six lethal and four sterile chromosomes were excluded from the experiment, although they represent the most extreme loss of fitness with homozygosis.

The frequencies of the Star heterozygotes in the experimental populations are given in Table 2. The name of each population is derived from the single natural chromosome it contained, excepting the control populations C1, C2, and C3, formed from a mixture of all

TABLE 1

RESULTS OF CROSSES TO TEST THE VIABILITY EFFECTS OF SECOND CHROMOSOMES FROM NATURAL POPULATIONS OF 'DROSOPHILA WILLISTONI'

Chromosome	Progeny of cross S/+ ♀♀ × S/+ ♂♂		
	+/+	+/S	% +/+
SP6	162	319	33.68
RP1	286	588	32.72
RP3	130	389	25.05**
RP4	106	139	43.27**
SJ1	73	147	33.18
SJ5	105	213	33.02
SJ6	235	440	34.81
SJ7	115	219	34.43
M7	57	94	37.75
M11	43	82	34.40
M13	102	204	33.33
M18	141	276	33.81
M19	153	268	36.34
M22	96	238	28.74
M23	77	208	27.02*

*) and **) indicate statistically significant deviations of observed frequency of +/+ flies from the expected frequency of 33.33% at the .05 and .005 levels, respectively.

'M' and 'SJ' chromosomes. For each population, all frequencies between the last one given in the table and the last sample taken are zero. The Star marker chromosome is evidently detrimental to its carriers, for it was eliminated or greatly reduced in frequency in all the populations. Clearly, elimination was more rapid in the control populations C1, C2, and C3. It is also clear that selection has changed over time in some of the populations. The two populations with chromosome RP3 showed this variation most spectacularly. The marker chromosomes rose to its maximum of 50 percent (recall it is homozygous lethal) and then declined to extinction. We must use the rate of decline of the marker chromosome to estimate the selection in our populations.

On the hypothesis that the selection was constant, maximum likelihood estimates of the selection in each population were obtained according to the method of ANDERSON (1969). All the samples, in-

TABLE 2

PERCENTAGE FREQUENCIES OF STAR HETEROZYGOTES IN THE EXPERIMENTAL POPULATIONS

Time of sample in weeks	C1		C2		C3		RP1		SJ6		SJ7		M13		M18		M23	
0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0
4	4.4	26.1	3.97	24.5	3.0	24.3	8.9	54.8	14.9	34.2	6.7	52.4	43.5	58.0	4.8	41.0	6.5	20.7
6	0.0	7.4	0.0	7.3	0.0	4.4	8.0	15.3	7.1	21.6	6.3	20.9	33.5	57.8	0.0	5.4	3.2	15.4
8		0.0		0.7		0.0	2.0	7.7	4.3	15.1	2.4	13.7	7.7	6.4		2.1	0.8	4.8
10				0.0			0.0	5.9	4.4	10.5	0.0	6.2	4.7	5.7		0.0	0.7	5.9
12								1.1	0.0	3.2		2.6	0.8	1.6		1.2	0.0	*
14								0.0	1.8	2.3		1.2	0.7	0.7				*
16									0.0	0.0		0.0	0.0	0.9				3.2
18														0.0				0.0
Time of last sample	*40*	*18*	*40*	*18*	*40*	*16*	*48*	*26*	*50*	*24*	*48*	*24*	*44*	*24*	*46*	*24*	*50*	*18*

TABLE 2 (*cond.*)

Time of sample in weeks	RP4		SJ1		SJ5		M11		M19		M22		RP3		SP6		M7	
0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0	20.0	80.0
4	9.4	47.2	32.5	58.8	17.7	42.0	25.5	47.7	30.7	62.7	11.5	45.2	81.4	89.7	11.3	45.1	22.0	42.4
6	3.8	20.8	10.2	41.9	11.9	18.4	31.0	42.0	28.7	47.6	2.7	39.7	91.2	98.0	9.0	29.8	29.6	33.8
8	0.0	14.3	8.5	27.6	6.2	16.1	33.5	36.7	21.5	28.3	4.3	34.9	100.0	100.0	4.6	23.4	19.7	20.5
10	1.0	10.1	2.8	8.6	3.4	11.6	31.0	41.7	10.4	15.4	4.3	*	100.0	100.0	4.7	15.0	6.7	17.0
12	1.3	2.7	1.3	9.0	11.1	7.0	32.3	36.5	7.1	4.3	0.9	*	100.0	100.0	15.1	16.9	3.2	16.0
14	0.5	0.0	0.0	0.0	1.9	3.7	28.5	20.6	4.2	3.1	0.0	19.3	100.0	87.6	12.8	18.9	1.3	12.1
16	0.0	1.7		2.6	1.5	3.8	23.1	7.0	2.6	0.0	0.9	20.8	100.0	70.4	17.3	5.6	0.9	10.9
18		0.0		0.9	0.8	5.4	26.7	10.9	0.0	0.8	1.5	21.4	100.0	36.0	2.8	2.8	0.9	16.7
20				0.0	0.0	4.3	16.9	5.5	0.0	0.9	0.0	7.6	92.9	21.8	2.9	2.3	0.0	7.8
22					0.7	0.8	4.1	0.9	0.0	0.0	0.0	3.0	69.1	10.0	1.8	0.8	0.8	2.2
24					0.0		0.0		0.8		0.0	1.8	36.7	8.6	0.0	0.0		
26									0.0		0.7	0.0	10.9	9.6				
28											0.0		20.2	1.9				
30													9.0	*				
32													4.0	*				
34													*	0.0				
36													*					
38													0.0					
Time of last sample	*48*	*22*	*44*	*24*	*46*	*22*	*46*	*22*	*48*	*24*	*50*	*26*	*50*	*36*	*44*	*24*	*48*	*22*

*) sample not taken.

cluding those toward the end of the experiment when no Star heterozygotes were observed, were used in order to give proper weight to the trend towards elimination of the Star chromosome. Chi-square tests were employed to text the goodness of fit between the observed frequencies and those expected with the estimates of the selection. In less than half of the populations could the data be accounted for by constant selection. Variation in selection over time has been common in our populations; for instance, in those containing chromosomes RP3 and M11, an advantage of the Star heterozygotes has changed to a disadvantage which led to elimination of Star. Fluctuations in selection within experimental populations have been observed before (LEVENE et al., 1954; POLIVANOV & ANDERSON, 1969), and appear to be more the rule than the exception.

We wish to estimate the selection on homozygotes for single natural second chromosomes and on heterozygotes for randomly-chosen pairs of the same natural chromosomes. The importance to evolutionary dynamics of such selection depends largely on the net result of such selection, and much less on the pattern of variation of the selection with time. The selection which occurs in nature undoubtedly fluctuates as much, and probably more, than that which operates in the carefully controlled laboratory environment. Selective values are likely to be constantly shifting in response to environmental changes and to changes in the genetic milieu of the population. An overall measure of the selection which has acted in each population is desired. The crucial factors are the ultimate reduction in frequency of the Star chromosome toward elimination, and the length of time required to bring it about. We have incorporated these factors into a measure we call 'net fitness.' It is simply that fitness which, if constant, would bring about the observed change in chromosome frequency in the observed time. The net fitnesses should be equal, or nearly so, to the maximum likelihood estimates of selective values for data where the assumption of constant selection is justified.

The frequency of a lethal allele (or chromosome) at generation T in a population begun from frequency Q_0 is

$$Q_T = \frac{Q_0 W^T (W - 1)}{W - 1 + Q_0 (2W^{T+1} - W^T - 2W + 1)}, \qquad (1)$$

where W is the fitness, or selective value, of the lethal heterozygotes

relative to that of organisms carrying two nonlethal alleles (ANDERSON, 1969). This equation defines a polynomial in W:

$$[Q_0\ (2Q_T - 1)]\ W^{T+1} + [Q_0\ (1-Q_T)]\ W^T + [Q_T\ (1-2Q_0)]\ W + [Q_T(Q_0 - 1)] = 0. \quad (2)$$

We have used Newton's iterative method to solve for W. Although this equation may have many roots, there will generally be only one possible solution in the biologically meaningful range of W.

As Q_T we have used the last non-zero frequency of the Star chromosome observed for each population. Chromosome frequencies were scored every two weeks, while a generation corresponds to 4 weeks. Where Q_T fell between generations, we used T in fractional generations in solving equation (2) for W. This form of interpolation proved best of the several types we explored.

The estimates of net fitness may be compared to the maximum likelihood estimates in those populations where constant fitnesses adequately fit the observations. The coefficient of correlation between the two sets of fitnesses is 0.91 with 14 degrees of freedom; the true correlation is clearly quite high. The estimates of net fitnesses are close to the maximum likelihood estimates, where these can be properly determined. This close correspondence is to be expected, since the time to elimination or near-elimination of the Star chromosome is a crucial factor in both forms of estimation as we have performed it. Most importantly, the net fitnesses provide the proper overall measures of selection, ones which indicate the actual outcome of the selection. A simple arithmetic average of selective values in various generations will not necessarily do so, since it may be weighted in favor of a strong but transient heterosis.

The estimates of the net fitnesses of the wild-type flies in each population, relative to that of the Star heterozygotes, are given in columns A of Table 3. For convenience we have chosen to present the results as fitnesses of wild-type flies relative to those of the Star heterozygotes, and these fitnesses therefore correspond to 1/W. The higher fitnesses in the control populations reflect the greater speed with which the marker chromosome was eliminated. The members of each pair of populations were begun with the same chromosomes, but in different frequencies. The difference between replicates is fairly small in com-

TABLE 3

ESTIMATES OF 'NET FITNESSES'

([*A*] of homozygous [pops. SP6–M23] or heterozygous [pops. C1–C3] wild-type flies relative to Star heterozygotes; and [*B*] of homozygotes for each natural second chromosome relative to random heterozygotes for these chromosomes).

Population	Initial frequency of hetero-zygotes	Net fitness *A*	Net fitness *B*	Population	Initial frequency of hetero-zygotes	Net fitness *A*	Net fitness *B*
SP6	0.20	1.5	.20	M11	0.20	1.3	.17
	0.80	2.4	.31		0.80	2.3	.30
RP1	0.20	3.2	.43	M13	0.20	2.6	.34
	0.80	4.7	.61		0.80	3.3	.43
RP3	0.20	1.2	.15	M18	0.20	4.4	.57
	0.80	1.7	.22		0.80	4.6	.60
RP4	0.20	2.9	.39	M19	0.20	1.7	.22
	0.80	2.7	.36		0.80	2.6	.34
SJ1	0.20	2.5	.33	M22	0.20	1.6	.22
	0.80	2.9	.38		0.80	1.9	.24
SJ5	0.20	1.8	.24	M23	0.20	3.9	.51
	0.80	2.4	.31		0.80	2.3	.30
SJ6	0.20	2.0	.26	C1	0.20	4.8	
	0.80	2.9	.38		0.80	6.2	
SJ7	0.20	2.9	.39	C2	0.20	5.4	
	0.80	3.6	.47		0.80	13.0	
M7	0.20	1.8	.24	C3	0.20	7.1	
	0.80	1.9	.25		0.80	9.2	

parison to the difference between populations containing different chromosomes. In all but 2 of the 18 pairs, the wild-type flies had somewhat higher fitness in the population begun with 80 percent Star heterozygotes. That is, the Star heterozygotes were eliminated more rapidly when they were initially more frequent. Thus, there is some evidence that selection has varied with the frequencies of the chromosomes.

The fitnesses of the homozygotes and of the random heterozygotes for the natural chromosomes were all estimated relative to that of the Star heterozygotes. We may thus compare the fitnesses of the homozygotes and the random heterozygotes, assuming that the Star

heterozygotes performed reasonably alike in populations containing these two kinds of wild-type flies. The control populations are all replicates of the same mixture of chromosomes, and for comparison with the homozygotes we may use the average fitness of the random heterozygotes in the six control populations. These relative fitnesses are given in columns B of Table 3. They must be viewed as rough estimates, since a number of assumptions and approximations were involved in their derivation. Nevertheless, the results are consistent in indicating a strong reduction of fitness in homozygotes in comparison to the heterozygotes, for all 15 different natural second chromosomes.

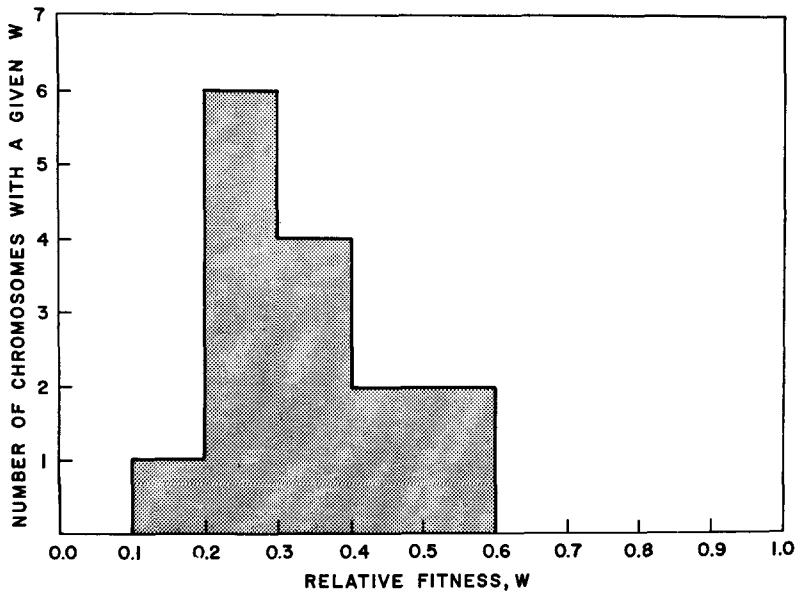

Fig. 2. Distribution of the estimates of fitness of homozygotes for natural second chromosomes, relative to the fitness of flies carrying random combinations of these same chromosomes.

The distribution of the estimates of net fitness for homozygous carriers of the 15 natural chromosomes is shown in Figure 2. Each chromosome is represented by the average net fitness of the two populations which contained it, since both high and low initial frequencies are thus included.

POPULATION SIZE AND PRODUCTIVITY

To measure population size and productivity we have used the 18 populations started with 80 percent wild and 20 percent heterozygous Star flies. These populations were continued after the elimination of the Star chromosome for a total of 44 to 50 weeks.

Flies start to emerge in the populations during the second week after oviposition. Owing to the addition of newly emerged flies, the populations increase in size rapidly and reach between weeks 8 and 14 peak sizes of about 2,500 flies or more. Thereafter, the numbers of flies in the populations gradually decrease, until an approximately

TABLE 4

MEAN POPULATION SIZE, PRODUCTIVITY, AND LONGEVITY, WITH THEIR STANDARD ERRORS, IN 18 EXPERIMENTAL POPULATIONS OF 'DROSOPHILA WILLISTONI' (The number of measurements was about 13 for population size and about 24 for productivity).

Population	Population Size			Productivity per Week			Longevity (days)
	Number	*Biomass (mg)*	*Individual wgt. (mg × 10³*	*Number*	*Biomass (mg)*	*Individual wgt. (mg × 10³*	
SP 6	1592±92	1041±53	654±12	1352±74	776±38	577±11	10.3
RP 1	1465±55	995±33	681±8	1104±36	664±21	606±12	11.4
RP 3	1689±66	1090±31	650±15	1219±44	702±22	578±14	11.8
RP 4	1882±57	1210±31	637±6	1376±41	790±24	578±9	11.7
SJ 1	1644±54	1035±26	632±12	1177±58	677±31	577±13	11.9
SJ 5	1635±62	1050±42	643±11	1134±56	665±30	592±10	12.2
SJ 6	1474±41	1003±26	681±8	1049±37	649±23	622±10	11.9
SJ 7	1539±80	1007±53	654±10	1210±66	696±37	578±10	11.0
M 7	1626±55	1012±30	626±12	1389±55	759±26	550±9	10.3
M 11	1332±62	912±41	625±57	1209±46	710±26	588±9	9.8
M 13	1474±64	992±42	676±15	963±47	608±28	639±16	12.8
M 18	1418±71	970±48	685±13	1046±59	630±33	610±16	11.6
M 19	1295±75	898±46	698±12	1026±55	643±32	630±10	10.9
M 22	1443±65	930±42	645±9	1110±45	661±27	598±10	11.2
M 23	1309±70	884±47	676±10	1041±57	627±33	602±11	10.9
C 1	1556±58	1044±37	674±12	1083±40	653±23	602±9	12.2
C 2	1495±74	1003±48	673±16	1192±54	714±31	601±13	10.9
C 3	1514±81	998±42	664±10	1168±41	700±24	604±12	11.2

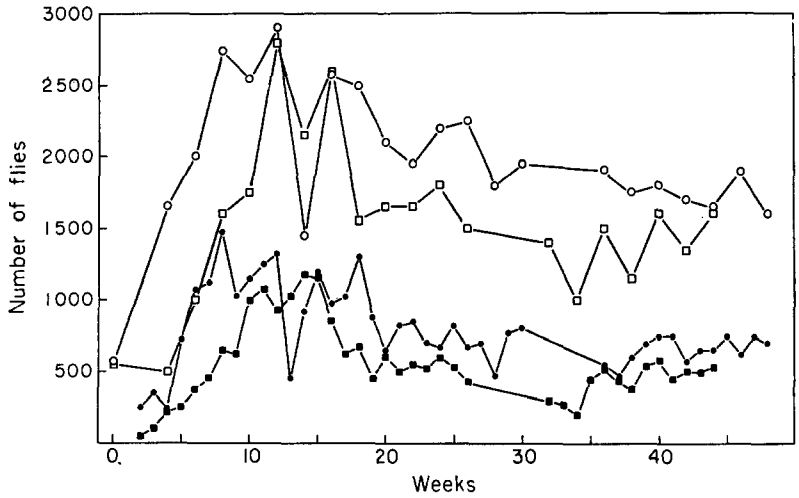

Fig. 3. Population size and productivity of two experimental populations of *Drosophila willisoni*. The productivity is given in number of flies produced per food unit. Circles=population RP4; squares=population M13. Open circles and squares=population size; solid circles and squares=productivity.

constant size is reached around week 20. The dynamics of two populations, RP4 and M13, are represented graphically in Figure 3. The pattern of oscillations in population size and in productivity are rather similar for all populations.

Table 4 gives for the 18 populations the mean number of flies, their biomass and the weight of an individual fly, for both population size and productivity per week. Mean longevity is also shown, estimated according to the method described in AYALA (1965). The means are calculated from week 20 or 22 until the end of the experiment. With the exception of population RP3, the Star chromosome had disappeared in all populations after week 24. Thus the means in Table 4 measure the average population size, productivity, and longevity of phenotypically wild flies.

It is apparent from Table 4 that the populations homozygous for the wild chromosomes differ from each other in their adaptedness to the experimental environment. The mean population size ranges from 1295 flies for population M19, to 1882 flies, or about 50 percent more, for population RP4. Similarly, the mean productivity ranges from 963

flies (population M13) to 1389 flies per week (population M7). The analyses of variance given in Table 5 show that there is significant heterogeneity among the populations, both for population size and for productivity. Number of flies has been used for the analysis in Table 5, but similar results are obtained if biomass rather than number is used. There is also significant heterogeneity in size and productivity among the 11 populations from São Jose and Mirassol and among the three populations from Ribeirão Prêto (in all cases, $P<0.001$); the three control populations, however, are not significantly heterogeneous ($P>0.20$).

TABLE 5

ANALYSIS OF VARIANCE OF POPULATION SIZE AND OF PRODUCTIVITY FOR 18 EXPERIMENTAL POPULATIONS OF 'DROSOPHILA WILLISTONI

Source	Degrees of Freedom	Mean Squares	F	Prob.
Population size:				
Genotype	17	271135	4.66	<0.001
Error	208	58169		
Productivity:				
Genotype	17	473443	6.87	<0.001
Error	409	68946		

The correlation between productivity and population size is shown in Figure 4. The correlation coefficient for all populations is 0.665 with 16 degrees of freedom, which is significantly different from zero and from one. Not unexpectedly, there is a fairly close association between productivity and population size. Those populations which produce more flies tend also to have larger population size. The correlation, however, is far from absolute. The size of population M11 is 1332 flies, one of the lowest, while its productivity per week is 1209 flies, well above the mean of all populations. The average longevity of the adult M11 flies is low, 9.8 days. The reverse situation occurs, for example, in population M13 which has the lowest productivity, 963 flies, but has a mean population size of 1474 flies, not much below the overall mean.

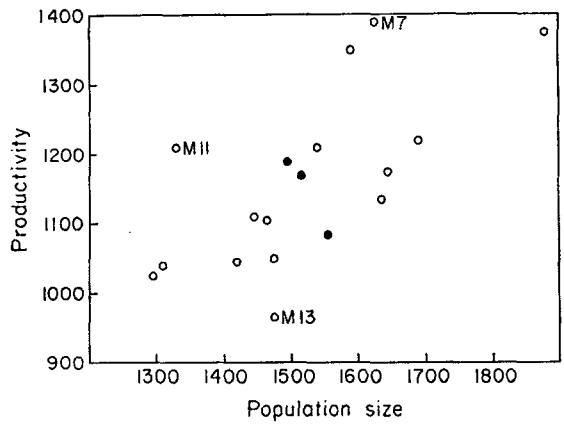

Fig. 4. Correlation between population size (abscissa) and productivity (ordinate). Solid circles indicate the control populations.

TABLE 6

AVERAGE MEAN POPULATION SIZE AND PRODUCTIVITY, WITH THEIR STANDARD ERRORS, OF THE POPULATIONS COMING FROM THE VARIOUS LOCALITIES

Locality	Number of Populations	Population size		Productivity	
		Number	*Biomass* (*mg*)	*Number*	*Biomass* (*mg*)
Ribeirão Prêto	3	1679±34	1098±18	1233±23	719±13
São José-Mirassol	11	1472±19	972±12	1123±16	666±9
Controls	3	1522±41	1015±25	1148±26	689±15

The mean longevity of the adult M13 flies is, in fact, the highest of all populations, 12.8 days.

The 11 chromosomes from the São José-Mirassol population were mixed to establish the three control populations. The average size and productivity of the 11 populations from this locality and for the three controls are given in Table 6. The control populations have larger average size and greater productivity than the populations homozygous for individual chromosomes. We have used the mean square error from the analyses of variance to test the significance of the difference between the averages of the control populations and the averages of the Mirassol-São José homozygous populations. The dif-

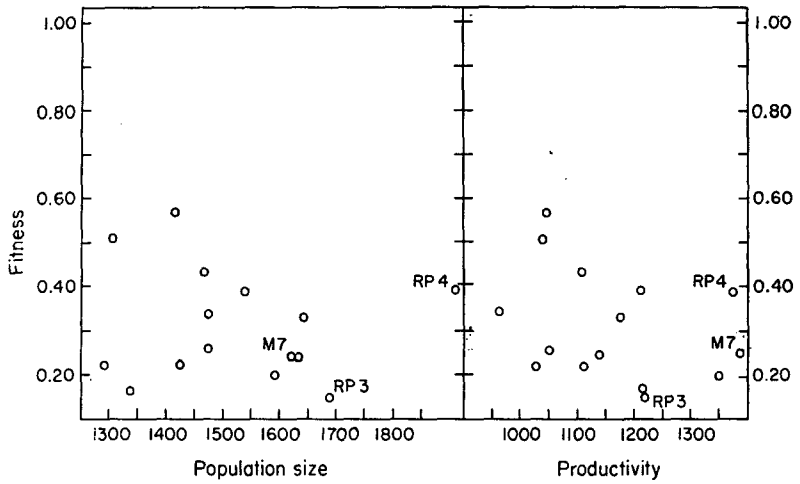

Fig. 5. Correlation between population size or productivity (abscissa) and Darwinian fitness (ordinate).

ference is statistically significant for mean number of flies in the population ($t = 2.18$, $P<0.05$), for mean population biomass ($t = 3.12$, $P<0.01$), and for mean biomass produced per week ($t = 2.65$, $P<0.10$), but not for mean number of flies produced per week ($t = 1.58$, $P<0.10$).

The correlation between Darwinian fitness and population size or productivity is shown in Figure 5. The Darwinian fitnesses used for this correlation are the net fitnesses of the populations started with 20 per cent heterozygotes (Table 3, under column B) since these are the populations in which population size and productivity were measured (see the beginning of this section). The correlation coefficient between fitness and population size is -0.16, and that between fitness and productivity is -0.35, both with 16 degrees of freedom. Neither correlation is significantly different from zero. In spite of the high fitness of the wild heterozygous flies in the control populations, their population size and productivity is only slightly above the average of the homozygous populations. Four of the 11 Mirassol-São José populations (SJ1, SJ5, SJ7 and M7) have larger mean size than their controls, and four other (SJ1, SJ7, M7, M11) produce more flies than the control populations.

We have also estimated the correlation between Darwinian fitness

and population size or productivity after excluding the one SP and the three RP populations, whose chromosomes were not introduced in the control populations. The correlation coefficient between fitness and population size is then 0.07, and that between fitness and productivity os 0.05, both with 12 degrees of freedom. Neither correlation is signicantly different from zero. Finally we have estimated the same correlations using only the SJ and M populations excluding the controls. The correlation between fitness and population size is in this case −0.13, and the correlation between fitness and productivity is −0.33, both with nine degrees of freedom. Once again these correlations are not significantly different from zero.

Discussion

Making the second chromosomes homozygous greatly reduced Darwinian fitness, as the values in Table 3 show. Estimates of the losses in fitness for the fifteen chromosomes ranged from 39 to 83 percent, with an average of 66 percent. The individual estimates are, of course, only approximate. Enough chromosomes were tested, however, and the results for all of them are consistent enough, to assure that the true average loss of fitness on homozygosity is large. It is important to note that we have estimated the overall fitness, including its components of viability, mating success, and fecundity.

The loss of fitness could come about through the effect of homozygosity for one or a few deleterious genes, or through the cumulative effects of homozygosity at many loci with small individual effects. The data of Table 1 indicate that the chromosomes when homozygous are, with the possible exceptions of RP3 and M23, normal in viability by the usual test of segregation frequency in crosses where larval competition is not very severe. We might have expected major viability effects of one or a few loci to appear in such tests, and it was on this basis that chromosomes causing lethality, semilethality, or sterility were excluded from the experiment. But the effects of some genes may depend strongly on density or on interaction between the genotypes, and such effects could appear in the populations but not in the separate rests reported in Table 1. Differences in fecundity and mating ability are not included in the viability test, of course, although they may be

major factors in the overall selection. Thus, our experiments do not allow a choice between the influence of a few, or of many, genes in producing the loss in fitness with chromosomal homozygosity.

The reduction of fitness in *D. willistoni* when nonlethal second chromosomes are made homozygous is clearly compatible with an important role for heterosis in this species, since homozygosity for heterotic genes of small individual effect should be accompanied by a lowering of fitness proportional in some way to the extent of heterosis. While this experiment can provide no firm conclusion on the extent to which heterosis may be involved in maintaining genetic diversity in nature, the results do fit quite well with those of SVED & AYALA (1970), who estimated that homozygotes for the second chromosome of *D. pseudoobscura* were on the average 60 to 70 percent less fit than organisms carrying random combinations of these same chromosomes. They reasoned that these results were consistent with a major role of heterosis. SPERLICH & KARLIK (1970) and SVED (1971) obtained similar results with *D. melanogaster.*

Populations homozygous for the natural second chromosomes differ in their adaptedness as measured by the productivity or the average size of the populations. Moreover, populations homozygous for chromosomes of different geographical origin differ in their adaptedness. The averages of size and productivity of the three Ribeirão Prêto populations are significantly higher than those of the eleven São José-Mirassol populations ($t = 10.5$, $P<0.001$, for population number; $t = 7.7$, $P<0.001$, for number produced per week; population biomass and biomass produced differ also at the 0.001 level of significance; the t values are estimated using the mean square errors from the analyses of variance). Although only three chromosomes from the Ribeirão Prêto population were tested, it is worth noticing that two of them, RP3 and RP4, have larger population size than any of the chromosomes from São José-Mirassol.

The eleven chromosomes from São José-Mirassol were mixed to establish the three 'control' populations. These controls are superior in population size and productivity to the average of the single-chromosome populations. This result supports previous observations (BEARDMORE, DOBZHANSKY & PAVLOVSKY, 1960; DOBZHANSKY & PAVLOVSKY, 1961; CARSON, 1961; AYALA, 1965, 1966) showing that increased genetic variability generally results in increased adaptedness

to the environment. It should be pointed out, however, that in some of the experiments just quoted the more variable populations were superior to every one of the parental ones. In the present experiment, the control populations are superior to the average of the single chromosome populations, but a few of the latter are superior to the controls. Thus, the higher adaptedness of the controls may not be due, at least not exclusively, to favorable interactions between the introduced genes, but also, and perhaps mostly, to the elimination of unfavorable genes or groups of genes coming from the less adapted chromosomes. That an increase in genetic variability may frequently result in an increase in the rate of adaptation of the population as measured by its size and productivity has been shown by AYALA (1966, 1968, 1970).

The 'adaptedness' of a population is not a single property of the population. First, it is clear, although not always explicitly acknowledged, that the adaptedness of a population is not an abstract property but rather exists only in relation to specific environments. Even relative to a specific environment, adaptedness may be measured in different ways, which measure different properties of the population. The average productivity per food unit and the average size are two closely related measures of the ability of a population to transform the available resources and energy into living matter. Indeed, average population size is a function of the productivity per unit time and the longevity of the adult flies. Yet these two measures give in some cases different estimates of relative adaptedness. Three examples, M7, M11, and M13, have been indicated in Figure 4. Population M11 ranks sixth among all 18 populations in productivity, but 16th in population size. A reverse situation exists in M13 which has the lowest productivity among all populations but ranks 11th in size. Individuals of the M11 genotype at the larval stage utilize the food resources efficiently but survive poorly as adults in the crowded cultures (average longevity = 9.8 days). Adults of the M13 genotype have the longest average life span (12.8 days). A lack of correlation between the adaptedness of larvae and of adults has been observed in other *Drosophila* populations (BIRCH, 1955; BEARDMORE, DOBZHANSKY & PAVLOVSKY, 1960; DOBZHANSKY & PAVLOVSKY, 1961).

The most interesting result of the present experiments is the lack of positive correlation between the adaptedness of a genotype and Darwinian fitness. The Darwinian fitness of the genotypes was measur-

ed by the rate of elimination of a marker second chromosome. The average fitness of six control populations was made equal to one. The difference in rate of elimination of the marker chromosome in the controls and in the homozygous populations gives an estimate of the relative fitness of the latter. Figure 5 shows that there is no positive correlation between either population size or productivity and relative fitness. This lack of correlation between adaptedness and Darwinian fitness in populations with *D. willistoni* alone stands in interesting contrast with the high positive correlation between the Darwinian fitness of four of the *D. willistoni* strains and their ability to compete with a strain of *D. pseudoobscura* (MOURÃO & AYALA, 1971).

Certain chromosomes (for instance RP3, RP4, M7) have in homozygous condition a very low fitness relative to other chromosomes, yet their carriers survive and reproduce in pure culture better than other genotypes with high Darwinian fitness. This result makes it clear that cartain population measures, such as 'genetic load' (CROW, 1958) and average Darwinian fitness, do not necessarily give an indication of how well adapted the population is to the environment. Consider, for instance, a population that at a certain point in time would consist of the RP4 chromosome and another chromosome of relative fitness 1.00, at frequencies of 0.9 and 0.1, respectively. Assume that the heterozygotes have also fitness 1.00 and that the three genotypes exist in Hardy-Weinberg frequencies (small deviations from Hardy-Weinberg frequencies would not substantially affect the argument). The mean fitness of this population would be 0.538 and its genetic load 0.462. Yet this population might be able to maintain a larger size and produce more flies than another population with the RP4 and the other chromosome at frequencies of 0.1 and 0.9, respectively, although the latter population would have a mean fitness of 0.994 and a genetic load of only 0.006.

The situation described by the hypothetical example given above is formally equivalent to that obtaining when gene substitutions occur by natural selection. A new gene or gene complex with high Darwinian fitness may arise which occurs first in low frequency, but gradually increases in frequency until it eventually eliminates the alternate, less fit gene or gene complex. It has been argued, for instance by HALDANE (1957) and KIMURA (1960, 1961), that there is a relatively low upper limit to the possible rate of gene substitution, because most

species could not tolerate a depression in fitness of more than about 10 percent per generation due to this cause. This kind of argumentation makes an unwarranted inference as to the adaptedness of a population based on estimates of mean Darwinian fitness. As the results presented in this paper illustrate, populations having genotypes with very low Darwinian fitness relative to other genotypes may nevertheless survive and reproduce quite well.

The relationships between Darwinian fitness and adaptedness deserve futher investigation. It is unfortunate that experimental studies have almost completely neglected this problem, and that theoretical investigations often assume that the average Darwinian fitness of a population is also a measure of its ability to survive and reproduce. This is clearly not so. Nevertheless, Darwinian fitness and adaptedness must frequently be positively correlated. If this were not so, natural selection would ultimately lead to the production of ill-adapted genotypes and therefore to extinction. This, of course, may be one reason why many species became extinct through evolutionary history.

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