Selection in experimental populations of *Drosophila pseudoobscura* with different initial frequencies of chromosomal variants*

T. WATANABE,† W. W. ANDERSON, Th. DOBZHANSKY, and
O. PAVLOVSKY

The Rockefeller University, New York and Yale University, New Haven

SUMMARY

Eight experimental populations of *Drosophila pseudoobscura* were made in laboratory population cages. All were polymorphic for ST, AR, CH, and PP gene arrangements in their third chromosomes, and all the chromosomes were of the same geographic origin. The initial frequencies of the gene arrangements were, however, different in the different populations. Natural selection has caused frequency changes in all the populations, from which we have attempted to infer the adaptive values of the different karyotypes. There was no evidence that the adaptive values were different in the populations begun from different initial frequencies of the inversions, although our ability to demonstrate such differences is severely limited. The selection process in these populations has been complex, and the simple model of constant adaptive values does not fit.

1. INTRODUCTION

Experimental populations of *Drosophila pseudoobscura*, polymorphic for various naturally occurring gene arrangements in their third chromosomes, can be maintained for several to many generations in laboratory population cages. Wright & Dobzhansky (1946) and Dobzhansky (1948) showed that, depending on the initial frequencies of the gene arrangements in such populations, more or less rapid changes in these frequencies are observed from generation to generation. The usual result of these changes, at least with chromosomes derived from the same natural population, is establishment of stable equilibria. The equilibria are maintained by a heterotic balancing selection, because the adaptive values of the heterokaryotypes are generally superior to those of the homokaryotypes. As expected, the equilibrium can be approached from both sides—the same karyotype increases or decreases in frequency, depending upon its having been below or above the equilibrium value in the founder population. The situation is, however, complicated by

several factors. The same karyotype has different adaptive properties in geographically different populations, evidently because the gene complexes locked up in the inverted chromosomes are subject to different selection pressures in these populations. Levene, Pavlovsky & Dobzhansky (1958) found that the relative fitness of two karyotypes may be changed by the presence of a third, a fourth, etc., karyotype in the same medium. The carriers of the karyotypes seem somehow to interact with each other, and a population of Drosophila in a population cage is an ecological system rather than a sum of independent individuals. Pavlovsky & Dobzhansky (1966), as well as Anderson et al. (1968), observed in some populations relationships suggesting, although not unambiguously proving, that the adaptive values of some karyotypes may also change depending upon these karyotypes being frequent or rare in the populations. The experiments described in the present article were designed to obtain information on this point. A series of eight populations were made, polymorphic for the same four gene arrangements, but with different initial frequencies of these arrangements.

2. MATERIALS AND METHODS

Drosophila pseudoobscura strains with Standard (ST), Arrowhead (AR), Chiricahua (CH) and Pikes Peak (PP) gene arrangements in their third chromosomes furnished the material for the present study. These are the same strains which were used by Pavlovsky & Dobzhansky (1966) and by Anderson et al. (1968).

Crosses were made (by O. Pavlovsky) of all the available strains, thus:

ST♀ × PP♂	AR♀ × CH♂	AR♀ × PP♂
PP♀ × ST♂	CH♀ × AR♂	PP♀ × AR♂
ST♀ × CH♂	ST♀ × AR♂	CH♀ × PP♂
CH♀ × ST♂	AR♀ × ST♂	PP♀ × CH♂

When the progenies hatched, groups of 1000 flies, about equally females and males, were used (by T. Watanabe) to start six experimental populations, and 996 flies in the remaining two. The numbers of the founding flies, all of them heterokaryotypes to insure hybrid vigour, were chosen to make the following initial frequencies of the gene arrangements (%):

Population	ST	AR	CH	PP
IA and IB	30	5	50	15
IIA and IIB	15	5	30	50
IIIA and IIIB	5	50	15	30
IVA and IVB	25	25	25	25

Plastic population cages (Takashima, Tokyo, manufacturers) were used; they were kept in a constant temperature room at 25 °C. Samples of the eggs deposited in the cages were taken on 6 to 7 successive days (the middle dates shown in Table 1); the larvae developing from them were grown under near-optimal conditions, avoiding crowding and with extra yeast added. With few exceptions

indicated in Table 1, a total of 300 chromosomes were studied per sample (six subsamples with 25 larvae in each). Until August 1968, the preparations (acetic orceine smears) were made, and the chromosomes were determined by T. W., and thereafter the preparations were made by O. P., and scored by Th. D.

Table 1. *Percentage frequencies of chromosomes with different gene arrangements in the experimental populations. Numbers of the chromosomes studied are* 300 *per sample, except when specified otherwise*

		Initial 25. ii. 68	27. ii. 68	1. iv. 68	1. v. 68	1. vi. 68	1. vii. 68	30. vii. 68	19. x. 68	15. ii. 69
I A	ST	30·0	29·3	27·7	49·3	60·0	72·7	67·0	81·3	82·7
	AR	5·0	6·7	6·3	7·0	7·3	11·0	12·7	9·7	15·7
	CH	50·0	56·7	53·3	31·3	27·3	10·0	14·7	5·3	1·3
	PP	15·0	7·3	12·7	12·3	5·3	6·3	5·7	3·7	0·3
I B	ST	30·0	29·9	33·7	47·0	56·0	65·7	70·7	76·0	78·0
	AR	5·0	3·3	5·0	6·7	10·3	16·3	12·7	18·0	20·3
	CH	50·0	51·3	41·0	30·0	23·0	12·3	10·3	2·3	1·0
	PP	15·0	16·3	20·3	16·3	10·7	5·7	6·3	3·7	0·7
II A	ST	15·0	19·7	22·0	36·7	47·0	66·7	74·0	63·7	78·7
	AR	5·0	5·3	3·7	7·7	13·3	12·7	10·3	22·0	18·0
	CH	30·0	30·3	34·0	30·0	18·3	8·7	5·0	6·0	2·7
	PP	50·0	44·7	40·3	25·7	21·3	12·0	10·7	8·3	0·7
II B	ST	15·0	22·3	23·0	38·7	62·7	60·7	71·7	67·0	72·3
	AR	5·0	5·7	7·3	10·3	9·7	15·7	14·0	21·7	25·0
	CH	30·0	26·7	32·0	21·3	14·0	13·7	8·7	5·3	2·7
	PP	50·0	45·3	37·7	29·7	13·7	10·0	5·7	6·0	0
III A	ST	5·0	5·7	11·7	9·3	18·0	17·3	24·0	23·0	36·7
	AR	50·0	51·3	53·7	70·0	62·3	74·7	63·7	72·7	63·0
	CH	15·0	9·3	19·0	6·7	6·7	3·3	3·7	1·0	0
	PP	30·0	33·7	15·7	14·0	13·0	4·7	8·7	3·3	0·3
III B	ST	5·0	8·0	10·2	8·3	13·0	14·0	22·0	28·0	34·0
	AR	50·0	44·7	56·4	67·3	70·3	76·3	70·7	70·0	65·0
	CH	15·0	10·3	11·4	9·3	7·3	3·7	3·7	1·3	0·7
	PP	30·0	37·0	22·0	15·0	9·3	6·0	3·7	0·7	0·3

		Initial 4. iii. 68	8. iii. 68	7. iv. 68	7. v. 68	7. vi. 68	12. viii. 68	19. x. 68	22. ii. 69
IV A	ST	25·0	29·0	34·3	46·4	61·3	51·4	52·3	63·0
	AR	25·0	24·7	32·0	32·0	28·3	37·6	42·3	36·3
	CH	25·0	22·3	16·7	15·2	6·3	7·6	3·0	0
	PP	25·0	24·0	17·0	6·4	4·0	3·3	2·3	0·7
					n. = 250		n = 210		
IV B	ST	25·0	30·0	38·0	37·0	55·7	57·8	60·7	64·3
	AR	25·0	22·3	29·3	33·7	30·0	32·6	34·0	35·0
	CH	25·0	24·0	20·7	13·3	5·0	3·9	1·0	0·3
	PP	25·0	23·7	12·0	16·0	9·3	5·7	4·3	0·3
							n = 230		

3. THE DATA

Percentage frequencies of the gene arrangements in the different samples are reported in Table 1. The 'Initial' frequencies were not determined by microscopic examination but deduced from the numbers of the founders of different karyotypes. Control egg samples were taken, and the chromosomes scored in the preparations, within several days after the populations were started. As expected, the controls agreed fairly well with the theoretically anticipated composition (see below, Analysis of the Data). Thereafter the relative frequencies of the gene arrangements changed greatly. CH and PP chromosomes declined in all eight populations, apparently regardless of their initial frequencies. When the experiments were terminated, approximately a year after the start, these chromosomes were rare or absent in all populations. The frequencies of ST increased in all populations; except in IIIA and IIIB, ST chromosomes became the most frequent type, although not reaching fixation in any population.

The behaviour of AR chromosomes was most interesting. In the four populations in which AR were initially rare, 5%, their frequencies tripled to quintupled, but remained well below ST (populations IA, IB, IIA and IIB). In IVA and IVB, where AR and ST were equally frequent at the start, AR increased but remained well below ST. In IIIA and IIIB, there were few ST but many AR among the founders; here both AR and ST increased in frequencies. Approximately 4 months after the start, in the samples with the middle date 1 July 1968, AR chromosomes reached peak frequencies, about 75% of the total. Thereafter their frequencies declined, while those of ST continued to rise. It is probable that, if these populations were continued longer, the AR's would have declined below the level of ST.

4. ANALYSIS OF THE DATA AND DISCUSSION

To get some idea of the selection in the eight populations, we have estimated the adaptive values of the karyotypes according to the method of DuMouchel & Anderson (1968), assuming that the selection acting on each karyotype was constant. The initial frequencies of the inversions in the model used for estimation are those among the newly formed zygotes, and they will not necessarily be identical to those among the adults used to initiate the populations. In particular, if the founder karyotypes had different fertilities, then the frequencies in the initial egg sample will depart from those among the parental karyotypes. The initial, 'control', egg sample allows us to determine whether there was appreciable selection among the founder karyotypes. Chi-square tests for goodness of fit of the observed frequencies of the four inversions in the control sample to those among the founder karyotypes are presented in Table 2. In four of the eight tests the chi-square was significantly larger than would be expected by chance deviation alone. The initial frequencies of the inversions should, then, be estimated from the initial sample of eggs. The computer program for analysis of selection was modified to provide joint maximum-likelihood estimates of the initial frequencies and of the

adaptive values. The procedure yields those estimates which together give the maximum probability of observing the particular frequencies which we obtained.

The maximum-likelihood estimates of the initial frequencies of the inversions and of the adaptive values are given in Table 3. These estimates were used to generate the expected numbers of each inversion at the various sampling times. The observed and expected numbers were compared in the chi-square tests reported in Table 3. In the majority of cases the observed and expected frequencies were significantly different, although the fits were comparable to those in previous, studies of multiallelic populations (Levene, Pavlovsky & Dobzhansky, 1954; Pavlovsky & Dobzhansky, 1966; and Anderson *et. al.* 1968). In population IIIB the fit was quite good, and in population IB the deviations were just significant at the conventional 0·05 level. In population IVB the deviations were significant at

Table 2. *Chi-squares for goodness of fit between the expected initial frequencies of the inversion types and those observed in the 'control' sample. Each total chi-square has 3 degrees of freedom*

	Population							
	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB
ST	0·0	0·0	4·4	10·7	0·3	5·4	1·9	3·0
AR	1·7	1·7	0·1	0·3	0·1	1·7	0·0	0·9
CH	2·7	0·1	0·0	1·1	6·5	4·4	2·1	0·1
PP	11·9	0·3	1·7	1·3	1·4	4·9	0·1	0·2
Total χ^2	16·3	2·2	6·2	13·4	8·3	16·4	4·1	4·2
Probability	< 0·005	0·5–0·75	0·1–0·25	< 0·005	0·025–0·05	< 0·005	~ 0·25	0·1–0·25

the 0·025 level. The contribution of each inversion type to the total chi-square varied from population to population. The total contributions of ST, CH, and PP over all eight populations were similar, while the contribution from the AR chromosome was noticeably lower. The stable equilibrium frequencies predicted with the estimates of the adaptive values are given in Table 3. In all the populations except IA, an equilibrium with ST and AR at intermediate frequencies is indicated; in population IA, only AR would remain at equilibrium.

It is clear that in most of the populations selection has not been constant, although a comparison of observed and expected inversion frequencies shows no consistent pattern to the variation in selection. In the hopes of getting some idea of how the selection varied, the adaptive values were estimated over the first and last halves of the data for each population. The new estimates did not fit the data materially better than those estimated from the total data. And the sampling variances became so large that there was no hope of extracting meaning from the differences between the populations; the estimation becomes unreliable when, as in this case, the minimum number of samples is used. Table 3 contains about as much information concerning the selection in the eight populations as we can extract from the data. Keeping the limitations of our analysis in mind, we may use the

estimates of the adaptive values in Table 3 as a rough guide to the selection in our populations. We should remember that in sets I, III, and IV there were populations which fitted the model of constant selection reasonably well, particularly in view of the multitude of factors which can cause selection to vary. There are no consistent differences evident between the sets of adaptive values in the four groups of populations begun from different initial inversion frequencies. With the exception of population I A, the predicted equilibrium frequencies are rather alike. These experiments, then, provide no evidence that selection has proceeded differently in the four sets of populations begun from widely different frequencies of the four

Table 3. *Joint estimates of the initial frequencies of the inversion types and of the adaptive values in experimental populations of* Drosophila pseudoobscura, *under the assumption that the selection was constant. Also given are the chi-squares for goodness of fit between the observed and expected frequencies of each inversion, and the predicted equilibrium frequencies*

	Populations							
	I A	I B	II A	II B	III A	III B	IV A	IV B
Initial frequencies								
ST	0·270	0·279	0·152	0·166	0·060	0·064	0·275	0·273
AR	0·056	0·037	0·042	0·052	0·495	0·470	0·254	0·243
CH	0·557	0·514	0·322	0·297	0·139	0·130	0·235	0·254
PP	0·119	0·170	0·484	0·485	0·307	0·336	0·237	0·230
Adaptive values								
ST/ST	0·94	0·96	0·86	0·81	0·83	0·57	0·83	0·79
ST/AR	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
ST/CH	0·65	0·58	0·65	0·68	0·67	0·70	0·72	0·63
ST/PP	0·74	0·62	0·73	0·65	0·88	0·23	0·81	0·70
AR/AR	1·08	0·94	0·66	0·66	0·83	0·71	0·77	0·61
AR/CH	0·55	0·65	0·76	0·74	0·47	0·53	0·56	0·46
AR/PP	0·23	0·92	0·43	0·31	0·60	0·54	0·46	0·62
CH/CH	0·36	0·26	0·04	0·18	0·77	0·47	0·55	0·48
CH/PP	0·36	0·60	0·51	0·32	0·55	0·28	0·01	0·03
PP/PP	0·96	0·01	0·07	0·18	0·00	0·15	0·18	0·42
Chi-squares								
ST	13·9	2·8	18·5	15·8	10·1	5·0	12·4	5·6
AR	5·1	5·3	15·7	3·1	8·1	1·2	6·2	1·7
CH	20·0	4·5	25·5	7·5	17·8	2·2	10·9	5·9
PP	8·3	9·0	11·4	20·5	15·6	1·8	9·4	10·5
Total	47·4	21·5	71·1	46·7	51·5	10·2	38·8	23·6
D.F.	12	12	12	12	12	12	9	9
Probability	< 0·005	~ 0·05	< 0·005	< 0·005	< 0·005	0·5–0·75	<0·00	~ 0·025
Predicted equilibrium frequencies								
ST	0·00	0·60	0·71	0·64	0·50	0·40	0·58	0·65
AR	1·00	0·40	0·29	0·36	0·50	0·60	0·42	0·35
CH	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
PP	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00

inversion types. Our ability to detect such differences, however, is admittedly weak, and we expect only large differences to be demonstrable. But we can say that large differences were most likely not present, and that the selection process in these populations is a great deal more complex than our simple model allows.

REFERENCES

ANDERSON, W. W., OSHIMA, C., WATANABE, T., DOBZHANSKY, TH. & PAVLOVSKY, O. (1968). Genetics of natural populations. XXXIX. A test of possible influence of two insecticides on the chromosomal polymorphism in *Drosophila pseudoobscura*. *Genetics* **58**, 423–434.

DOBZHANSKY, TH. (1948). Genetics of natural populations. XVIII. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographic regions. *Genetics* **33**, 588–602.

DUMOUCHEL, W. H. & ANDERSON, W. W. (1968). The analysis of selection in experimental populations. *Genetics* **58**, 435–449.

LEVENE, H., PAVLOVSKY, O. & DOBZHANSKY, TH. (1954). Interaction of the adaptive values in polymorphic experimental populations of *Drosophila pseudoobscura*. *Evolution* **8**, 335–349.

LEVENE, H., PAVLOVSKY, O. & DOBZHANSKY, TH. (1958). Dependence of the adaptive values of certain genotypes in *Drosophila pseudoobscura* on the composition of the gene pool. *Evolution* **12**, 18–23.

PAVLOVSKY, O. & DOBZHANSKY, TH. (1966). Genetics of natural populations. XXXVII. The coadapted system of chromosomal variants in a population of *Drosophila pseudoobscura*. *Genetics* **53**, 843–854.

WRIGHT, S. & DOBZHANSKY, TH. (1946). Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. *Genetics* **31**, 125–156.