THE DARWINIAN FITNESS OF POLYMORPHIC AND MONOMORPHIC POPULATIONS OF *DROSOPHILA PSEUDO-OBSCURA* AT 16° C.

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1. INTRODUCTION

NATURAL populations of many species of *Drosophila* show a chromosomal polymorphism which is due to inversions of block of genes. Over the range of environments common in the natural habitats, this polymorphism is adaptive and balanced; the heterokaryotypes possess an adaptive value superior to that of the homokaryotypes.

As shown by Dobzhansky (1948, 1957), Levine (1955) and Levene, Pavlovsky and Dobzhansky (1958), experimental populations of *Drosophila pseudoobscura* containing third chromosomes with AR and with CH gene arrangements are subject to strong selection, which at 25° C. conduces to an equilibrium of about 70 per cent AR and 30 per cent CH. Beardmore, Dobzhansky and Pavlovsky (1960) have made an attempt to compare the fitness of experimental populations of *Drosophila pseudoobscura* polymorphic and monomorphic for the above gene arrangements. They estimated certain parameters which were considered significant for evaluation of fitness. The results indicated that the polymorphic populations studied were superior to the monomorphic ones. However, since the experiments were carried out at 25°, it was not possible to decide whether the differences between the polymorphic and the monomorphic populations were due to a superiority of the heterozygotes or to a mutual facilitation between the carriers of the different karyotypes. It was conjectured that both factors were involved.

In order to throw more light on this question, we repeated the experiments with the same populations but kept them at 16° C., a temperature at which heterozygotes are known not to be superior to homozygotes. This is the scope of the present work.

2. MATERIAL AND TECHNIQUES

The three experimental populations studied were studied by Beardmore *et al.* (1960) at 25° C. Population No. 180 was monomorphic for AR, No. 181 was polymorphic for AR and CH, No. 182 was monomorphic for CH. In August 1958, the three population cages were transferred from 25° to a constant temperature

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room at 16°. The populations were sampled between 9th September 1958 and 3rd March 1959. From time to time a cup with food in which pupæ were beginning to form was withdrawn from each cage, attached by adhesive tape to a glass "chimney" provided with a cotton stopper and when the adults began hatching, they were collected daily, etherised, counted and weighed individually on a misco quartz helix balance. Then they were preserved in alcohol. Details of the methods adopted and of the foundation stocks of the experimental populations, are given by Beardmore, Dobzhansky and Pavlovsky (1960).

Ten random samples were taken from each population for the purpose of measuring wing length. The number in each sample was fifty flies of each sex, or the total emergence if this was less than fifty flies. Both wings were removed from each fly and mounted under cover-glasses, right and left wings forming separate rows.

The measurements were made using the Promar (Seibert-Wetzlar) projecting microscope of the Zoology Department, University of Padua, Italy. This permitted easy measurement with the aid of a ruler of the image of the wing projected onto a sheet of white paper. The wing length was measured from a point near the posterior end of the humeral crossvein to the tip of the wing near the end of the third vein.

3. RESULTS

(i) *Numbers of flies produced*

Table 1 shows the means, variances and variation coefficients of the numbers of the flies which hatched from the cups taken from the

TABLE 1
Number of flies hatching per cup in the three populations

Population	Sex	Mean	Variance	Variation coefficient	Cups examined
180, AR	Female	258.00 ± 36.2	18,651	52.7	14
181, AR + CH	Female	304.78 ± 44.8	28,345	55.1	14
182, CH	Female	270.14 ± 29.6	12,249	41.5	14
180, AR	Male	202.28 ± 28.2	11,251	52.4	14
181, AR + CH	Male	268.28 ± 34.9	17,287	48.8	14
182, CH	Male	234.07 ± 29.3	12,036	47.0	14

three population cages. For the reasons stated by Beardmore, Dobzhansky and Pavlovsky (1960), the number of the adults that hatch is an indication of the ability of the different genotypes in a given population to exploit successfully the available environmental opportunities.

The comparison of the polymorphic (181) and the two monomorphic populations (180 and 182), which have been sampled at the same time, indicates that the number of flies hatching per cup is higher in the polymorphic population in both sexes. However, these differences are not statistically significant. The variances and the coefficients of variation indicate that the variability in the polymorphic population is not lower than in the monomorphic ones. It seems that population 182 (CH) is least variable, but apparently the differences are not significant.

(ii) *Weight and biomass*

The flies hatching in each of fourteen cups in the three populations studied were weighed individually. The mean weights of the flies of both sexes are given in tables 2 and 3, together with the number

TABLE 2

Weight (in mg.) of flies in the three populations. Females

No. 180, AR			No. 181, AR+CH			No. 182, CH		
Mean	s^2	Flies	Mean	s^2	Flies	Mean	s^2	Flies
0.754	0.055	233	0.800	0.020	243	0.720	0.032	226
0.800	0.037	168	0.636	0.029	293	0.737	0.020	234
0.622	0.021	436	0.577	0.033	561	0.675	0.026	329
0.700	0.042	251	0.552	0.026	528	0.685	0.028	389
0.760	0.047	498	0.657	0.027	571	0.658	0.036	462
0.696	0.031	290	0.652	0.028	490	0.723	0.043	294
0.591	0.036	467	0.593	0.026	386	0.607	0.018	440
0.924	0.012	114	1.165	0.023	124	0.767	0.030	340
0.974	0.037	67	0.952	0.020	204	0.751	0.036	257
1.058	0.053	132	0.996	0.057	122	0.958	0.039	155
0.854	0.084	306	0.946	0.090	161	1.133	0.020	108
0.620	0.032	288	0.689	0.055	186	0.784	0.070	182
1.128	0.059	105	0.978	0.053	155	1.156	0.045	135
0.669	0.084	257	0.688	0.024	243	0.536	0.022	231

TABLE 3

Weight (in mg.) of flies in the three populations. Males

No. 180, AR			No. 181, AR+CH			No. 182, CH		
Mean	s^2	Flies	Mean	s^2	Flies	Mean	s^2	Flies
0.693	0.047	175	0.732	0.019	233	0.706	0.026	181
0.754	0.027	154	0.603	0.020	275	0.689	0.021	160
0.583	0.014	413	0.531	0.011	497	0.625	0.042	318
0.661	0.081	125	0.516	0.018	460	0.602	0.023	311
0.676	0.034	387	0.573	0.020	442	0.605	0.022	482
0.616	0.030	224	0.603	0.018	435	0.667	0.024	285
0.549	0.029	223	0.547	0.026	218	0.539	0.012	318
0.817	0.013	125	1.085	0.014	148	0.718	0.020	313
0.852	0.028	46	0.893	0.014	173	0.719	0.025	261
0.943	0.026	117	0.854	0.045	127	0.869	0.018	130
0.830	0.076	230	0.883	0.063	154	1.024	0.014	86
0.575	0.032	292	0.635	0.042	185	0.700	0.033	140
0.978	0.046	98	0.896	0.038	171	1.020	0.026	142
0.609	0.043	223	0.647	0.017	238	0.526	0.027	150

of flies in each cup and the variances of the individual weights (s^2). The intra-cup variances, *i.e.* the average variances of the individual flies emerging in the same cup, and the inter-cup variances, *i.e.* the

variances of "means", for each population and sex, are given in table 4.

In contrast to what has been observed in the experiments of Beardmore *et al.*, in our experiments the variances, both intra-cup and inter-cup, do not differ significantly in the three populations. The same is shown by the coefficients of variation, which have been computed in order to eliminate the effects of the differences in the average weights. The coefficients of variation range for females from 13.0 to 34.1 in the polymorphic population AR+CH, from 11.9 to 43.2 in the population monomorphic for AR, and from 12.3 to 33.8 in the population monomorphic for CH. For males, they range from 10.9 to 32.3 in the polymorphic population, from 13.9 to 42.9 in the population AR, and from 11.6 to 32.6 in the monomorphic CH. Considering only the variances of individuals and the coefficients of variation,

TABLE 4

Mean weight (in mg.) of a fly, and intra-cup variances (of individuals) and inter-cup variances (of means), in the three populations

Population	Sex	Mean	Intra-cup variance	Inter-cup variance
180, AR . . .	Female	0.797	0.045	0.029
181, AR+CH . . .	Female	0.777	0.036	0.038
182, CH . . .	Female	0.778	0.033	0.033
180, AR . . .	Male	0.724	0.037	0.020
181, AR+CH . . .	Male	0.714	0.026	0.031
182, CH . . .	Male	0.715	0.024	0.024

the population 182 (CH) seems to be the least variable. This, however, is not confirmed by the variances of the means which are not in agreement with the intra-cup data. The absence of a regular trend in the three series of data (intra-cup variances, coefficients of variation, inter-cup variances) makes the differences observed not significant.

There is no significant difference between the weights of flies belonging to the polymorphic population and the weights of those belonging to the two monomorphic ones. However, there is a slight inverse correlation between the number of flies produced in a cup and their weights. Flies developing at 16° C. are somewhat lighter than those produced by the same populations at 25° C., from which hatch much smaller numbers of flies. There is nevertheless some indication (table 2) that, with equal population densities, the flies are slightly heavier in polymorphic than in monomorphic populations.

Let us now consider the total weights of the flies emerging in the different populations with the same amount of food (the amount of food contained in a cup). These values (table 5) correspond to the biomass, and are obtained by multiplying the numbers of the flies hatching in the different cups by their mean weights. The data show

that the biomass is greater in the polymorphic population (181); this is true also for females and males considered separately. The data reported in tables 1, 4 and 5 show that the polymorphic population produces more flies, which are not much lighter than those produced in smaller numbers in the monomorphic populations.

TABLE 5

Total weights (biomass) in mg. produced per cup in the three populations

Sex	Population		
	180, AR	181, AR+CH	182, CH
Females	189.41	212.85	196.75
Males	137.51	175.51	158.01
Total	326.92	388.36	354.76

Thus at a temperature of 16° C., just as at 25° C., the polymorphic population, 181, is more productive than the monomorphic ones, 180 and 182.

(iii) *Wing length*

Table 6 gives the mean wing lengths of the flies, together with the intra-cup and inter-cup variances. The coefficients of variation are shown in table 7. The data indicate no difference in wing length

TABLE 6

Mean wing length (in mm.) and intra-cup variance and inter-cup variance in the three populations

Population	Sex	Mean	Intra-cup variance	Inter-cup variance
180, AR	Female	2.309	0.024	0.017
181, AR+CH	Female	2.298	0.019	0.026
182, CH	Female	2.223	0.020	0.010
180, AR	Male	2.116	0.022	0.010
181, AR+CH	Male	2.118	0.011	0.017
182, CH	Male	2.069	0.014	0.007

between the polymorphic and monomorphic populations. However, the flies, females and males, in population 182 (CH) have significantly shorter wings than the flies in populations 180 (AR) and 181 (AR+CH). This may partly depend upon environmental factors, such as the degree of crowding in the population cages.

Comparison of the "individual" variances (intra-cup) of wing length shows that the polymorphic population is less variable than the monomorphic ones. In fact, the females in the population 181 have

the variance of 0.01921 and in the populations 180 and 182, 0.02447 and 0.01988; the males show a variance of 0.01148 in the polymorphic and 0.02224 and 0.01403 in the monomorphic populations. These differences in variability become even clearer in the coefficients of variation (table 7).

TABLE 7
Mean wing lengths. Coefficient of variation for individuals

Population	Sex	
	Females	Males
180, AR	6.60	6.91
181, AR+CH	5.96	4.93
182, CH	6.23	5.52

The variances of means (inter-cup) on the other hand behave differently, being greater in the polymorphic population than in the monomorphic ones, as in the case of the inter-cup variances of the fly weights. Here again we can say that the disagreement between the two types of variances greatly reduces the significance to be attached to the differences observed.

(iv) Asymmetry

The data on wing asymmetry, *i.e.* the mean intra-fly difference between right and left wings, calculated for each population and reported on table 8, are more informative. They clearly show that flies

TABLE 8
Mean asymmetry of wings in microns in flies of different populations

Sex	Population		
	180, AR	181, AR+CH	182, CH
Females	11.505	9.671	10.235
Males	11.705	9.121	10.893

from the polymorphic population (181) have lower average values of wing asymmetry than flies from monomorphic populations (180 and 182). A statistical comparison of the means (table 9) shows that the differences are highly significant in three cases out of four. The only exception is in the case of the females of the comparison $182 > 181$. A similar result was observed in the population of Beardmore, Dobzhansky and Pavlovsky (1960) at 25° C. The same explanation can be given

in our case, namely that the flies from population 182 have shorter wings than those from population 181 (see table 6) and the "asymmetry value is therefore correspondingly lower than it would have been had the mean wing length of flies from 182 been more nearly equal to that of the 181 flies". As pointed out by Thoday (1958) the degree of asymmetry of bilaterally paired structures appears to depend in part on the absolute size of the structures themselves. We have converted the absolute asymmetry values into relative values, dividing them by the wing lengths. On doing so, the difference in wing asymmetry between the females of the populations 182 and 181 becomes

TABLE 9
Comparisons of mean values of wing asymmetry in the three populations

Sex	Populations	<i>t</i>	P
Females	180>181	3.162	<0.01
	182>181	1.007	0.4-0.3
Males	180>181	4.379	<0.001
	182>181	3.055	<0.01

statistically significant. This clearly shows that, at 16° as at 25° C., developmental homeostatic mechanisms are more efficient in the polymorphic than in the monomorphic populations.

4. DISCUSSION

At the time when our experiments were carried out, the genetic composition of the three populations studied, 180, 181 and 182, was approximately the same as in the experiments by Beardmore *et al.* The two monomorphic populations, 180 and 182, consisted respectively of flies homozygous for the AR and the CH gene arrangement of the third chromosome, whereas the polymorphic population, 181, consisted of flies belonging to the three karyotypes AR/AR, CH/CH and AR/CH, with AR being about twice as frequent as CH and, consequently, with slightly fewer than half of the flies belonging to the heterokaryotype AR/CH. At 16° the three karyotypes appear to have approximately the same adaptive values, as shown by the absence of changes in the frequencies of the gene arrangements.

The data reported in the present paper indicate that the polymorphic population produces more and, with equal population densities, slightly heavier flies, and consequently a greater biomass, than the monomorphic populations. As far as the variability is concerned, the polymorphic and monomorphic populations show inconsistent differences. Beardmore *et al.*, working with the same populations kept at 25°, observed lower variances in the polymorphic than in the monomorphic populations.

The absence of clear-cut differences between the variabilities in

polymorphic and monomorphic populations may be a reflection of the fact that the temperature of 16° is a less demanding environment than 25°, as shown indirectly by the far greater numbers of flies surviving at the lower temperature.

It may be of some interest to note that the difference in biomass between 25° and 16° data is accounted for by a greater number of flies and not by larger flies, at 16°; in fact the mean weight of a fly at the lower temperature is lower than it is at the higher one, though the figures are, for many reasons (time of sampling, etc.), not strictly comparable.

The significance of the differences in wing asymmetry, found at 16° as well as at 25°, is perhaps due to the more precise method of wing measurement adopted in the present work. It clearly shows that flies belonging to the polymorphic population have more symmetrical wings. May this be taken as an indication of a higher homeostasis in the polymorphic population? Many facts suggest that asymmetries are often the result of an imperfect homeostasis. A higher degree of homeostasis, as indicated by the lower variability of certain quantitative traits, is a property of heterozygous genotypes as compared with the homozygous ones. Now observations made by Mather (1953) and Tebb and Thoday (1954) in *Drosophila melanogaster* show that the degree of asymmetry of certain traits such as the numbers of sternopleural bristles on the two sides of the body, is greater in homozygotes than in heterozygotes. Moreover, according to Beardmore, Dobzhansky and Pavlovsky (1960), "it is hardly an accident that heterozygous genotypes, at least those heterozygous for co-adapted gene complexes derived from the same population, show both low variance in traits concerned with fitness and low asymmetry".

Thus, even disregarding, because of the lack of statistical significance, the ability to produce a greater number of flies and a greater biomass shown by the polymorphic population in our experiments, other results show that the polymorphic population is superior in fitness to populations monomorphic for AR or for CH.

The persistence of the advantage, though reduced, at approximately optimal environmental conditions, without any superior fitness of the heterozygotes, such as is observed at 25°, would indicate that the higher fitness of the polymorphic population is, at least in part, to be attributed to phenomena of mutual facilitation between different genotypes. This is in agreement with the view expressed in a previous work by Beardmore *et al.* (1960).

Experiments by Levene, Pavlovsky and Dobzhansky (1954, 1958), Spiess (1957) and Lewontin (1955) have shown that the viability of a genotype is a function of the other genotypes coexisting with it in the same medium. In our case, the two karyotypes, AR/AR and CH/CH, would actually benefit by living side by side and together with AR/CH. The ecological mechanism which underlies this peculiar interaction remains, however, to be clarified.

5. SUMMARY

The observations reported here concern three experimental populations of *Drosophila pseudoobscura* kept at 16° C. One of them was polymorphic for the AR and CH gene arrangements in the third chromosome, one was monomorphic for the AR, and one for the CH chromosome. At the temperature employed, the heterozygotes AR/CH are not heterotic, as they are at 25° C. Thus the composition of our populations was the same as it was at 25° C., the temperature at which the three populations had been kept previously, namely, 70-75 per cent AR and 25-30 per cent CH, with about half of the individuals belonging to the heterokaryotype AR/CH.

Samples were taken from each of the three populations, at more or less regular intervals, in order to estimate certain parameters which were considered significant for evaluation of fitness.

The results show that (a) the polymorphic population produces more, and with equal population densities, slightly heavier flies than do the monomorphic ones; (b) the polymorphic population produces also a greater biomass; (c) the mean length of the wings does not differ significantly in polymorphic and monomorphic populations; (d) the variabilities of the numbers, weights, and wing lengths in the flies produced in the three populations show no consistent differences; (e) the asymmetry of the wings is significantly greater in monomorphic populations than in the polymorphic one.

The conclusion, mainly based on points (a) and (e), is that the polymorphic population is superior in fitness to the monomorphic ones. The persistence of such advantage, though to a lesser degree, at 16° C. as well as at 25° C., favours the view that the superior fitness of the polymorphic population is due at least in part to mutual facilitation between the carriers of the different gene arrangements.

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