HOW STABLE IS BALANCED POLYMORPHISM?*

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Natural populations of many species of organisms are polymorphic, i.e., are composed of two or more distinct kinds of individuals. Several genetic mechanisms may participate in the maintenance of polymorphism in populations.¹⁻⁴ One of the most widespread and interesting ones is balanced polymorphism. It has long been known⁵⁻⁸ that if the heterozygotes for a pair of alleles, A₁A₂, are superior in adaptive value to the corresponding homozygotes, A₁A₁ and A₂A₂, natural selection will, in sexually reproducing and cross-fertilizing populations, establish an equilibrium state at which the two alleles, A₁ and A₂, will continue to occur in the gene pool of the population with certain predictable frequencies. This will happen even if one or both of the homozygotes are deficient in fitness, or are inviable, provided only that the heterozygote enjoys the advantages of hybrid vigor.

The mean adaptive value of the population is maximized when stable equilibrium frequencies of the alleles A₁ and A₂ are achieved. Nevertheless, the poorly adapted homozygotes continue to be born generation after generation in populations under balanced polymorphism. In other words, the population fails to reach the level of adaptedness which it would have if it were monomorphic for a genotype with an adaptive value equal to that of the A₁A₂ heterozygotes. Suppose, then, that a mutation produces an allele A₃ such that the fitness of the homozygote A₃A₃ is equal to that of the heterozygote A₁A₂. Natural selection is expected to lead to gradual elimination of A₁ and A₂, and to establishment of a population monomorphic and homozygous for A₃A₃.

These considerations have led to the view⁹⁻¹⁰ that balanced polymorphisms "... are to be regarded as temporary makeshifts that arose in the stress of comparatively rapid evolutionary flux and that are due to be rectified ultimately, when a long-term natural selection repairs its short-term imperfections and miscarriages."
The flaw in the above argument lies in the implied assumption that whatever level of adaptedness may be reached by a heterozygote $A_1A_2$, it can always be equalled, if not exceeded, by mutations yielding a superior allele $A_3$. Experimental verification or invalidation of this assumption is evidently difficult, especially if it is not specified just how "temporary makeshift" the balanced polymorphisms are expected to be. Natural populations of many species of Drosophila are balanced polymorphic systems of several gene arrangements, differing in inversions of sections of chromosomes. Although some early speculations which ascribed to inversion polymorphisms an antiquity ascending to Eocene and to Cretaceous times\textsuperscript{11} appear to have been not well founded, there is good evidence that balanced polymorphs may not be easily replaceable. Thus in *Drosophila willistoni*, which is a species not closely associated with man, some inversion heterozygotes occur over vast territories, including much of the intertropical zone of the Western Hemisphere. A genetic variant must have existed assuredly for a long time to have spread to, and become incorporated in the gene pools of populations so numerous and so remote.

Laboratory experiments on balanced polymorphisms cover time intervals obviously small compared to the persistence of such polymorphisms in natural populations. There are two considerations, however, which make experimental approaches to the problem hopeful. In the first place, experiments are carried in laboratory environments which are of necessity unlike the natural ones. Experimental populations are thus faced with environmental challenges, to which they may respond by genetic changes adapting them to the experimental environments. A serviceable monomorphism may then be substituted for a balanced polymorphism. Secondly, what is sometimes described picturesquely as a "genetic revolution," may be produced by making experimental populations derived from founders obtained by hybridization between geographically distinct (allopatric) natural populations or races. Segregation and recombination of the genes in which the races differ supply an abundance of genetic variance. Natural selection in the experimental environments is then called upon to construct a new adaptive system, which may or may not include balanced polymorphism.

Many experimental populations of Drosophila flies, polymorphic for naturally occurring inversions, have been studied by various investigators since 1946. In most of these populations apparently balanced equilibria were attained, provided that the foundation stocks of these populations were of geographically uniform origin. It is, of course, arguable that experiments were usually discontinued too soon, before substitution of monomorphism for polymorphism could take place. The few recorded exceptions are therefore interesting. In experiments with *Drosophila pseudoobscura*, a population containing third chromosomes with ST and TL gene arrangements became, in about a year after its foundation, almost monomorphic for ST. It may be noted that TL chromosomes are relatively rare in the natural population from which the foundation stock came.\textsuperscript{12}

In an experiment with the same species, in which chromosomes of Texas origin with AR and PP gene arrangements were involved, two experimental populations were started with equal frequencies of the two kinds of chromosomes. Some 605 days later the populations contained about 95 per cent AR and 5 per cent PP chromosomes.\textsuperscript{13} It may be noted that in the natural populations of Texas, PP
chromosomes are considerably more frequent than AR chromosomes. This shows how different may be the relative fitness of the same karyotype in natural and in experimental environments. Lewontin's experiments with similar populations brought confirmatory results.\textsuperscript{14, 15} But in addition, Lewontin observed that the progress of the selection in the experimental populations was such as to indicate either that the environment, or that the relative fitness of the karyotypes were changing during the experiments. If the latter possibility is correct, it would follow that an evolutionary process was here enacted which led to the abrogation of the balanced polymorphism in favor of monomorphism.

That this is not a general rule for experimental populations of geographically uniform origins, is shown by the data in Table 1. These populations, of \textit{D. pseudo-}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Days & Generations & 173 & 176 & 181 & Chi Square \\
\hline
0 & 0 & 20.0 & 20.0 & .. & .. \\
35 & 1.4 & 39.3 & 40.0 & 40.7 & 0.12 \\
70 & 2.8 & 45.0 & 48.3 & 41.3 & 2.92 \\
105 & 4.2 & 50.0 & 50.3 & * & 0.15 \\
118 & 4.7 & 5.7 & 56.7 & * & 1.21 \\
140 & 5.6 & 57.3 & .. & 67.7 & .. \\
175 & 7.0 & .. & .. & .. & .. \\
264 & 10.6 & .. & .. & .. & .. \\
275 & 11.0 & .. & .. & .. & .. \\
302 & 12.1 & .. & .. & .. & .. \\
365 & 14.6 & .. & .. & .. & .. \\
488 & 19.5 & .. & .. & .. & .. \\
583 & 23.3 & .. & .. & .. & .. \\
610 & 24.4 & .. & .. & .. & .. \\
649 & 26.0 & .. & .. & .. & .. \\
790 & 31.6 & .. & .. & .. & .. \\
980 & 39.2 & .. & .. & .. & .. \\
\hline
\end{tabular}
\caption{Frequencies (in per cents) of AR Chromosomes in Populations of \textit{Drosophila pseudoobscura} Containing AR and CH Chromosomes Kept at 25°C}
\end{table}

\textit{obscura}, contained AR and CH chromosomes from southern California (Piñon Flats). In experimental, as well as in natural populations, AR chromosomes have an advantage over CH; an equilibrium is therefore established with AR outnumbering CH. The progress of selection in three populations, Nos. 173, 176, and 181, though they were started at different times, was very similar for at least a year. This is shown by the small chi-square values in the rightmost column in Table 1; (these chi-squares have mostly 2 degrees of freedom; where samples were not taken at similar ages, interpolation was made between the successive samples; such interpolations are marked in Table 1 by asterisks). The experimental populations were kept at 25°C, but the population No. 181 spent several months at 15° when it was about 1\frac{1}{2} years old, and was later returned to 25°. The sojourn at 15° has apparently altered its genetic properties, and it reached an equilibrium at a lower frequency of AR chromosomes than did populations Nos. 173 and 176. The chi-squares computed for the 583 day and 980 day stages indicate significant differences between No. 181 and the other two populations. All samples contained 300 chromosomes.

The progress of natural selection in populations Nos. 173 and 176, and in No. 181 during the first year of its career, can be accounted for on the assumption that the adaptive values of the three karyotypes were as follows:
The course of selection predicted on the basis of these values gives a satisfactory fit, thus indicating that the adaptive values of the karyotypes have remained constant during the course of the experiment. The aberrant behavior of No. 181 after it was exposed to changes in the temperature regimen shows, however, that even in populations of uniform geographic origin, genetic variance may be available which may be used to alter the equilibrium position of the balanced polymorphism. And the alteration was, in the present case, towards making the two gene arrangement more nearly equal in frequencies than they were in other populations, not at all towards monomorphism.

Some geographic populations of the sibling species, Drosophila willistoni, D. paulistorum, and D. tropicalis are remarkable in having more than 50 per cent of the individuals heterozygous for certain inversions in their chromosomes. In these populations, one half of the zygotes formed in every generation are handicapped genetically enough to suffer a differential mortality, even under the near-optimal conditions in laboratory cultures. It would seem that natural selection in populations of this sort should be especially likely to lead to a monomorphic condition that would free them from the necessity of paying the “costs” of balanced polymorphism. In August, 1956 we started two experimental populations of D. willistoni, using in one (No. 168) progenies of the flies collected some months earlier near Recife, Brazil, and in the other (No. 169) flies from Guaramiranga, Ceará, Brazil. The results obtained are reported in Table 2.

About 60 per cent of the individuals in the Recife population were originally heterozygous for an inversion J, in the third chromosome (IIIJ), which was present in 55 per cent in the Ceará population (Table 2). An inversion E in the right limb of the second chromosome (IIRE) was, however, heterozygous in about 65 per cent of the individuals in the Ceará population, but in only 14 per cent at Recife. Moreover, about 32 per cent of the Recife population was heterozygous for the inversion P in the third chromosome (IIIP), which did not occur in Ceará at all. Samples of 200 larvae were examined when the populations were 140, 835, and 1,110 days old from the start. The 420-day samples were obtained by testing the zygotic constitution of 200 adult flies per population; these samples are not entirely comparable with the rest. Table 2 shows that, when the populations were slightly more than three years old, the balanced polymorphism was retained. The frequencies of the heterozygotes have, however, undergone changes; the frequencies

<table>
<thead>
<tr>
<th>Days</th>
<th>IRE</th>
<th>IIIJ</th>
<th>IIIP</th>
<th>IRE</th>
<th>IIIJ</th>
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<tbody>
<tr>
<td>0</td>
<td>14</td>
<td>61</td>
<td>32</td>
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<td>140</td>
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<td>?</td>
<td>59.5</td>
<td>?</td>
<td>59.5</td>
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</tr>
<tr>
<td>835</td>
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</tr>
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<td>1110</td>
<td>51.0</td>
<td>44.0</td>
<td>9.0</td>
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</tr>
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</table>
of IIRE and IIIJ heterozygotes decreased somewhat in the Ceará population, as did the IIIJ and especially the IIIP heterozygotes in the Recife population. The frequency of IIRE heterozygotes in the Recife population has, however, increased quite strikingly—it has more than trebled.

An unpremeditated experiment of a much longer duration, up to 211 generations, was performed by maintaining laboratory strains of *Drosophila pseudoobscura*, the wild progenitors of which were heterozygous for certain chromosomal inversions. Although the maintenance of laboratory stocks involves some inbreeding, only 3 of the 27 strains which were originally polymorphic became monomorphic in the course of time. The adaptive advantage of the chromosomal heterozygotes in these populations was evidently strong enough to counteract the effects of inbreeding and genetic drift. At the same time, no well-adapted homozygotes have developed which would have permitted replacement of the polymorphism by monomorphism. It may, of course, be objected that inbred strains do not possess sufficient genetic variance to enable such evolutionary changes to take place. This stricture does not apply to experiments described below.

Chromosomally polymorphic experimental populations of *Drosophila* have been made using for the foundation stocks hybrids between strains of flies coming from different geographic regions. Such populations of geographically mixed origin often behave quite differently from those of geographically uniform origin. The process of natural selection in populations of mixed geographic origin is characteristically erratic, and often quite different in replicate experiments. The balanced chromosomal polymorphism is sometimes retained and sometimes lost. The selective processes which are enacted in such populations lead to compounding of new adaptive systems from genetic fragments yielded by the hybridization of the geographic races. The replacement of the balanced polymorphisms present in the parental races by chromosomal monomorphisms certainly proves that acquisition in evolution of a balanced polymorphic condition need not be irreversible; the population is not, so to speak, committed to remain polymorphic forever. On the other hand, retention of the polymorphism shows that it may be advantageous even when the adaptive system in a population undergoes reconstruction. It has been cogently argued that balanced polymorphism is most likely to be retained in populations living in variable environments, and replaced by monomorphism in uniform environments. Although the environmental conditions in the experimental populations are not quite uniform, yet they are almost certainly more nearly so than in the natural habitats of *Drosophila*.

Levine and Beardmore have maintained an experimental population of *Drosophila pseudoobscura* of mixed geographic origin (California + Mexico) for about 53 months (66 generations). In about 20 generations from the start, the population established an equilibrium of about 30–35 per cent CH and 65–70 per cent AR chromosomes, and kept it till the end of the experiment, with occasional fluctuations up to 40 per cent and down to 25 per cent of CH.

We have considered most suitable for testing the stability of balanced polymorphism the populations of *Drosophila paulistorum* and *D. willistoni* which contain, in nature, more than 50 per cent of heterozygotes for certain inversions (see above and the references 16 and 17). Two experimental populations of *D. paulistorum* were set up, with the foundation stocks of mixed geographic origin. In about 10
months, corresponding to 12–15 generations, the balanced polymorphism broke down and the populations became virtually monomorphic. A different outcome was, however, observed in the populations Nos. 178 and 179 (Table 3), the founda-

<table>
<thead>
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<th>Days</th>
<th>Population No. 178</th>
<th>Population No. 179</th>
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<tr>
<td></td>
<td>HIRE</td>
<td>IIIJ</td>
</tr>
<tr>
<td>0</td>
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<td>52.0</td>
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<td>27.5</td>
<td>45.0</td>
</tr>
<tr>
<td>400</td>
<td>33.75</td>
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</tr>
<tr>
<td>640</td>
<td>40.5</td>
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<tr>
<td>820</td>
<td>45.5</td>
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</tr>
<tr>
<td>980</td>
<td>44.0</td>
<td>46.0</td>
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tion stocks of which were hybrids between the experimental populations of *D. willistoni* Nos. 168 and 169 (see Table 2).

As shown in Table 3, the frequencies of the heterozygotes for the inversion HIRE decreased at first in both populations; instead of progressing towards monomorphism, the heterozygotes became, however, more frequent again, and, in fact, reached frequencies higher than they had in the foundation stocks. The incidence of the heterozygotes for the inversion IIIJ also declined in both populations. In No. 179 a new equilibrium was established at a level lower than in the foundation stock; in No. 178 the frequency of heterozygotes diminished at first, but later recovered a part of this initial loss, and became stabilized. These populations apparently did not possess genetic materials from which monomorphic genetic systems conferring high adaptedness could be built.

**Conclusions and Summary.**—As a method of adaptation, balanced polymorphism is a costly one, since it entails production of some relatively unfit homozygotes. Balanced polymorphism is nevertheless widespread in natural populations of *Drosophila* and probably in other sexually reproducing organisms as well. There is every reason to think that the chromosomal balanced polymorphisms in *Drosophila* are stable. This is shown by the wide geographic distribution and the apparent antiquity of many natural polymorphisms, and also by their retention in experimental populations in which opportunities are offered for adaptive reconstruction of the genetic system. On the other hand, establishment of balanced polymorphism in a population is not an evolutionary blind alley, as shown by the emergence of secondary monomorphisms in some experimental populations.

From a long-range view, the evolutionary advantages of genetic systems based on balanced polymorphisms may lie, as suggested by Lerner and others, in their great adaptational plasticity and in the possession of the property of genetic homeostasis. It may also be that the fitness conferred upon their carriers by heterozygosis for balanced supergenes is not easily equalled in homozygotes. How important are balanced polymorphisms in the genetic systems of man and of other higher organisms with limited fecundity is an open question. Chromosomal inversions of the kind known in *Drosophila* are unlikely to be common in mammals, but other genetic mechanisms which form and maintain balanced supergenes may exist. The losses of the homozygotes of lower fitness are more difficult to put up with, but they can be reduced by development of numerous heterotic supergenes acting like multiple alleles of single loci.
THE OCCURRENCE OF PARTIALLY FERTILE TRIPLOID METAFEMALES IN DROSOPHILA MELANOGASTER*

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Bridges\(^1\) was the first to discover the existence of triploid females (3X3A) in Drosophila melanogaster and the occurrence of intersexes (2X3A), superfemales (3X2A), and supermales (1X3A) among their offspring. It was also in these triploid lines that Bridges\(^2\) discovered the occurrence of tetraploid females (4X4A). From his studies of these sex types Bridges concluded that sex determination in Drosophila was an example of genic balance, the X chromosomes carrying a preponderance of female determining factors, the autosomes carrying a preponderance of male determining factors. The sexuality of an individual could be expressed by the ratio between the number of X chromosomes and the number of sets of autosomes it possessed, an X:A ratio of 1.0 being the norm for femaleness, an X:A ratio of 0.5 being the norm for maleness. Values between these two figures produce intersexes, values above or below these two figures produce the supersexes.

The terms “superfemale” and “supermale” are actually inappropriate since the excess of genetic determinants for femaleness or maleness results developmentally in weak individuals with sterile, underdeveloped gonads. The prefix “meta” (be-