EFFECTS OF DISRUPTIVE SELECTION

II. POLYMORPHISM AND DIVERGENCE WITHOUT ISOLATION

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1. INTRODUCTION

EVIDENCE that supports the view that disruptive selection can promote genetic diversity within a population has been presented in a previous paper (Thoday, 1959). Disruptive selection, resulting from heterogeneity of environmental conditions, can therefore be regarded as a significant cause contributing to the genetic diversity that is such a striking property of natural populations of outbreeding species.

Mather (1955) has argued that disruptive selection, when the different forms selected are interdependent, may be expected to produce polymorphic populations, and the evidence already put forward helps to support this argument in so far as it is legitimate to regard polymorphism as but a special extreme form of this general genetic diversity. Mather also pointed out that only experimental study of disruptive selection can inform us of the relationship between selection differential and gene-flow, which must in part determine the quantitative importance of isolation in evolution.

One of the lines described in the previous paper (D^+) provides relevant information. Some of this information has already been published in a preliminary report (Thoday, 1958).

2. MAINTENANCE OF THE LINE

Details concerning the maintenance of this line under disruptive selection with positive assortative mating were given in the previous paper. It is only necessary to reiterate here that each generation was represented by four single-pair cultures, two of which were selected for high and two for low sternopleural chaeta-number (selection of one fly in 20). Each culture was assayed by counting 20 flies of each sex. The mating system was described in the previous paper in terms of the four component female sub-lines. It is recast in terms of the four male sub-lines in table 1 of this paper as this renders it more readily intelligible from the present point of view. There are two high male-lines and two low male-lines. Each selected high male is mated in each generation to a female selected for high chaeta-number but taken from a culture of a low male-line. Likewise a low male is mated to a low female from a culture of a high male-line.

There are therefore in each generation two high and two low cultures, but crossmating ensures that they are all parts of the same

population, which is subjected in every generation to selection for both extreme chaeta-numbers.

The population can also be considered in terms of its component high and low sub-populations. These are subjected to divergent directional selection but are not isolated in any way. On the contrary there is between them " forced gene-flow," for all flies are progeny of crosses between the two sub-populations. One-half of the autosomal genes and, as migration from one sub-population to the other is through females, two-thirds of the X-linked genes are exchanged in each generation. Only the differential segments of the Y chromosomes are isolated. The mean chaeta-numbers of the two sub-populations may therefore be used to test the efficacy of this type of disruptive

TABLE 1

Mating and selection system

Parents of Generation	Culture: *i.e.* male sub-line			
	1(H)	2(H)	3(L)	4(L)
	♀ ♂	♀ ♂	♀ ♂	♀ ♂
n	H3 × H1	H4 × H2	L1 × L3	L2 × L4
n+1	H4 × H1	H3 × H2	L2 × L3	L1 × L4
n+2	H3 × H1	H4 × H2	L1 × L3	L2 × L4
n+3	H4 × H1	H3 × H2	L2 × L3	L1 × L4

The entries designate the flies used to produce the culture in the generation shown in the first column. H indicates the highest and L the lowest chaeta-number fly found in the appropriate culture. 1, 2, 3, 4 indicate the culture from which the fly was taken.

selection, and to test whether maximal gene-flow can prevent divergent selection pressures from causing the two sub-populations to diverge.

As reported earlier, an error of selection was made in generation 21 when the whole population was selected for low chaeta-number.

3. RESULTS

(i) Divergence of mean chaeta-numbers

The differences between the mean chaeta-number of the two high cultures and that of the two low cultures are illustrated in fig. 1. The first generation of selection produced a positive divergence of mean, but in this generation, of course, there was no gene-flow. This generation is not really relevant to the experiment but sets a standard showing the responsiveness of the line to one generation of divergent directional selection. In the following generation the gene-flow between the two sub-populations was introduced and the difference of mean was reduced as a consequence. Thereafter it fluctuated widely, sometimes having negative values, until generation 9, after which there was a steady fall until generation 13. In this generation (13), however, some

change clearly occurred which permitted the population to maintain a consistently positive difference between its high and low components. This difference, though it fluctuated, was of the order of that produced by directional selection in generation 1. The difference was maintained until generation 21 when the selection for low chaeta-number in the whole population reduced it to zero. Further disruptive selection, however, rapidly restored the difference (at the expense of within-sex-and-culture variance which fell from 2·5 to 1·5 : see Thoday,

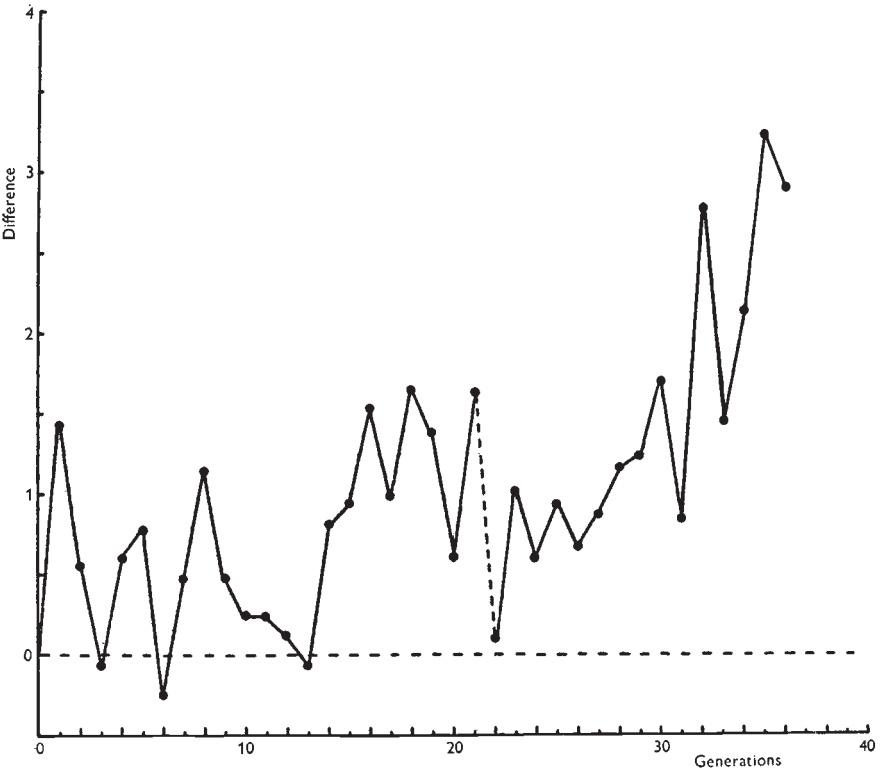


FIG. 1.—Differences, in chaetæ per fly, between the mean of the two high male-lines and the mean of the two low male-lines. The broken line indicates the error of selection at generation 21. (It should be noted that 9 must be added to the number of generations when this figure is compared with Figure 1 of Thoday, 1959).

1959). The difference has since increased (and the within-culture variance also : see Thoday, 1959), and is now at a level considerably higher than that attained in generation 1. The error in selection was unfortunate, but it at least serves to show that the population could recover from such an error.

(ii) The individual male-lines

Fig. 2 represents the deviations of each of the four male-lines from their joint mean and reveals information concerning the changes that have occurred.

It is clear from the figure that, until generation 14, there was no tendency for any one male-line to stand out consistently as High or Low. The highest male-line is more often H2 and the lowest male-line is more often L3, but no consistent behaviour of the different male-lines could be postulated. Since generation 13, however, L4 has been almost consistently the lowest of the four male-lines and H1 has been the highest except for a period, after the selection error in

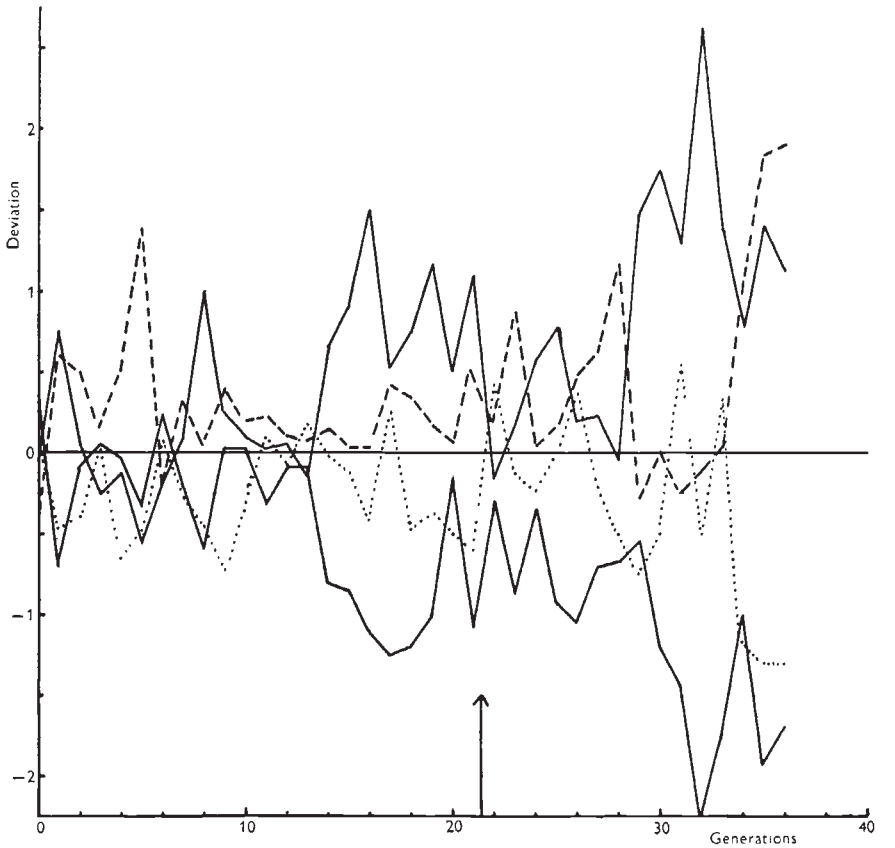


FIG. 2.—Deviations, in chaetæ per fly, of each male-line from their joint mean. Solid lines H1 and L4. Broken line H2. Dotted L3. The arrow indicates the selection error.

generation 21, during which H2 differentiated temporarily. H2 was consistently higher than L3 from generation 14 to 21 and from 23 to 28, but the order of difference between them was much less than that distinguishing H1 and L4, until generation 35 when H2 and L3 also differentiated strikingly.

The first significant event which led to the development of the consistent distinction of the high and low sub-populations therefore occurred at generation 13, in H1, or in L4, or in both. As it led to the immediate separation of both H1 and L4 from the other two male-lines, it clearly involved both the production of a high genetic factor in H1 and the production of a low genetic factor in L4.

The coincidental production of the two factors suggests that recombinations were involved, producing the two coupling products from a repulsion linkage of relevant genetic factors (*cf.* Mather, 1943). Thereafter (until the error was made in generation 21), these would be maintained as " effective factors " (Mather, 1949) in the two male-lines and could not break down in the absence of recombination in males. They could only with difficulty escape the male-line in which they originated, because selection of the migrating females would tend to eliminate them. In generation 21 the high factor would be lost from H1 when it was in error selected for low chaeta-number. Thereafter H1 did not really recover until in generation 28 a new event seems to have occurred, this time having effects of greater magnitude. After this second event H1 and L4 differed in mean chaeta-number considerably, but H2 and L3 had similar means. A third event seems to have occurred in generation 33 leading to the separation of H2 and L3. This third event will not be discussed here; it is too recent to be considered in the present analysis.

(iii) The differences distinguishing male-lines H1 and L4

General considerations would lead us to expect H1 and L4 to be distinguished by autosomal factors. It is conceivable that H1 and L4 might be distinguished solely by their Y chromosomes, but that this is not so is clear. Y chromosomes are usually confined to males and, if so, could only account for a distinction between the males of the subpopulations. But the observed differences that distinguish male-line H1 from male-line L4 occur in the females as well as the males. Females, of course, can carry Y chromosomes, but that the lines differ in supernumerary Y chromosomes seems unlikely. There has been no evidence, in crosses made with females from the lines, of sons with paternal X chromosomes such as occur when supernumerary chromosomes are present.

The cytoplasm is ruled out as a possible explanation by the design of the mating system and we must look to the other chromosomes for an explanation.

The X chromosomes can hardly be responsible. A brief consideration of the effect of the mating and selection system on a heterozygous X-linked locus shows that such heterozygosity could not maintain a consistent differential. Any X-linked allele or effective factor distinguishing a high (or low) male-line would be lost in one generation. X-linked effects would therefore have to be complex to explain the situation.

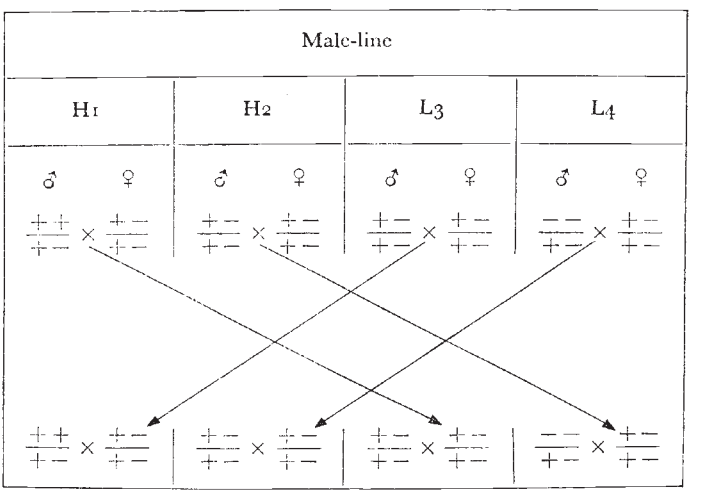
Autosomal factors could, on the other hand, readily provide an explanation. It has already been suggested that the high and low male-lines H1 and L4 were, until generation 21, distinguished by effective factors produced by recombinational events that converted repulsion complexes of genes into the corresponding coupling complexes. Such a system involving a pair of autosomes and assuming

only additive effects of the genes involved could well operate in the mating system by which the line is maintained as is indicated in table 2, which assumes homozygosity of the line except for the heterozygous " effective factors ".

This system could work even with the loosest possible linkage and with $--++$ as well as $++--$ intermediate chromosomes, for not only does lack of recombination in the male ensure its permanence, but also recombination, were it to occur, could not do more than reconstitute the existing chromosome types. It could therefore work

TABLE 2

Proposed simple model of the genetic system distinguishing male-lines H1 and L4. (Compare mating system in table 1)



equally well (and perhaps be set up more readily) in a population in which it was the males instead of the females who migrated from one side to the other. This system is formally equivalent to a three-allele polymorphism.

If such were the system operating in the population after generation 13, then of course the high " allele " will have been lost at generation 21. A new high allele seems to have been produced in male-line H1 in generation 28. If this were also an " effective factor ", produced by recombination, then it would not be likely to be " allelic " to its predecessor. If anything we might expect it to be in a different chromosome or chromosome arm.

The result would be a double test-cross system. H1 would be heterozygous for high and intermediate alleles at one " locus ", L4 would be heterozygous for low and intermediate alleles at another " locus ".

According to this view high chaeta-number flies taken from H1 should be heterozygous for a high factor and an intermediate factor, and low chaeta-number flies from L4 should be heterozygous for a

low factor and an intermediate factor, the high and low factors being non-allelic. Test crosses have been made to determine whether this is so.

(iv) Test-cross results

The test crosses were made in generation 33 using extreme high females from H1 and extreme low females from L4. These were mated to males, all with 18 chaetæ (9 on each side) selected from a slightly inbred stock homozygous for *y*, *bw* and *st*. Ten F1 males from the progeny of each of the four females were test-crossed to *y bw st* females, and the chaetæ were counted in 5 flies of each sex of each of the four classes of test-cross progeny, making 1600 flies in all.

The test crosses assess all three major chromosomes. Effects of II and III are tested by segregation of chaeta-number from the markers *bw* and *st*. Effects of the X (or supernumerary Y) chromosomes would be indicated by differences of sex difference, for the test-cross females contain an X from the tested male-line, whereas the test-cross males do not.

The test-cross data are summarised in fig. 3. Of the 20 L4 genomes tested, 11 were distinguished from the *y bw st* tester stock by a low chaeta-number effect attributable to chromosome II. Of the 20 H1 genomes tested, 4 were clearly distinguished from the *y bw st* stock by a high chaeta-number effect attributable to chromosome III. Two others, referred to as dubious in the figure, seemed as if they might belong to the same class, for they showed a smaller effect, also attributable to chromosome III. The remaining genomes are scattered round intermediate values for both chromosomes though, as must be expected, there are residual differences, attributable to both chromosomes, distinguishing the H1 and L4 genomes. No differences attributable to the X chromosomes emerged. A full analysis of variance of the *bw st* homozygotes obtained in the test cross gave no significant differences except the overall sex difference. Thus the differences illustrated in fig. 3 depend on the genomes extracted from H1 and L4 and not on variation of the autosomes from the *y bw st* standard against which the test was made. This analysis also shows that the sex chromosomes are not contributing to the difference between the H1 and L4 genomes tested.

The results of the test crosses show that half of the L4 genomes tested contained low chaeta-number second chromosomes. The L4 females tested were therefore heterozygous for a low and an intermediate chaeta-number factor on chromosome II.

The H1 females segregated high and intermediate third chromosomes, but there are fewer high segregants than the 10 expected, and it is not so clear that the H1 genomes fall into two distinct classes. Six of the derived chromosomes were therefore retested by test-crossing derived *y bw +/y bw st* males to *y bw st* standard females. Two of these derived chromosomes were of the high class, two were those of the dubious class, and two were of the intermediate class (see fig. 3).

Two cultures of each chromosome were obtained and 10 flies of each sex and genotype were counted in each culture (480 flies in all).

The results of this further test cross are summarised in table 3. The analysis of variance suggests that at least three classes of third chromosome were extracted from the H1 females, for each of the four degrees of freedom concerned with class means and with class × +/*st* interactions are associated with significant mean squares. Repre-

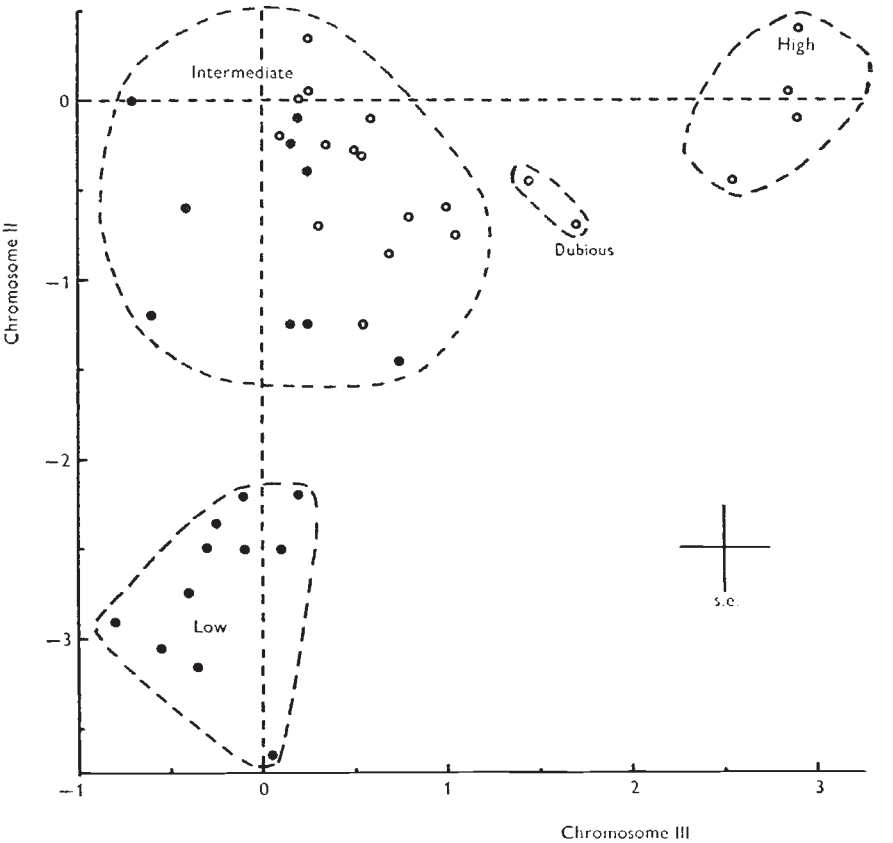


FIG. 3.—Deviations, in chaetæ per fly, from homozygous *y bw st* standards, produced by chromosomes extracted from extreme H1 and L4 females. Points L4 genomes. Circles H1 genomes. The mean standard error of a single entry is indicated.

sentatives of all three classes were obtained among the progeny of one of the two females originally extracted from H1. It thus seems that third chromosomes of the " dubious " class are recombination products in which the effective factor distinguishing H1 has partially broken down. This evidence supports the view that recombination rather than mutation is the key factor in the responses that occurred in the line, though it also indicates that the genetical model proposed on p. 210, is, as might be expected, too simple.

Despite the results for H1 being less clear-cut, the test crosses show unequivocally that L4 and H1 are heterozygous for genetic factors

giving the extreme chaeta-numbers, and allelic factors giving intermediate chaeta-numbers that can migrate from one part of the population to another without reducing the differences distinguishing the different male-lines. These factors, though they are probably linked

TABLE 3

Results of second test cross of H1 third chromosomes. (Entries are the differences in mean chaeta-number between +/st *and* st/st *flies. All flies homozygous* y *and* bw)

Chromosome tested	Class of Chromosome III		
	Intermediate (I)	Dubious (D)	High (H)
A	−0·1750	1·2250	2·4500
B	0·5250	1·1250	2·5250
Joint mean deviation	0·1750	1·1750	2·4875

Analysis of variance

Source	Mean Square	*n*
H/(D+I)	22·8750	1 †
D/I	11·8125	1 *
+/*st*	196·3521	1 ‡
Sex	46·2521	1 ‡
Sex × +/*st*	3·5021	1
HDI × sex	2·9646	2
H/(D+I) × +/*st*	88·1667	1 ‡
D/I × +/*st*	19·4374	1 †
HDI × +/*st* × sex	2·2145	2
Cultures and chromosomes	1·0521	9
Cultures and chromosomes × +/*st*	1·6354	9
Other interactions	2·5632	18
Individuals (error)	2·6891	432

* P<0·05 † P<0·01 ‡ P<0·001

complexes, are behaving as genes. The population is therefore polymorphic.

The present indications are that further response of the population is likely to occur. This may involve the further development of the existing effective factors by further recombination, the development of additional effective factors, or the selection of a background that enhances the effects of the existing loci, perhaps by dominance modification. The line is being maintained and it should be possible to determine which of these means is used. At present there are no indications that selection of modifiers has been involved in the development of the polymorphism, for none of the relevant interactions in the test-cross data are clearly significant.

4. DISCUSSION

The results of this experiment are clear-cut. The two sub-populations of the line, though there is 50 per cent. gene-flow between them, have diverged significantly as a result of the divergent selection pressures imposed upon them. At first they were unable to diverge consistently, but after a time they developed a genetic constitution permitting them to do so. Continued selection has increased the difference distinguishing them.

This divergence has been made possible by the development of a low chaeta-number second chromosome factor in the low sub-population with an allele of intermediate effect, and of a high chaeta- number third chromosome factor in the high sub-population also with an allele of intermediate effect. The two sub-populations are able to maintain

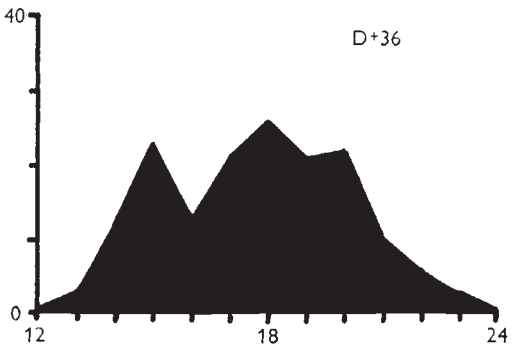


FIG. 4.—Distribution curve for the 160 flies assayed in generation 36.

a difference because the intermediate factors can migrate from one to the other without greatly affecting the difference of chaeta-number.

Whether the factors are genes in the more limited sense, or are relatively large chromosome regions (the evidence suggesting one of them can break down by recombination indicates the latter), they behave as genes in the system and are maintained at high frequencies by the selective pressures acting on the system. The population is therefore formally polymorphic even though, because these genes are influencing a continuously varying character, the population does not, as yet, show phenotypic discontinuity. Genetically the population is polymorphic. Even if it is not polymorphic by the strictest definition of Ford (1953), it is at least cryptomorphic in the sense of Huxley (1955). In fact the latest generation gives a bimodal distribution (fig. 4) which is significantly platykurtic, so that there is evidence suggesting phenotypic discontinuity.

It follows, therefore, that Mather (1955) was fully justified in his arguments that disruptive selection could in appropriate circumstances give rise to polymorphism. All that is required for the population to become strictly polymorphic is the selection of a background enhancing the effects of the " switch " genes.

It also follows that two component parts of a population can diverge despite lack of isolation barriers limiting gene exchange between them. The two pairs of cultures in each generation of this experimental line are, in a formal sense, in the same relative situations as would be the two parts of a population in a mosaic environment consisting of two distinct habitats arranged, for example, as a chequer-board. In such a situation a population could in principle develop two different forms, one adapted to each of the component environments even if there were forced (50 per cent.) gene-flow between the two forms (ecotypes). It is to be presumed that they could diverge more readily under random mating (25 per cent. gene-flow). They could do this by becoming polymorphic as the experimental population has done. It must be pointed out, however, that even if the individuals were mobile and therefore could choose to live in the environment to which they were adapted, the population would adapt to both environments at a cost. In the extreme situation envisaged a considerable proportion of the offspring would be intermediate and ill-adapted to both environments. Sessile forms would adapt in this way at greater cost as half the offspring would fall in the environment to which they were not adapted. However, the reproductive potentials of many species may leave a margin that would meet this cost, and habitats will often intergrade.

Biological "races" (*e.g.* Thorpe, 1956) are at the outset in situations quite similar to that under which the present population has diverged. The females remain and lay their eggs in the environment in which they were reared, having a pronounced preference for the species of host plant on which they have fed. By analogy with the present experimental population we must presume that, in these circumstances, two female lines on two host plants could diverge genetically in adapting to those host plants even if random mating occurred.

It is not suggested that this *is* the way in which genetic differences between biological races arise. It seems more likely that some measure of isolation would be involved, especially as there is a possibility that differing foods may of themselves cause isolation. Riley (1950) reared *Drosophila* on normal food and on food containing peppermint, and obtained some evidence that females not only laid eggs preferentially on the food on which they had been raised but also mated preferentially with males reared on the same food. If substantiated, this would involve an acquired isolation mechanism which could aid divergence of biological races.)

Nevertheless it *is* suggested that there is no *theoretical need* that there should be an isolation barrier before two such races may diverge genetically. Mayr (1942) clearly thinks there is such a theoretical need for he states that "it seems as if nothing would prevent the random mating by males, in other words, swamping. . . ."

The concept that, in the absence of isolating factors separating two components of a population, genetic differences between the two

components must be "swamped", derives from the concept of panmictic populations and from the mathematical consequences of panmixis. But that "interbreeding forms that are interfertile cannot diverge if they are able to breed together freely" (Carter, 1951), though it has become almost a text-book dogma, does not necessarily follow from the mathematics of panmixia, for we may have random mating of two kinds. Mating may be random with respect to the genotypes of zygotes, or it may merely be random with respect to the genotypes of adults. The first is panmixia but probably never occurs as natural selection will always ensure that the adults which mate are a non-random sample of zygotes. The second is not panmixia unless the adults represent a random sample of zygotes.

The statement that "interbreeding forms that are interfertile cannot diverge if they are able to breed together freely" is therefore not necessarily true, if, as it seems to do, it refers to adults. Neither would random mating by males necessarily lead to "swamping" as Mayr suggests. Selection operating between zygote and adult could ensure that random mating by males would not lead to swamping.

It is therefore in principle possible for biological "races" and ecological "races" to originate in the absence of isolating factors. If, thereafter, changed circumstances set a premium on further divergence of the forms or on more precise adaptation to the local environments (*cf.* Mather, 1943), selection for isolation could occur essentially as Dobzhansky (1941) has argued it will when two geographically isolated forms meet. This process would be sympatric speciation.

The same arguments raise the question whether Mayr (1954) is necessarily right in suggesting that the failure of peripheral populations of a species to adapt successfully to the local environment is explained "if we assume that this process of adaptation by selection is annually disrupted by the infiltration of alien genes and gene-combinations from the interior of the species range which prevents the selection of a stabilized gene-complex adapted to the conditions of the border region". There seems no reason in principle why a locally adapted population should not be formed even if one-way gene-flow into the local area were so great that all progeny were hybrids. The local individuals would need only to produce a dominant effective-factor conferring adaptation. A perpetual test-cross system would result and half their progeny by crossing with the migratory inflow would be adapted to the local environment. That it would be only half the progeny would set a premium on the subsequent development of isolation. Again the result could be speciation by a process in which divergence could precede isolation.

It may be objected that these arguments are not fully supported by the evidence put forward here, as, in the present population, the migrators are selected for characters appropriate to the sub-population *to* which they migrate. Though this may sometimes happen in nature, the reverse might happen more frequently. However, in nature the

maximum gene-flow will be that involved in random mating. Experiments now in hand indicate that two sub-populations under divergent selection pressures, with random mating permitted by migrators selected for characters appropriate to the sub-population *from* which they migrate, can also maintain genetic differences.

5. SUMMARY

1. Further results obtained with the population exposed to disruptive selection with positive assortative mating are described.

2. The population consisted of four male-lines, two selected for high sternopleural chaeta-number and two for low. Males of a high male-line were always mated to females from a low male-line selected for high chaeta-number. Males of a low male-line were mated to females from a high male-line selected for low chaeta-number. The population thus had a high and a low component, but the two were always crossed so that the gene-flow between them was maximal.

3. This gene-flow at first prevented the divergence of the two sub-populations, but after generation 13 they developed consistently and increasingly different chaeta-numbers.

4. Analysis of the data showed that for a long period only one high and one low male-line had diverged. The high male-line is distinguished by a factor in chromosome III and the low by a factor in chromosome II. The population is polymorphic. The factor in chromosome III can break down by recombination.

5. The line therefore demonstrates:

(i) that disruptive selection can produce polymorphism;

(ii) that two components of a population can diverge without any isolation limiting gene-flow between them.

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