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GENETIC ASSIMILATION OF THE *BITHORAX* PHENOTYPE

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INTRODUCTION

Some years ago it was suggested (Waddington, 1942) that if selection was practised for the readiness of a strain of organisms to respond to an environmental stimulus in a particular manner, genotypes might eventually be produced which would develop into the favoured phenotype even in the absence of the environmental stimulus. A character which had originally been an "acquired" one might then be said to have become genetically assimilated. An experimental investigation of the suggestion was carried out on *Drosophila melanogaster,* using a heat shock applied to the pupa as the environmental stimulus, and a crossveinless phenotype as the modification for which selection was practised. As has been described earlier (Waddington, 1953a), a genetic assimilation of the crossveinless phenotype was achieved in this case.

It seems possible that considerable general importance should be attached to processes of this kind, which appear able to provide a satisfactory explanation of the evolution of certain types of adaptation which have in the past been difficult to explain convincingly. It was therefore thought desirable to investigate the genetic assimilation of other characters, so as to broaden the observational basis on which the theory rests. Mrs. K. G. Bateman in this laboratory has studied a number of rather mild developmental modifications, produced by temperature shocks applied to the pupa. These can perhaps be considered as of the same general type as the crossveinless phenotype previously investigated. The present communication deals with experiments on a phenocopy of a rather different character. This is the bithorax-like modification which can be produced by ether treatment of the young embryo (Gloor, 1947). The phenotype involves a profound modification of the normal appearance of the animal. The meta-thoracic disc, which normally gives rise to the halteres, becomes changed so as to develop into structures resembling the normal mesothorax, including the wings. If such a change occurred during phylogenesis it would certainly be accounted a macro-evolutionary phenomenon. It was felt that, if such a fundamental modification as this can be genetically assimilated, then one would have some grounds for confidence that the process was powerful enough to be invoked to explain quite far-reaching evolutionary changes.

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Materials and Methods

The stock used was a mass bred Oregon *K* wild type. Eggs were collected over a one-hour period. At the age of 2½–3½ hours after laying they were given an ether vapour treatment by inverting the watch glass on which they were laid over an open dish of ether. The duration of the treatment was normally 25 minutes. The ether-treated eggs were allowed to hatch and develop into adults, all cultures being carried on at 25° C. On emergence, which was spread over a period of about two days, the flies were classified as wild type or bithorax phenocopies. In the up selection lines from the latter category sufficient males and females (virginity uncertain) were collected every 24 hours to produce the next generation of eggs. In most generations between 2,000–3,000 flies were classified, and to produce these several hundred parents were used in each generation. Parallel with the upward selection line in which the bithorax phenocopies were used as parents, a downward selection line was carried on, in which the parents were taken from the wild type flies. Two replicates were made of this two-way selection experiment, so that there were eventually four lines, i.e. Experiment 1, Upward Selection and Downward Selection, and Experiment 2, Upward Selection and Downward Selection.

Results

The two replicates were drawn from different initial stocks of Oregon R, and they started by showing rather different levels of phenocopy production. In Experiment I the first generation gave 24.5% of bithorax phenocopies, whereas Experiment II gave 48.8%. From these two initial points both the upward and the downward selections were effective. There

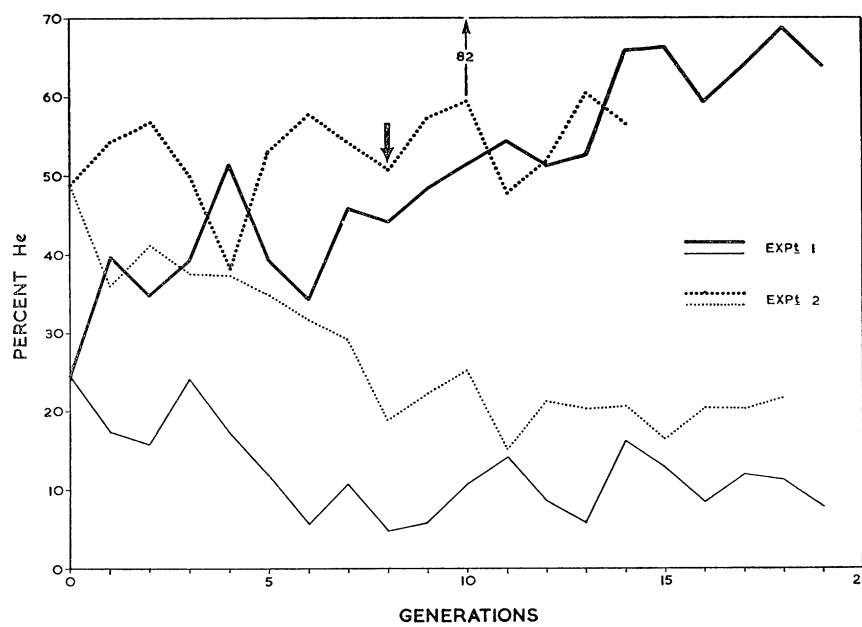

Fig. 1. Percentage of He (= bithorax-like) phenocopies in successive generations of upward and downward selection, following ether treatment of the eggs. The figures relate to flies which emerged from the puparia. At generation 10 in Experiment II, a count was made of all flies in a sample, including those failing to emerge and a percentage of 82 He found. The arrow at gen. 8 in Experiment II indicates the point at which the first assimilated stocks appeared.

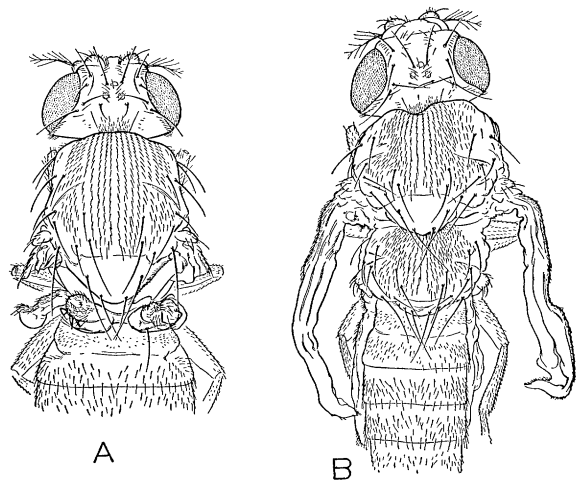

FIG. 2. Two typical phenocopies (medium and extreme in grade). Mesothoracic wings removed in 2a, metathoracic wings partly concealed by the abdomen in 2b.

is, however, not much point in quoting in detail the numbers of bithorax phenocopies which emerged in later generations, particularly in the upward selected lines. This is because a considerable proportion of high-grade phenocopies failed to emerge from the pupa cases. For instance, in generation 10 of Experiment II the proportion of phenocopies among the hatched individuals of the upward selection line was 59.5%, but from a sample of pupae in which the flies which failed to emerge were dissected out and examined, the proportion was about 82%. Normally such dissections were not done and the percentages of the phenocopies among the hatched adults, which are shown graphically in figure 1, give only a rough indication of the changes which the selection produced. It was also noted that in the upward selection lines the average grade of expression of the phenocopies rose, so that eventually a large number of very extreme bithorax types appeared (figure 2). It was in particular these most extreme types which had difficulty in emerging. The figures for the downward selection lines are probably more reliable, since in these, as the proportion of phenocopies was reduced, so also was the grade of expression, and there was a comparatively low incidence of failure of emergence. It will be noted that after many generations of downward selection the strains still produced a considerable and fluctuating number of phenocopies. A similar situation was found in the previous experiment with crossveinless (Waddington 1953a), where again selection did not succeed in rendering the stock completely resistant to the environmental stimulus.

The First "Assimilated" Stock

a. First set:

In each generation, besides the eggs which were collected over a timed interval for ether treatment, a number of others were collected and allowed to develop without treatment. In the early generations, as would be expected, no signs of bithorax-like phenotypes appeared, the flies being normal Oregon K wild types. However, in the eighth generation of the upward selection line of Experiment II, one fly from an untreated egg was found to show slightly enlarged halteres. In the next generation about 10 such flies were found among the untreated indi-

viduals. These were mated together in pairs and a number of "haltere-effect" or "He" lines were started. Each of these lines from the beginning threw a fairly large proportion of He flies, i.e. individuals with somewhat enlarged halteres. Selection was made amongst the lines to try to produce one in which the frequency of the haltere effect rose to 100%, or in which the phenotype was more extreme. In neither respect was success achieved. In the phenotype of an untreated individual it is very rare to find in these lines anything more extreme than the comparatively slight enlargement of the haltere illustrated in figure 3a, although in the first generation a few flies of slightly more extreme type appeared (fig. 3b).

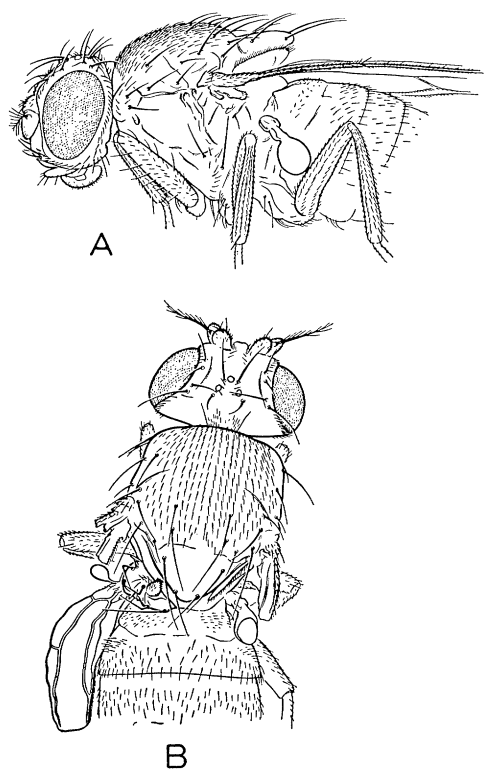

FIG. 3. Two individuals from the first set of "assimilated" stocks (He 17). Most flies of this stock show only the slightly enlarged halteres seen in 3a (cf. *Bxl*); a few have a bithoraxoid-like enlargement of the metathorax, as in 3b (from which the mesothoracic wings have been removed).

These were bithoraxoid-like; i.e. the metathorax represented the posterior part of the mesothorax (cf. Lewis, 1951). The frequency of the low grade phenotypes in one or two lines rose to 80% or slightly over, but never further.

Crosses with a *Cl*B;Cy:Me stock showed that the haltere effect is due to a gene or genes on the third chromosome. In fact, the genetic condition can be perfectly balanced against Me, when it behaves exactly as though one were dealing with a single dominant gene with recessive lethal effect. Crosses to stocks containing Bxl show that the He/Bxl compound is lethal. All this is consistent with the hypothesis that the He effect is due to an allele of Bxl. The only thing which causes one any hesitation in adopting this hypothesis is the fact that in some of the "best" He stocks, and in their crosses with wild types, the numbers of He flies are higher than would be expected if He/He flies died. Thus in the selected He stocks, instead of the He individuals making up two-thirds of the progeny, they usually formed from 75% up to 85%; and in crosses and backcrosses with Oregon K and other wild types there was a consistent though not very large excess of He progeny (table 1). It was at first suspected that there might either be some polygenic tendency in the He stocks towards the enlargement of the haltere, cooperating with the major He gene, or that the lethality of the He homozygote was not absolute. Fairly extensive tests failed to confirm either suggestion. When the wild type individuals of an He strain were bred together no He's were ever produced in their progeny; nor could any He individual be found which gave rise to 100% He offspring. It was concluded therefore that the He effect is, in fact, produced by a single dominant with recessive lethal effect, but that, particularly in the strains which had been selected for a high proportion of expression, the viability of the heterozygote is somewhat greater than that of normal wild types.

TABLE 1. *Crosses of first He stocks*

	Parents ♀	Parents ♂		He's of 1st set (He 5–He 16) He	Total	He %	He's of 2nd set (He 17) He	Total	He %
1.	He	He	Gen. 1.	2639	3436	76.8	551	753	73.4
			Gen. 2.	1713	2232	76.7			
			Gen. 3.	871	1143	76.2			
2.	OrK	He		778	1476	52.7	406	822	49.4
2a.	He/OrK	He		2114	3072	68.8	702	1070	65.6
2b.	He	He/OrK					412	602	69.0
3.	He	OrK		748	1287	58*	389	767	50.6
3a.	He/OrK	He		1453	2026	72*	494	743	66.3
3b.	He	He/OrK					308	484	63.6

* The wild types used in these crosses were not OrK, but were from the downward selected line of Experiment II.

b. The second set:

These facts show that, in this first group of stocks in which the bithorax phenotypes seem to be to some extent assimilated, the "assimilation" has not proceeded in the normal way by the selection of many minor genes acting in the required direction, but occurred by the fixation of a single major gene mutation which presumably arose *de novo* by chance. Further selection for phenocopy production was therefore continued. Experiment II was carried to the 14th generation; Experiment I was taken to generation 20, when the duration of treatment was reduced to 20 minutes till generation 26, after which it was reduced again to 15 minutes. No further sign of assimilation occurred until generation 29 in the upward selected line of Experiment I. At this time a few untreated flies were found which showed indications of a bithorax-like phenotype. In most of these the abnormal phenotype was again confined to a slight enlargement of the halteres. By breeding these together and selecting amongst their progeny another haltere-effect stock known as Hel7 was produced. This behaved genetically exactly like the previously obtained haltere-effect stocks, the phenotype being due to a dominant with recessive lethal effect, which is indistinguishable from that previously described.

The Second "Assimilated" Stock

Among the untreated bithorax-like flies of generation 29 in Experiment I, one or two showed not only enlarged halteres but also extra pieces of thorax-like material formed on the dorsum by the metathoracic bud. These were bred together, and selection made amongst their offspring for the development of extra thorax. A few generations of such selection produced a stock known as He*,

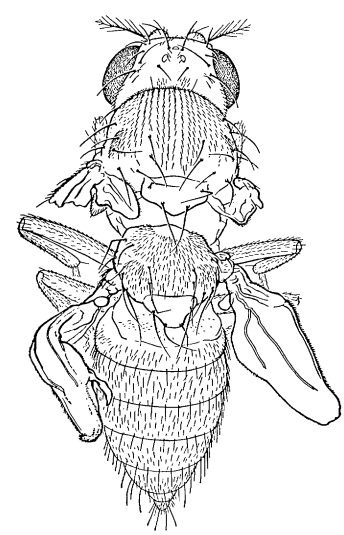

FIG. 4. An extreme, though fairly frequent, type from the second assimilated stock He*. Normal mesothoracic wings removed to show the bithorax-like metathorax.

which in mass matings gave a frequency of bithorax-like phenotypes of about 70–80%. These bithorax-like flies (fig. 4) frequently showed a large amount of extra thorax material as well as enlarged halteres. This extra material was of bithorax type, i.e. represented the anterior section of the mesothorax. The flies thus differed considerably in appearance from those of the previously described haltere-effect stocks. The difference persists even when eggs of the two stocks are given ether treatments and their phenotypic abnormalities thus exaggerated.

As a first step towards an analysis of the genetic situation in the He* stock a cross was made of an He* male to a ClB_1. Cy : Me female. No He* phenotypes appeared in the F_1. CyMe males and females from the F_1 were intercrossed and He* flies of rather weak grade were found in the F_2 in numbers which suggest that the character has a polygenic basis, the genes concerned being located both on the 2nd and 3rd chromosomes (table 2). There is no sign of any effect of recessive sexlinked factors.

This result falls in line with what one might expect if the assimilation were due to the accumulation of minor genes acting towards the bithorax phenotype. The results of crosses and back-crosses to wild types were, however, somewhat anomolous. The results of the first set of such crosses are given in table 3. It will be seen than when He* females were crossed with Oregon K males (of the stock used for Experiment II) there was a large percentage of He* individuals in the F_1,

TABLE 2. *ClB; Cy; Me* ♀ × *He** ♂ *gave no He** *phenotypes in F1.* $\frac{Cy;\ Me}{He^*}$ *mated* inter se *gave in F2:*

Offspring	He* ♀	He* ♂	non-He*	Total	He*%
+	31	23	106	160	33.7
Cy	24	31	179	234	23.5
Me	15	16	262	293	10.6
Cy; Me	15	8	506	529	4.3

TABLE 3. *He*: first set of crosses to OrK (1a, 1 bare back-crosses from 1; 2a, 2b from 2)*

	Parents ♀	Parents ♂	He*	Total	He*%
	He*	He*			70–80
	Non-He* ♀ and ♂ from He* stock				10–14
1.	He*	OrK	184	398	46
1a.	He*/OrK	He*	5	419	1.2
1b.	He*	He*/OrK	98	174	56
2.	OrK	He*	0	539	0
2a.	He*/OrK	He*	3	416	0.7
2b.	He*	He*/OrK	48	276	17.4

whereas in the reciprocal cross no He* individuals appeared. The sexes were approximately equally represented in the He* F_1 individuals, from the He* female by Oregon K male cross, so that one cannot attribute the difference between the reciprocal crosses to a direct effect of recessive sex-linked factors. One must rather suppose that there is some influence of the mother, either by way of a maternal effect or possibly of a cytoplasmic nature. The series of crosses are, however, somewhat inconsistent with one another, in that many fewer He* flies were produced by the back-cross of a heterozygous He* male derived from an Oregon K mother on to an He* female (see line 2b) than had been produced when a pure wild type male had been used on an He* female (see line 1, F_1). The explanation of this was presumably to be found in the fact that there was considerable heterogeneity between the different cultures, which was taken to indicate that mass-mated He* stock was far from uniform in genotype.

An attempt was therefore made to purify the He* stock by carrying it for several generations in pair matings, amongst which those which gave the highest percentage of He* phenotypes were selected. After some generations of this procedure, a new series of matings were set up, both between the various phenotypes in the He* stock, and between the He* stock and an Oregon K wild

TABLE 4. *He*: second set of crosses to OrK*

	Parents' genotype		Parents' phenotype		Groups of pairs	Progeny		
	♀	♂	♀	♂		He*	Total	He*%
1.	He*	He*	He*	He*	10	264	387	68
					2	16	84	19
2.	He*	He*	+	He*	8	362	854	80
					1	11	85	13
3.	He*	He*	He*	+	12	429	658	63
4.	He*	He*	+	+	6	225	358	63
5.	He*	+	He*	+	—	90	592	15
5a.	He*	He*/+ ex 5	He*	He*	7	143	254	56
					1	3	29	10
5b.	He*	He*/+ ex 5	He*	+	7	91	247	37
					1	9	88	10
5c.	He*/+ ex 5	He*	He*	He*	—	7	653	1
5d.	He*/+ ex 5	He*	+	He*	—	3	358	1
6.	ex 5c	ex 5c	+	He*	3	117	187	63
					2	31	179	17
					4	14	225	6
7.	ex 5c	ex 5c	+	+	6	193	346	56
					6	9	517	2
8.	+	He*	+	He*	—	0	674	0
8a.	He*	+/He* ex 8	He*	+	—	298	610	49
8b.	+/He* ex 8	He*	+	He*	—	17	859	5
9.	ex 8b	ex 8b	+	He*	5	156	255	61
					4	8	311	2
10.	ex 8b	ex 8b	+	+	2	55	104	53
					9	14	300	3

type. As will be seen from table 4, lines 1–4, there is still some heterogeneity within the He* stock, but this had been very considerably reduced. Matings between individuals of this stock which had wild type phenotypes produced nearly the same number of He* offspring as did matings between individuals of He* phenotype, but a few pairs gave considerably fewer He* offspring than did the majority of matings. Thus it appeared that the stock had become more uniform for a genetic constitution which had produced He* phenotypes in a proportion of between 60% and 70%.

The results of crosses with wild type and back-crosses carried out at this stage are also given in table 4. It will be seen that once again the reciprocal crosses with the wild type give different results; those with a wild type mother giving no He* phenotypes in the F_1 (line 8), whereas in the opposite cross such phenotypes do appear (line 5), though in a considerably lower proportion than was found in the earlier series of crosses with the less purified strains. In the back-crosses of the F_1 males to He* females, the numbers of He* phenotypes appearing are now in reasonable agreement with one another (lines 5a, 5b, 8a). The most interesting result appears from the back-crosses of the F_1 females to He* males and the subsequent generations. The back-crosses themselves gave rise to very few He* phenotypes (lines 5c, 5d, 8b). However, when the female offspring of these back-crosses (wild type in phenotype) were themselves crossed to their brothers (either to wild type or He* in phenotype), the families seemed to fall into two distinct groups (lines 6, 7, 9,

TABLE 5. *Hatchability in unselected wild type and selected lines of Experiment I (generation c. 35), following 25 mins. ether vapour at 2½ hrs.*

Stock	Eggs treated	Hatched	Hatched %
Or.K.	400	270	67.5
Expt. I, down selected	500	408	81.6
Expt. I, up selected	500	418	83.6

10). One group gave about 55% to 60% of He* offspring, while another group gave a low percentage of the order of 2–5%. This result can be simply explained if we suppose that an important factor in the production of the He* phenotype in these stocks and crosses is a recessive gene which has no obvious effect on males but which causes females homozygous for it to produce eggs with a strong tendency to develop into He* phenotypes. This maternal effect gene must be supposed to co-operate with a polygenic tendency towards He,* dependent on minor genes scattered throughout the second and third chromosomes.

Other Effects of the Assimilation Procedure

The ether treatment necessary to produce the bithorax phenocopy causes a certain reduction in the percentage hatch of the eggs subjected to it. This was particularly the case at the beginning of the experiment. As shown in table 5, hatchability improved in both the upward and downward selected lines. Since in both cases the selection involved breeding from survivors, this improvement is not unexpected.

It is interesting to inquire whether selection for a high rate of phenocopy production by a certain environmental stimulus produces a specific sensitivity in the selected strain, or whether one finishes with a stock which is highly sensitive against any type of abnormal environment. This question has not been studied in detail, but the He* lines were given the hot shock treatment of the pupae which had previously been used to produce the crossveinless phenocopy, and the assimilated crossveinless strain, together with the wild Edinburgh stock from which it had been derived, were given the He treatment. The results are shown in table 6. It is clear that all the He lines had become rather more sensitive to the temperature shock to the pupae than was the wild type from which they originated. There is no evidence that the lines selected for low sensitivity to ether treatment are more resistant to the hot shock than the lines selected for high susceptibility: in fact the low line of Experiment I is the most sensitive of the group. The *cvl* line, selected for high susceptibility to hot shock, had reacted in the opposite way, and became considerably more resistant to the ether treatment than the Wild Edinburgh from which it originated. It seems then that the selections, which had certainly produced specific sensitivities or resistances to particular environmental stimuli, had not regularly resulted in corresponding changes in general developmental buffering.

TABLE 6. He *lines given* cvl *treatment (4 hrs. at 40° C.). Percentage of* cvl *phenocopies produced*

Stock	Time treatment applied after pupation: 19–21 hrs.	21–23 hrs.
He Expt. I		
Up selected	2.3	11.8
Down selected	9.3	24.3
He Expt. II		
Up selected	2.6	13.7
Down selected	6.9	12.9
Or.K.		8.1

Lines from *cvl* expt. given *He* treatment. Percentage of *He* phenocopies produced.

cvl assimilated line	6.0
Wild Edinburgh	21.1

Discussion

Selection for the ability to respond to ether treatment of the eggs by the production of bithorax-like phenocopies has on three at first sight separate occasions resulted in the appearance of stocks which exhibit this phenotype even in the absence of any special environmental stimulus. There were, firstly, the *He* stocks which arose in generation 8 of Experiment II, giving a *Bxl*-like adult; secondly, the similar stock (He 17) which arose in generation 29 of Experiment I; and thirdly, the *He** stock which appeared at the same time in Experiment I, but which gave a more *bx*-like phenotype, which was inherited in a different manner.

Such genetic assimilation of the abnormal phenotype might be due, in the first place, to the occurrence of a single new mutation which produces the character in question, and this mutation might be either spontaneous, or in some way caused by the treatment; or, in the second place, to the assembling together, by the selection pressure exerted, of already-present minor allelic differences tending in the appropriate direction; or finally, to a combination of these two mechanisms. The evidence strongly suggests that it is to the occurrence of new mutations that one must attribute the appearance of both the first group of *He* stocks and the second such stock, *He* 17. In both cases, the phenotype is dependent on a single gene, which produces its effect even in normal wild-type backgrounds, and there is no evidence that the accumulation of minor gene differences has played any significant role. Moreover, the genes are dominant in phenotypic expression, and cannot have been present in the initial population from which selection was begun.

Since the first lot of He stocks originated at quite a different time to *He* 17, and in a different selection line, the question arises whether we are dealing with two independent mutations; and if so, whether the occurrence of two apparently identical mutations in the populations involved would suggest that the treatment had caused them. As a matter of fact, since the selected stocks were basically wild-type in character, there is no absolute safeguard against the possibility that *He* 17 was a contaminant from the earlier *He* stocks which were of course present in the laboratory at the time it was found. However, concurrent experimental work has been little troubled by contamination, and there is no specific reason to suspect it in this case. If one adopts the alternative possibility, that two independent mutations to a *Bxl*-like allele have occurred, it is of interest to inquire whether the numbers of animals involved makes it all reasonable to postulate such a double event without supposing that the mutations were induced by the treatment. Unfortunately the records which were kept do not make it possible to give a completely accurate figure for the numbers. We are only concerned with the upward selected lines, since if such a mutation occurred in the downward lines, it would have been classified as a phenocopy and excluded from breeding. In Experiment II, rather over 17,000 flies had been treated and examined before the first *He's* were found; and to these must be added a fairly considerable, though unrecorded, number of untreated sibs which were examined (and amongst which the *He's* occurred). One can take it that at least some 20–25,000 flies had been inspected by the time the *He* mutation was found. Selection was then carried on for a further six generations in Experiment II, involving 14,000 flies examined. In Experiment I, some 87,000 flies had been inspected before *He* 17 was found in generation 29. If one makes allowance for examined untreated flies, one can conclude that a total of the order of 150,000 individuals were inspected in the whole upward selected lines of both experiments. The occurrence of two similar mutations amongst this number would give a mutation rate for the locus rather nearer 10^{-5} than 10^{-4}. Such a spon-

taneous mutation rate, though undoubtedly high, does not seem to be so excessive so that one is driven to postulate a direct inducing action by the environmental treatment.

In the other stock which shows assimilation, He*, the genetic changes are more far-reaching in their effects. The abnormal individuals produced by this stock are much nearer to the phenocopy, showing all grades of bithorax-like appearance up to an extreme type with a large second pair of wings and a considerable extra mesothorax. The assimilation has therefore been rather thorough. Its genetic basis certainly does not depend on the chance occurrence of only a single mutation producing this effect; the variation in the proportion of abnormal phenotypes in the various crosses clearly demonstrates that the character is affected by a polygenic system.

Since the phenocopy is induced by an environmental stimulus on the early egg, the effectiveness of which may well be supposed to depend on characteristics of the egg cytoplasm, it is perhaps not unexpected to find that an important part in the genetic constitution of the assimilated stock is played by a maternal effect. The full genetic analysis of this effect is still in progress and will be reported on later. The present data show that the genetic determinant or determinants of it are recessive. The relatively clean-cut segregation of the daughters of the back-cross of an F_1 female to an He* male into two classes (table 4, lines 9 and 10) suggests that the genetic basis of the character lies on one chromosome. It may indeed be a single gene, but it might be two or more fairly closely linked genes. If it is single, the question will arise whether it could have existed in the initial population before selection started. In crosses of He* females to wild-type males the maternal effect, operating on eggs which are heterozygous for the rest of the He* genotype, produces 15% of bithorax-like offspring. It is not yet known what effect, if any, the maternal effect genes can produce in a genotype which lacks any of the rest of the He* complex. If even in these circumstances it produces some bithorax-like phenotypes, one would have to conclude that the condition had not been present in the initial population but had arisen by mutation or recombination during the course of the selection. As has been pointed out, the number of individuals runs into six figures, so that an origin by mutation would not be anything out of the way.

Whatever the origin of the maternal effect gene or genes turns out to be, there can be no doubt that the selection has brought together and concentrated a considerable number of minor alleles tending in the bithorax direction. Many if not all of these were presumably present in the original population. The attempt to assimilate the bithorax character was originally undertaken with the idea that from the evolutionary point of view it was a very bizarre aberration and would provide a good test of the powers of the mechanism of assimilation. The fact that the assimilation has been successfully carried out in a number of generations, which although long in the laboratory are very short in the time scale of nature, suggests that the mechanism can in fact be an extremely powerful one. It seems likely that any modification produced by the environment could, if it were favourable to the animal, be genetically assimilated in a relatively short time.

It is instructive to compare the process of genetic assimilation as it has occurred in the He stocks with the "organic selection" of Baldwin (1902) and Lloyd Morgan (1900). They argued that if an animal subjected to any environmental stimulus is able to respond to it in an adaptive manner, the animal and the population of which it is a member will be able to continue existing in the region where the environmental stimulus operates, until such time as a chance mutation produces a phenotypic effect which mimics the adaptive response. Now this is what seems to have happened in the first two

sets of He assimilated stocks, in which a dominant allele of Bxl has appeared and produces a phenotype which has at least the lowest grade of the phenocopy appearance. The development of the full assimilation of the character in the He* stock can not, however, be accounted for in these terms. In the first place, one of the components of the genetic system, namely the maternal effect, is recessive. If we are to account for its appearance by some process other than selection for the capacity to respond to the environmental stimulus, we would have to take account not only of the low frequency with which such a mutation might be expected, but of the small probability that it would have emerged in homozygous state in these experiments, in which the inbreeding was very low. Moreover, there is no doubt that the maternal effect does increase the readiness with which the eggs respond to the ether treatment by developing bithorax phenocopies. It must surely be to this fact that its selection and concentration must be attributed, and the same applies to the other components of the He* genotype. We must be dealing with an "assimilation," produced by selection of capacity for response, rather than an "organic selection" of a chance mutation mimicing the response.

In a recent discussion by Underwood (1954) of the relevance of experiments on genetic assimilation to evolution as it occurs in Nature, some misunderstanding seems to have arisen as to what is actually being claimed for this process. It is quite true, as Underwood points out, that animals in Nature are rarely subjected to such extreme environmental stimuli as the temperature shocks used in the experiments on crossveinless phenocopies, while the ether treatment used here is even less "natural." Again it may be conceded that neither of these environmental treatments did, or could in the nature of the case, produce phenotypic changes which were adaptive. All this, however, does not in any way make it impossible that such experiments should reveal a general mechanism, relating genotypes to selective forces acting on environmentally-influenced phenotypes, which may be of general significance, and which may operate in an essentially similar manner when the environmental stimuli are milder in character and their phenotypic effects adaptive.

Another point raised by Underwood is the consideration that if a genotype can respond adaptively to the environment it normally meets, genetic assimilation of the adaptive phenotypic characters will be unnecessary. On *a priori* grounds, the argument may seem plausible; but the fact is that most adaptive features of animals are genetically fixed, and do not alter much when development takes place in somewhat unusual conditions. It is not entirely clear why this should be so; possibly it is because it is difficult to build up a genotype which develops with the right degree of adaptive modification to the whole range of environments it has to meet, and that it "pays better" to sacrifice something of the flexibility of the developmental system and to assimilate genetically the adaptation to the most usual environment.

Underwood suggests that "we should distinguish as *primary* (genetically) *fixed adaptations* those which we believe to have arisen as such, and as *secondary fixed adaptations* those which have arisen by fixation of a facultative adaptation." This implies that the environmental modification of development is quite irrelevant to the origin of the primary fixed adaptations; and the same view is implicit in Underwood's argument "if it be necessary in the end to invoke a change of genotype to establish the suitable embryonic inductor (of callosities in the ostrich) then it may surely be invoked in the first place." Now it is certainly very usual at the present time to discuss the evolution of new characters without ever referring to the effects of environment on development, or at best to bring this in at a late stage in the discussion, as a secondary factor in the way suggested by Under-

wood. But in my opinion such an approach is theoretically inadequate. All phenotypes are modified, to a greater or lesser extent, by the environment. All genotypes under natural conditions will be subject to selection pressures relating to the manner in which their development is modified by the environment. The phenotypic effect of any new gene mutation must therefore be to some extent influenced by the kind of developmental flexibility which has been built into the rest of the genotype by selection for its response to the environment. The effect of such specific developmental flexibilities may sometimes be rather slight (as it seems to have been in the first set of assimilated He stocks), or considerably greater, (as it was in respect of the maternal effect factor in the He* stock); but it must always be there. The distinction between primary and secondary fixed adaptations is therefore not a real one, as Underwood has drawn it.

There is not space here for a full discussion of all the implications of the views advanced above. It is sufficient to point out that the experiments on genetic assimilation have been designed to maximise the influence of selectively-determined developmental instabilities, since this was the aspect in the total situation to which attention was being particularly directed. The fact that they have succeeded in producing a change of macro-evolutionary magnitude in the short space of 30 generations of selection suggests that such instabilities may on occasion be of considerable importance.

Summary

1. Two replicate experiments were made, in which treatment by ether vapour was given to the eggs of Drosophila melanogaster 2½–3½ hours after laying, and selection practised either for the tendency to produce bithorax-like phenocopies ("upward selection"), or against it ("downward selection"). The two foundation stocks were both Oregon-K wild types, but had been bred separately for some considerable time.

2. Selection was effective in both directions, but its progress was not followed in detail owing to the death of many extreme phenocopies in the puparia.

3. In the 8th and 9th generations of the Up selected line of Experiment II, flies which showed a slight enlargement of the halteres appeared among the untreated individuals. These gave rise to a series of stocks, which were found to contain a dominant allele of *Bxl* with recessive lethal effect.

4. In the 29th generation of the Up selected line of Experiment I, similar flies were again found among untreated individuals. These gave rise to a stock (He 17), which contained a factor which was indistinguishable from that mentioned in para. 3. It might, indeed, have arisen through contamination by flies carrying that gene; but the numbers involved in the experiment (c. 150,000) make it conceivable that both occurrences of the gene were due to independent chance mutations.

5. In the 29th generation of the Up selected line of Experiment I there also occurred, among untreated individuals, some showing the metathorax partially converted into a mesothorax. These gave rise to a stock He*, in which the bithorax phenotype appears in high frequency and extreme grade. The genetic basis of the character is partly a recessive gene which causes females to produce eggs developing into bithorax phenotypes (a maternal effect), and partly a number of minor genes on both the second and third chromosomes.

6. Selection for sensitivity to one type of environmental stimulus does not necessarily produce susceptibility to stimuli of a different type.

7. The fact that such a bizarre phenotype as bithorax can be assimilated, with high grade expression, in less than 30 generations, suggests that the genetic assimilation mechanism is a very powerful

one, which could have far-reaching effects during evolution.

Literature Cited

Baldwin, J. M. 1902. Development and Evolution. New York and London.

Gloor, H. 1947. Phänokopie Versuche mit Aether an Drosophila. Rev. Suisse Zool., **54**: 637–712.

Lewis, E. B. 1951. Pseudo-allelism and gene evolution. Cold Spring Harbor Symp. Quant. Biol., **16**: 159–174.

Lloyd Morgan, C. 1900. Animal Behaviour. London.

Underwood, G. 1954. Categories of adaptation. Evolution, **8**: 365–377.

Waddington, C. H. 1942. Canalisation of development and the inheritance of acquired characters. Nature, **150**: 563–565.

——. 1953a. Genetic assimilation of an acquired character. Evolution, **7**: 118–126.

——. 1953b. The "Baldwin Effect," "Genetic Assimilation," and "Homeostasis." Evolution, **7**: 386–387.