Selection for developmental canalisation

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It was shown some years ago (Waddington, 1960) that in stocks of *Drosophila melanogaster* containing the mutant Bar the effect of temperature on eye size is under genetic control. In normal laboratory stocks the eyes are larger at 18°C. than at 25°C., but it was possible by selection to produce a line in which the difference at these two temperatures had been reduced to a small fraction of what it is in the unselected founder stock. However, noteworthy success in improving the canalization of Bar against the effect of temperature was only achieved by the somewhat artificial method of family selection, which must also have involved a considerable amount of inbreeding. It was therefore decided to carry out another experiment in which selection would be applied both to improve and to reduce canalization, using other breeding procedures. The experiments also give some information about the effects of submitting a population to two different selection processes simultaneously ('disruptive selection').

1. PLAN OF THE EXPERIMENTS

A base population was set up as follows: From a laboratory culture of Bar ten new bottles were set up by merely shaking over flies from the stock bottle, these parents being removed after a few days. When the new generation emerged, from each of these ten bottles six small-eyed males were taken and mated with six large-eyed females and separately six large-eyed males were mated with six small-eyed females. After allowing 24 hours for mating the two groups originating from each bottle were placed together and allowed to lay. This gave rise to ten stocks, each of which should have contained a large fraction of the total genetic variation available in the initial population. The later selection was carried out in the same manner in each of these ten replicate stocks.

To ascertain the eye size in the foundation generation of the stocks, 400 larvae were collected from each stock; 200 of these were allowed to develop at 25°C. while the other 200 were kept for 48 hours at 25°C. and then transferred to 18°C. until they had completed their development. From the flies emerging from these two temperature treatments a random sample of twenty males and twenty females were used

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for facet counts. The flies were digested in 10% NaOH, the corneas then removed, stained in 1% pyrogallic acid, and squashed under a coverslip, tearing if necessary to ensure flattening, and the facet numbers counted under a compound microscope. The facets on both eyes were counted, but only the means of both sides will be quoted here since no additional information emerges from consideration of the sides separately.

From each of the ten replicate founder stocks, two selected lines were set up: a 'canalizing' and an 'anti-canalizing' line. Canalizing lines were set up as follows. From the part of the stock treated with low temperature (which should enlarge the eye) the five males and five females with the smallest eyes were selected from a sample of 100. This selection was not based on precise facet counts but on the judgement of eye size, flies being inspected with a binocular microscope at a magnification of $15 \times$. From the part of the stock cultured throughout at 25°C., the five males and five females with the largest eyes in a sample of 100 individuals were selected. These ten males and ten females were put together and allowed to mate at random. From the eggs produced by this mating, two batches, each of 150 larvae, were again subjected to the two temperature treatments, and when the adults emerged the selection procedure was repeated.

The selection procedure for anti-canalization was essentially similar except that individuals with the largest eyes were selected from the low-temperature treatment and those with the smallest eyes selected from the high-temperature treatment. In all these lines selection was practised on the right eye only.

Precise counts of facet numbers were made at the 4th and 9th generations of selection. Throughout the work, the 18°C. temperature fluctuated within a range of ± 1°C. During most of the selection the 25°C. temperature fluctuated within a range of ± 2°C. but in the 4th and 9th generations the flies to be used for facet counts after 25°C. treatment were grown in an incubator in which the temperature was controlled to less than half a degree.

With this breeding procedure it will be apparent that the canalization and anti-canalization lines were kept separate from the beginning of the experiment with no gene flow between them. However, within each of these lines there was no attempt to control the mating between the parts of the stock which had been cultivated at high and at low temperatures, the selected flies drawn from these two treatments being mixed and allowed to mate at random.

Within each of these two lines, some individuals were selected for large eyes, others for small eyes. This amounts to the practice of 'disruptive selection'. The form this has taken in the present experiments is rather different from the way disruptive selection has been carried out in other experiments on the subject, such as those of Thoday and his collaborators (1959–64), since in the present work selection for two optima was performed on a population which was also subjected to controlled environmental variations which affect the phenotypes under selection. We will return to this point in the discussion; in the meantime it may be pointed out that selection of a population for two different optima would be expected, on the face of it, to lead to an increase in phenotypic variance.

2. RESULTS

The effectiveness of selection for and against canalization

Table 1 shows the mean numbers of facets in the eyes of flies in the foundation population and in the canalization and anti-canalization lines at generations 4 and 9, under the two temperature treatments, 18°C. and 25°C. We should expect the canalizing selection to reduce the difference in facet number between the two temperature treatments and anti-canalizing selection to increase it. It is clear that these

Table 1. *Mean number of facets per eye (right eyes only, 20 eyes counted per replicate, averages of 10 replicates)*

	18°C.	25°C	Range 18–25°C.	Difference in range	t.
Males					
Foundation stock	163·9	98·5	65·4		
F_4					
Anti-canalization lines	185·8	97·4	88·4 ⎫	29·3	5·45**
Canalization lines	175·7	116·6	59·1 ⎭		
F_9					
Anti-canalization lines	164·1	88·7	75·4 ⎫	20.6	2·62*
Canalization lines	197·4	142·6	54·8 ⎭		
Females					
Foundation Stock	137·8	83·1	54·7		
F_4					
Anti-canalization lines	142·4	87·1	55·8 ⎫	11·4	2·75*
Canalization lines	145·3	100·9	44·4 ⎭		
F_9					
Anti-canalization lines	133·8	71·9	61·9 ⎫	21·4	3·88**
Canalization lines	157·5	117·0	40·5 ⎭		

* Significant at $P = 0·05$
** ,, ,, $P = 0.01$

expectations are, in general, fulfilled, although in males of the anti-canalizing line the increase does not progress steadily, but is larger in F_4 than in F_9.

In the canalization line selection was for small eyes at 18°C. and for large eyes at 25°C. The results show that the size of the eye at 25°C. has actually increased, but it also increased, though to a lesser extent at 18°C. Thus what has occurred is a general improvement throughout the whole population of one of the two favoured phenotypes.

In the anti-canalization line the results were more complex. Selection was for increased size at 18°C. and in the first four generations this was effective. However, between generations 4 and 9, the response was reversed and at the end of the experiment the eye size was very slightly less than it had been in the foundation stocks at 18°C. The selection for small eyes at 25°C. had, in the meantime, made slow

progress with a slight fluctuation upwards at generation 4 in the females. Thus in the early generations there was an improvement in the adaptation at 18°C. with little overall change (averaging males and females) at 25°C., but at the end of the experiment there had been a slight improvement in the 'adaptation' to 25°C. accompanied by a slight deterioration of it for 18°C.

The effect of the disruptive selection on the phenotypic variance of the various lines was investigated by calculating the coefficients of variation of the various populations. The data are given in Table 2. The main body of the table gives the

Table 2. *Coefficients of variation (In each group, the upper figures under F_4 and F_9 apply to the canalization line, the lower two figures to the anti-canalization line.)*

| | | 18°C. | | | | | | 25°C. | | | | |
| | | ♂ | | | ♀ | | | ♂ | | | ♀ | |
Family	P	F_4	F_9	P	F_4	F_9	P	F_4	F_9	P	F_4	F_9
1	18·5	11·6	10·7	14·9	17·5	10·8	14·9	17·7	18·1	12·7	14·0	14·9
		18·4	14·7		20·6	15·9		21·7	33·3		19·6	25·5
2	12·0	21·3	13·4	19·5	20·7	15·6	14·3	26·6	18·7	11·9	22·9	15·7
		13·6	14·8		20·1	15·7		18·5	23·6		27·1	23·5
3	6·7	16·1	12·8	11·7	13·9	12·0	11·1	17·7	13·1	14·0	18·9	12·6
		20·4	16·6		19·8	22·2		14·3	19·3		27·8	32·3
4	14·3	13·7	38·6	10·2	20·9	45·4	18·2	14·6	17·6	19·7	26·3	42·1
		21·7	20·1		29·8	22·9		37·7	19·1		23·6	23·5
5	13·3	12·4	16·1	16·3	18·1	21·9	16·0	15·4	29·9	16·4	20·0	22·1
		16·0	21·6		21·4	18·6		31·0	25·3		25·1	14·2
6	11·0	15·3	17·0	10·0	23·4	15·4	15·4	17·5	15·9	11·2	20·3	19·1
		11·4	30·9		20·6	28·9		16·9	38·6		19·5	34·9
7	14·2	15·2	26·3	15·4	28·3	24·7	14·5	19·6	23·3	18·4	15·7	21·3
		17·5	26·6		22·1	28·9		21·6	28·2		16·3	25·6
8	17·7	20·8	27·0	18·1	20·1	13·4	22·8	24·6	22·0	11·1	21·6	14·5
		14·4	54·0		47·7	37·9		35·9	48·2		42·6	50·2
9	13·2	18·5	13·6	17·9	15·0	14·5	21·7	17·1	15·7	18·7	18·7	22·0
		19·1	17·9		28·7	12·5		30·9	22·9		26·9	23·3
10	13·5	20·6	13·2	15·6	22·7	16·9	14·3	12·7	18·7	14·8	18·1	13·7
		13·2	36·6		23·4	33·1		35·9	29·2		31·7	25·3
Means	13·0	16·2	16·3	14·4	19·5	17·4	15·8	17·9	20·3	14·4	19·4	18·6
		17·3	23·4		24·4	22·3		23·2	27·3		25·1	26·5
S.D.	1·33	1·24	1·47	1·27	1·26	1·52	1·26	1·25	1·37	1·26	1·20	1·43
Factors		1·30	1·53		1·32	1·44		1·40	1·34		1·31	1·39

coefficients of variation within the measured individuals (twenty males and twenty females) in the ten families at the original or parental generation (P) and at F_4 and F_9 for both the canalization and anti-canalization lines. The lower two horizontal panels of the table give the means of these coefficients, and 'standard deviation factors' suitable for assessing the significance of differences between the means. These two items have been calculated as follows:

'*Means.*' The standard error of a coefficient of variation V based on N readings is

approximately $V/(2N)^{1/2}$. That is to say, the standard error is proportional to the coefficient. A simple arithmetical average of the coefficients would therefore be biased and misleading. A transformation to $\log V$ will however approximately stabilize the standard errors for cases such as this, where N remains constant. The 'means' given have therefore been calculated by taking the antilog of the mean of the logs of the coefficients for the ten families in each line.

'*Standard deviation factors*.' The standard deviations of the logs of the coefficients was calculated, and then converted to the antilog. The confidence limits of the 'means' can be found by multiplying and dividing the 'mean' by the appropriate multiple of the 'standard deviation factors' given in the table.

It will be seen from the upper part of the table that in all cases the mean coefficients of variation in the selected lines (of both kinds) had increased over that in the foundation stocks. The increases were considerably greater in the anti-canalization line than in the other. In both lines most of the increase had occurred already at generation 4, but, as the S.D. Factors show, there is between generations 4 and 9 a continued increase in dispersion of the means. This is brought about mainly by a further increase in coefficient of variability in a few families, e.g. the canalization line No. 4, the anti-canalization No. 6 and 8.

DISCUSSION

The results of the main experiment on canalization confirm those of the previous work; the sensitivity of the Bar stock to temperature differences is under genetic control and selection for greater sensitivity (against canalization) or lesser sensitivity (for canalization) is quite successful. This is so also in conditions which involve random mating and individual selection, as they did here, instead of the family selection which was practised in the previous work.

The selection for increased canalization not only reduced the sensitivity of the line to temperature differences but also brought about a general increase in facet number. This is the character which was selected for in the high temperature and against in the low temperature. Selection of this kind must be expected not only to increase the frequency of genes which reduce temperature sensitivity but also to bring about changes in the frequency of genes which tend either to increase or decrease eye size at all temperatures. The relative importance of these various effects in any particular course of selection will depend on the distribution of the various kinds of genetic variation available in the base population. The results of these experiments on canalizing selection lead to the conclusion that the founder population in this case contained a good deal of easily utilizable genetic variation tending to increase eye size at all temperatures. In the anti-canalization experiments, in which there was selection for increased eye size at low temperature and for reduced size at high temperature, there was an increase in eye size in the first four generations for males and females at low temperature and for females at high temperature, while the males at high temperature declined very slightly in facet number. These changes were probably produced by utilizing the same genetic variation as played such a large

part in the canalization experiments. In the later generations of the anti-canalization line, the effect was reversed and there was some decline in eye size throughout the whole population.

In both the canalization and anti-canalization lines, in fact, the final result at the end of the experiment was an improvement in the character selected for at 25°C., whether this was increased size (canalization line) or decreased size (anti-canalization line). This presumably indicates either that heritabilities of eye sizes in general are higher at 25°C. than at 18°C.; or, perhaps more plausibly, that rearing at the higher temperatures gives the flies some reproductive advantage in a panmictic population, perhaps in readiness of males to mate, or in the numbers of eggs laid by females.

Before discussing the results of the 'disruptive selection' that operated in the two lines, it will be as well to consider in general terms the importance of this type of selection. As Thoday has often pointed out (e.g. 1964) laboratory experiments on disruptive selection owe their main interest to the light they may throw on the conditions under which sympatric species divergence may occur in nature. In a natural population, disruptive selection would occur if the habitat, which will usually be to some extent heterogeneous, contains two or more different environmental niches which differ from one another in selection optima. Individuals inhabiting one niche will then be selected by a criterion which is not the same as that applying to individuals in the other niche. The evolutionary consequences of these disruptive selection pressures will be affected by a number of other factors. In the first place, the rate of flow of genes between the sub-populations in the two niches will depend on the degree to which successive generations of a sub-population return to the same niche as that inhabited by their parents, that is to say, on the development of continuing habitat-preferences within the sub-populations. Again, the gene flow will be affected if there is any development of mating preferences, which lead members of a sub-population to mate with each other more frequently than with members of the other sub-population. A final factor, with which the present experiments are particularly concerned, is the possibility that the niches exert differential effects on the developing phenotypes of a kind which is relevant to the selection optima. Since the niches with which we are concerned are different enough to exert different selection pressures, and since most organisms notoriously show some capacity for phenotypic adaptation, this last factor would be expected to be of importance in most natural situations.

The largest body of experimental work on disruptive selection is that which has been carried out for some years by Thoday and his collaborators (1959–64). The plan of the earlier experiments corresponded to a natural situation in which habitat-preferences were more or less strongly developed. Separate lines were kept (e.g. a 'high' line and a 'low' line for bristle number) with different selection pressures exerted on them, and various controlled degrees of gene flow allowed between them. In these circumstances, it was usually found that the lines diverged. This would seem not unexpected, although Thoday expresses some surprise at the result. It is, however, obvious that divergence would occur if there was no gene flow between the

lines; whether it occurs at any given rate of gene exchange will depend on the values of various genetic parameters in the population, such as the heritabilities of the characters, selection intensities, dominance and epistatic effects etc. The basic theory of the situation does not appear to have been worked out, nor have these parameters been estimated. However, it is not clear that such situations can have much relevance to the possibility of sympatric divergence in nature, since they depend on the existence of some previously developed system of habitat-preferences, without which the whole population could not be regarded as separated into two sub-populations between which there is gene exchange. The relevance of laboratory experiments on disruptive selection to situations in nature has also been discussed by Maynard Smith (1962) with particular reference to the stability or instability of any polymorphism produced.

In a recent experiment, which has been rather briefly reported, Thoday & Gibson (1962) (Thoday, 1964 *a, b*) have dispensed with anything corresponding to habitat preferences. In each generation, flies selected either for high or for low bristle number were all placed together and allowed to mate panmictically; and from their progeny, selection was again made for high or low bristle number, with no reference to the character of the parents. This experimental set-up is comparable, in its use of panmixis and of purely individual selection unrelated to the parental characters, to that of the present experiments; and both experiments would seem to provide a good model of a natural population faced with two environmental niches in relation to which no habitat-preferences have been developed.

The results of the two sets of experiments have, however, been somewhat different. In the work reported here there has been rather slight increase in the phenotypic variance exhibited by populations from the canalization lines when kept in a constant environment, while in the anti-canalization lines the increase was only about twice as great, Moreover, the selection has not led to a simultaneous increase in adaptedness to the two selection criteria applied. In the canalization line, the population as a whole became better adapted to one of the niches, i.e. the 25°C. environment in which selection operated for large eyes, and worse adapted to the other. In the anti-canalization line, the overall result was again a slight increase in adaptedness to 25°C., in which, in this case, selection was towards smaller eyes.

Thoday and Gibson's experiments, on the other hand, led to a great increase in phenotypic variance, brought about by progress in both directions towards the two selection optima of high bristle number or low bristle number. The difference between the results of the two experiments could, perhaps, find its explanation in differences in the rate of response to selection in the two directions in the early generations. In each generation, there will be a panmictic population of parents, equal numbers of which have been selected for criterion A and for criterion B. Matings will occur of types AA, AB and BB. The first of these will be expected to give offspring which approach more nearly the selection criterion A, and the last more offspring fulfilling criterion B. As soon as any noticeable progress is made towards either selection-optimum, it will clearly be reinforced in later generations, especially if the selection pressure is high enough to ensure that nearly all the

individuals selected for criterion A in any generation come from parents who were also selected for A. Thoday and Gibson's selection pressure was fairly severe; in each generation they selected eight high females from among a total of eighty females, of which forty were the progeny of eight parental high females which had had an equal chance of mating with high or low males. Even with completely random mating, one would expect four of these parental females to have mated with high males, so that the chances that the selected high offspring would come from a HH mating was very considerable. In so far as this occurred—and supposing a similar situation to hold for the selected low individuals—the two selection lines would in effect be quite distinct from one another even though an opportunity for cross-mating had been provided. Their divergence would then be no more unexpected than that between any other upward and downward selected lines in a two-way selection experiment.

But this mechanism could only operate in so far as the previous selection had led to important progress. The lack of divergence produced in our Bar experiments is probably due to low response to selection in the early generations, and to the fact that such response as did occur seems to have been asymmetrically in one direction, towards large eyes.

Thoday also claims that in his experiments a considerable degree of mating preference was developed, so that both high and low flies tended to mate predominantly with their like. Clearly any such tendency would increase the rapidity of divergence. It is surprising, however, that the experiments involved sufficient selection pressure in this direction to bring about the very striking effects Thoday claims. Other attempts to select directly for mating preferences (Wallace, 1954; Koopman, 1950; Knight, Robertson & Waddington, 1956) have made much slower progress. It is most desirable that Thoday's results, both on the achievement of rapid divergence, and particularly on the development of mating-preferences, should be repeated, with special attention to the strict virginity of the flies used, which is obviously a matter of crucial importance. In experiments on a similar plan, with selection for high or low expression of cubitus-interruptus-Dominant, Scharloo (1964) found that disruptive selection led to a considerable increase in phenotypic variance, but no divergence into two phenotypic groups, and he found no evidence of the development of mating-preferences.

The most interesting fact about disruptive selection which has emerged from the experiments reported here is the greater increase in phenotypic variance in the anti-canalization as compared with the canalization lines. In the canalization lines, selection has been exerted against the effects of the two different environments. It would therefore be expected that the partition of the phenotypic variance into genotypic and environmental components would be shifted in favour of the former, while in the anti-canalization line an opposite result would be anticipated. The phenotypic variances in constant (or nearly constant) environments which were estimated at the end of the experiment will be expressions of the genetic variation for large and small eye-size available in the population, the buffering of individual development against minor environmental fluctuations, and the buffering against

genetic variance, which may be expected to be closely comparable to that against environmental factors. Since in the canalization line, the environments were acting to suppress the differences in eye size which were selected, while in the anti-canalization line they were acting to produce them, it might, on the simplest assumptions, be expected that the canalization line would finish up with more genetic variation for eye size than the other. However, it would also be expected to be—and in fact has been found to be—better buffered against environmental variations, and therefore probably against genetic variation also. The actual result which has been described above seems to show that the latter effects have in practice proved more important than the former in determining phenotypic variance. It seems doubtful whether there is any theoretical reason why this should always, or even usually, be the case. A similar experiment with a different foundation stock might have led to the opposite result. In fact a comparison of canalization line No. 4 with anti-canalization line No. 9 in Table 2 provides an example of behaviour exactly the opposite of that shown by the means of all the lines.

There is, therefore, no major generalization to be drawn from the results of the disruptive selections; but these experiments are, perhaps, of significance in demonstrating that the theory of the process is considerably more subtle and complex than current treatments admit.

SUMMARY

Starting from a genetically variable stock homo- or hemi-zygous for Bar two selection lines were set up, one selected for decreased sensitivity to the effect of larval temperature on eye size ('canalization line'), the other for increased sensitivity ('anti-canalization line'). In each generation a sample of larvae was grown at 25°C. throughout life and another sample at 25°C. for the first 48 hours, followed by 18°C. until emergence. In the canalization lines a selection was made of individuals (five males and five females out of 100) least affected by the temperature treatment and the anti-canalization line for a similar number most affected by the treatment. These ten males and females were allowed to mate at random and from the eggs produced random samples were then treated in the next generation in a similar manner in the two temperatures. Precise counts of facet numbers on the right eyes were made at generations 4 and 9 and it was clear that selection had been effective both in decreasing and increasing temperature sensitivity.

The whole canalization line can be regarded as a population which has been subjected to disruptive selection for two different criteria: one (small eyes) being regarded as adaptive to the low-temperature regine or habitat; the other (large eyes) being regarded as adaptive to the high-temperature habitat. A similar type of analysis can be applied to the anti-canalization line in which, however, the selective values of the phenotype are regarded as reversed in value in the two habitats. The experimental procedure employed involved not only random mating between individuals selected according to these two criteria but also random allocation of the offspring of this panmictic population to the two habitats of the next generation.

This corresponds to a natural population in which there is disruptive selection exerted by two different habitats but no habitat preferences exhibited by members of the population. Under these circumstances, in our experiments, disruptive selection produced only rather slight increases in phenotypic variance, which were rather larger in the anti-canalization than in the canalization lines. Possible reasons for the difference between this result and those reported by Thoday and Gibson are discussed, and it is suggested that an important reason may be the slowness of the response to selection in the first few generations in our lines.

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