SELECTION FOR CANALIZATION OF THE SCUTE PHENOTYPE IN *DROSOPHILA MELANOGASTER*

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Summary

Selection for low variance of scutellar bristle number in scute flies resulted in canalization about a mean of two bristles. Selection for high variance appeared ineffective. The sensitivity of high selection lines to changes in temperature at which the flies were reared was much greater than the sensitivity of the low selection lines and the sensitivity of wild-type cultures.

I. INTRODUCTION

Waddington (1957) has argued the importance of canalization of developmental pathways. Canalization describes the tendency of a developmental process to hold to its normal course in the face of both genetic and environmental forces tending to deflect it into other channels. The existence of canalization in the development of the scutellar bristles of *Drosophila melanogaster* has been demonstrated by Rendel (1959*a*, 1959*b*). The presence of the sc^{sc} gene segregating in a set of selection lines reduced the number of bristles to about one in scute males and two in scute females. With the reduction in mean phenotype considerable variation appeared between scute flies. This disappeared again when, by selection, the phenotype of the sc^{sc} genotypes approached four. At the same time variation appeared in sc^{+} genotypes segregating in the selection lines, when flies with five and six scutellar bristles started to appear as a result of selection for increased bristle number in their sc^{sc} sibs. It was suggested that the developmental path was canalized at the level of four bristles; if development could be forced out of this path, either up or down, it became more variable. On the other hand, when forced back into a course leading to four as the mean number, variability disappeared again. This variability was at least in part genetic as it responded to selection. So far no account of the production of canalization has been published; it is of interest to show how rapidly canalization can be brought about by appropriate selection.

Falconer and Robertson (1956), by breeding from mice whose weights deviated most from the litter mean and mating heavy to light mice, failed to show that there were any genes controlling sensitivity to the environment. But they did show that when the mean weight started to rise at the eighth generation so did the actual variance. They discount this effect by using the coefficient of variation, which is justified by the correlation of variance with the mean, although, in fact, at low levels of mean this correlation disappears and is reversed. In the line in which they selected for intermediates there are signs of reduction of variance and the possibility that canalization has begun cannot be ruled out. On the assumption that mouse weight is to some extent canalized, one would expect a rise in mean to result in a rise

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in variance, because it takes the phenotype away from the canalized zone; but one would expect any mating system which selected against deviation from the mean to result in a lowered variance, not only because of increased homozygosis but because canalization would be strengthened.

Falconer (1957) also selected for intermediate number of abdominal bristles in *D. melanogaster* without any marked changes occurring in the phenotypic variance or its components. In addition he quoted Harrison (1954, *Drosophila Inf. Serv.* **28**: 122–3) as having obtained no change in variance between segments within flies after many generations of selection for both high and low difference between the fourth and fifth abdominal segments. Thoday (1959), who selected for extremes in sternopleural bristle number by disruptive selection, has found changes in variance accompanying changes of mean from the initial value.

In the following description an account is given of a selection experiment in which the character "scutellar bristle number" in *D. melanogaster* was successfully canalized at the level of two bristles. As selection proceeded, the line developed reduced phenotypic variance at 25°C and lowered sensitivity to extreme temperature changes. Attempts to increase variability in a parallel selection line and properties of the scute character in a random-bred Oregon laboratory stock are also described.

II. Experimental Procedure

The selection lines were derived from a stock homozygous for scute and the blood allele of white. Two lots of 10 single-pair cultures were set up as foundation material. One lot was designated the high line (HL) and in this line the selection procedure was designed to increase the variance of scutellar bristle number. The second lot of 10 cultures was designated the low line (LL) and the procedure for this line was designed to reduce the variance of scutellar bristle number. It was unfortunate that the progeny of the two lines in the first generation had a different variance, but this was in the right direction, so that unconscious selection of the original parents started LL off with a somewhat lower variance than HL.

As the object of the experiment was to see if selection could make a particular phenotype insensitive to both genotypic and environmental influences, the mean bristle number was kept at approximately two bristles. As males have a lower number than females this was not possible by any rigid system of selection. When the number of bristles seemed to be getting too low, males with two bristles were chosen as male parents; when the number got too high, males with one bristle were chosen as male parents. Females with two bristles were used throughout. As we were not primarily selecting for mean expression but for high or low variability of expression about a mean, matings were assessed by calculating the variance of their progeny. The HL parents for the next generation were taken from cultures with the highest variance and the LL parents from cultures with lowest variance. Any females with two bristles and any males with one or two, as the case might be, were selected from the chosen cultures, each of which was always the product of a single mating.

Three pairs of flies were taken from each of the four cultures in HL and LL with the highest and lowest variance respectively. These sets of matings became

lines A, B, C, and D with cultures 1, 2, and 3 in each. Matings in pairs were always made between A♀♀ and D♂♂, B♀♀ and A♂♂, C♀♀ and B♂♂, and D♀♀ and C♂♂. The most extreme culture out of three in each of the sublines A, B, C, and D was chosen on variance each generation. This paper covers the first 28 generations of selection.

From time to time a culture in a subline would fail, leaving only two or even one to be scored. This later became rather commoner in HL than in LL; to avoid losing the opportunity of selecting on variance due to such failures, five cultures

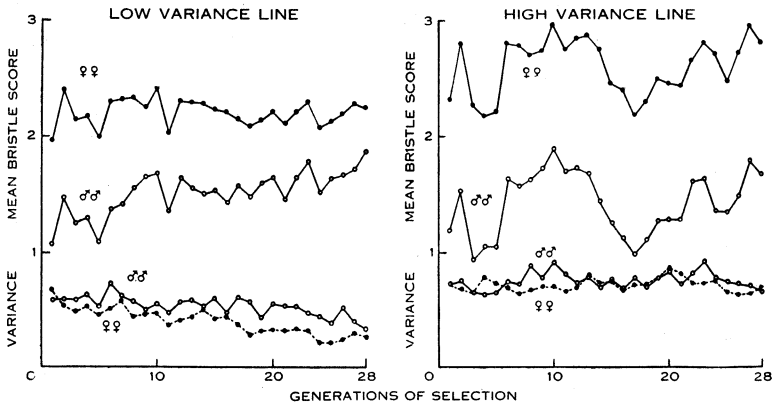

Fig. 1.—Means and variances of the selection lines plotted against generation of selection.

were set up of which the best three were scored. Even so HL sometimes failed to produce three fertile cultures in each subline. There has thus been some selection for fitness over and above selection for variance and this selection has, because of the weakness of HL in this direction, been more severe in HL than in LL. Since parents of intermediate phenotype have always been selected, there should have been a tendency towards homozygosis of additive genotypes making for intermediate bristle number.

After 24 generations HL and LL were compared at different temperatures. Fertile females were allowed to lay eggs for about 12 hr in standard bottles. They were then removed and the bottles put in incubators at 15, 20, and 30°C. As all the selection was carried out at 25°C, the results of the selection lines were taken as typical of this temperature. As flies lay very reluctantly at 15°C the egg laying was all done at 25°C and the temperature treatments lasted from about 12 hr after laying to occlusion. Some wild-type flies were treated in the same way for comparison. They came from an Oregon RC stock which is held in this Laboratory as a wild-type stock.

III. Results

Figure 1 shows how mean bristle number has changed during the 28 generations of selection. In LL the number has fluctuated about a mean of 2·20 bristles in females; these fluctuations have, if anything, steadied down towards the end of the

experiment. The mean in males has risen steadily from *c.* 1·20 to *c.* 1·80. It is remarkable how much the phenotypic difference between males and females has been reduced during selection. The two are not yet identical as they are in the presence of the sc^+ gene but whatever it is that causes the sex difference appears to have far less effect after selection for low variance. The high variance line started with a higher mean; this rose to 1·9 in males and nearly 3 in females at the tenth generation, then fell to 1·0 and 2·2, and rose again to 1·7 and 2·8. There is no sign of any decrease in the sex difference. The within-bottle variances of each generation are plotted in Figure 1 below the means. In LL there is a steady fall in variance to about half the original value in both sexes. Males are more variable

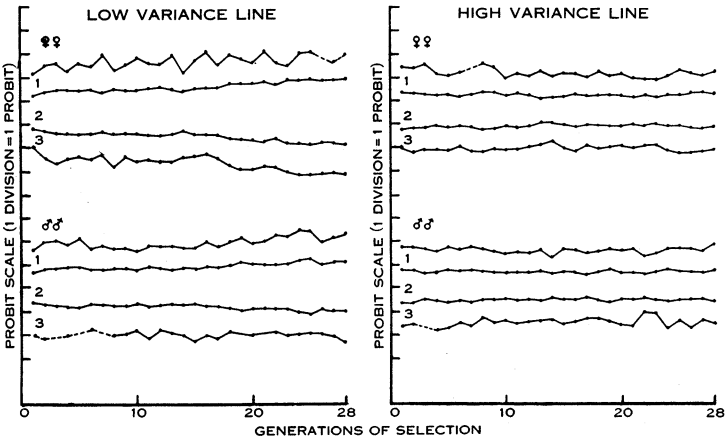

Fig. 2.—Probit distances spanned by the 1-, 2-, and 3-bristle classes in the selection lines plotted against generation of selection.

than females as a rule and there is no indication of variance being proportional to mean. In HL there is no clear indication of any effect of selection. Males tend to be more variable when the mean increases and females when the mean decreases. These tendencies are probably not very significant but are to be expected. As the mean decreases, a high proportion of males come to have 0 bristles and variance tends to drop as there is no class scored below 0; if the mean were reduced till all males had 0 bristles the variance would be 0. As the mean rises a high proportion of females have 4 bristles and, as none have 5, a drop in variance can be expected. The slight difference in mean between LL and HL may in part account for the differences in variance between the lines at the beginning of the experiment.

The effect of selection on the sensitivity of a particular bristle class to influences tending to alter bristle number can best be seen by estimating the distance subtended by the class in standard deviations. The standard deviation is of a distribution, which is described only in part by the bristle scores in most cultures. For if the mean of the distribution falls near the 0 or 4 class large portions of it will lie beyond these thresholds, and there will be no information about such portions since the 0 and 4 class boundaries are the most extreme crossed by either selection line. The method

TABLE 1

ANALYSIS OF VARIANCE OF SELECTION LINES AT BEGINNING, MIDDLE, AND END OF THE EXPERIMENT

Period	Source of Variation	High Line				Low Line			
		Females		Males		Females		Males	
		D. F.	M. S.	D. F.	M. S.	D. F.	M. S.	D. F.	M. S.
Generations 1, 2, 3	Generations	2	33·3	2	37·4	2	14·8	2	13·8
	Cultures within generations	28	3·8	28	3·4	29	2·9	30	2·6
	Within cultures	1386	0·67	1464	0·69	1143	0·50	1217	0·59
Generations 13, 14, 15	Generations	2	21·0	2	20·1	2	1·4	2	0·6
	Cultures within generations	30	6·9	30	9·8	31	3·2	30	9·0
	Within cultures	1382	0·78	1353	0·77	1576	0·45	1594	0·56
Generations 26, 27, 28	Generations	2	7·4	2	12·0	2	1·2	2	6·5
	Cultures within generations	32	3·8	32	7·4	32	0·6	33	1·8
	Within cultures	1523	0·65	1473	0·72	1708	0·28	1743	0·43

and justification of the analysis are dealt with in greater detail by Rendel (1959*b*). In brief, the distance of the 0/1, 1/2, 2/3, 3/4 cut-off points from the mean of the distribution to which the population measured belongs can be found by calculating the percentage of flies with 0; 0 and 1; 0, 1, and 2; 0, 1, 2, and 3 bristles, and looking for the corresponding probits in Fisher and Yates (1953) tables. By subtraction, the distance occupied by a bristle class in standard deviations can be estimated. Although the distance occupied by the 1-, 2-, and 3-bristle classes can be estimated, that of the 0 and 4 classes cannot as they are incomplete classes, there being no −1 or +5 classes in the selection lines. In the Oregon + stock, however, 3-, 5-, and 6-bristle classes do appear and estimates of the 4 classes can be made.

TABLE 2

MEAN SCUTELLAR BRISTLE NUMBER IN THE HIGH AND LOW VARIANCE LINES AT FOUR TEMPERATURES

Test at generation 24

Temperature (°C)	High Line		Low Line	
	Females	Males	Females	Males
30	0·75	0·06	1·75	1·12
25	2·71	1·35	2·08	1·52
20	3·16	2·25	2·36	1·89
15	3·74	2·92	2·38	2·12

Figure 2 shows how the distances have changed in the two lines as selection proceeded. The scores of all cultures in a line were pooled each generation before calculating the probit distances subtended by the bristle classes. The difference between culture means, though highly significant, was never very large and it was felt it would not affect the probit scores. The analyses of variance of generations 1, 2, and 3; 13, 14, and 15; and 26, 27, and 28 are shown in Table 1. In generation 26 the probit values were calculated from the mean of the probits of each culture as well as from pooled scores to check the effect of pooling. There was no appreciable difference. It was felt that more might be gained by pooling the data and using large numbers than averaging the probits based on the rather small numbers of each culture and that the time taken in calculating each culture separately was not warranted. In LL the sum of the distances spanned by the 1, 2, and 3 classes has increased steadily; the increase is due almost entirely to the increase in width of the 2 class. In HL there is little regular change. There is a tendency for the total distance spanned to decline in the middle generations. Towards the end of the run the distance increases again. The probit analysis is more informative than the analysis of changes in variance because it is not affected by the extent to which

the distribution tends to approach the 0 or 4 bristle threshold. We expect, therefore, that any effect of selection for high variance will show more clearly on the graph in Figure 2 than in Figure 1. The effect has not been marked and may have been counteracted by selection for fitness. If there is any advantage in a regular development of the scute character, selection for high variance will result in loss of fitness. HL has been far more difficult to keep going than LL. Despite the fact that a subline is represented by five and later six replicates, blanks occur through poor cultures.

TABLE 3

PROBIT DISTANCES SPANNED BY THE DIFFERENT BRISTLE CLASSES IN BOTH SELECTION LINES AT FOUR TEMPERATURES

Selection Line	Bristle Class	Temperature (°C)			
		30	25	20	15
High line					
Females	1	0·90	0·81	0·75	—*
	2	1·42	1·36	1·38	1·01
	3	—*	1·02	0·88	0·73
Males	1	0·87	0·93	0·81	1·10
	2	—*	1·38	1·08	1·21
	3	—*	1·09	1·04	0·88
Low line					
Females	1	0·90	1·16	0·66	—*
	2	2·90	2·71	1·99	2·05
	3	0·41	1·32	1·09	1·24
Males	1	0·87	1·29	0·82	—*
	2	—*	2·24	1·59	2·40
	3	—*	0·92	1·10	1·00

*Indicates incomplete classes.

Table 2 shows the effect of rearing HL and LL at different temperatures. At high temperatures both lines have fewer bristles than they do at lower temperatures but this is far more marked in HL than in LL. In LL there is a difference of about 0·7 bristles in females and 1·0 in males between the mean bristle number at 15 and 30°C, in HL the difference is about 3·0 bristles in both females and males. Obviously selection for invariability has been effective in stabilizing development in the face of a changed environment as well as in a relatively constant environment. An examination of the probit distances occupied by 1-, 2-, and 3-bristle classes at the different temperatures shows that there may be a tendency for canalization to be reduced at lower temperatures. The distances are given in Table 3.

The effect of temperature on Oregon + flies has been investigated and is given in Table 4. Two effects were noticed here. The first is the effect of high temperature

on bristle number which differs considerably from the effect found in scute flies. Extra bristles of the kind found in the earlier selection lines by Rendel (1959*a*) are large, usually at least half the size of normal ones, and usually near a normal bristle site though sometimes between normal sites. The mean number of such extra bristles is increased slightly rather than decreased at 30°C in the + stock. The second effect is apparently associated with crowding of cultures at 30°C, which results in very small extra bristles always situated between the posterior scutellars.

TABLE 4

FREQUENCY DISTRIBUTIONS FOR SCUTELLAR BRISTLES IN OREGON WILD-TYPE FLIES AT FOUR TEMPERATURES

Number of flies with very small extra bristles situated between the posterior scutellars is given in parenthesis

Temperature (°C)	Females				Males			
	No. of Bristles:				No. of Bristles:			
	3	4	5	6	3	4	5	6
30	—	557	6 (99)	4 (28)	1	612	5 (19)	— (5)
25	1	788	7	—	2	823	—	—
20	1	533	13	1	3	694	2	—
15	1	443	16	2	4	515	2	—

This was an unexpected finding and the details have not yet been worked out. The number of flies of this type are given in parenthesis in Table 4. Table 5 tends to confirm the suggestion that canalization is reduced at low temperatures. Non-scute flies, in general, are far less susceptible to the effect of temperature than either HL or LL, presumably because they are much more strongly canalized. The 4 zone in the ++ flies of the Oregon stock is about 5σ depending on temperature, whereas in LL the 2 zone is only $2{\cdot}9\sigma$ at best and in HL is about $1{\cdot}4\sigma$. In other words it takes nearly twice the effort to move across the LL 2-bristle zone that it does to cross the HL 2-bristle zone and four times the effort to cross the Oregon 4-bristle zone.

IV. DISCUSSION

Selection has effectively reduced variance in LL and has had little, if any, effect on HL. The change in variance in LL could be due to increased homozygosis; the lack of effect on HL by comparison would be due to selection of heterozygotes from segregating cultures. The experiment of raising the two lines at four different temperatures was undertaken to test this point and it shows that LL is less sensitive

to temperature change than HL. It is difficult to see how homozygosis of LL could account for insensitivity to temperature. If we suppose that the lesser spread of phenotypes in LL at 25°C is due to a higher frequency of homozygous genotypes making for phenotypes with 2 bristles, we must suppose the 0, 1, 2, 3, and 4 phenotypes of the two lines to be genetically similar, though of different frequency. In this case HL should overlap LL at both ends of the scale at all temperatures, as the selection programme has left it with a wider range of phenotypes than LL at 25°C.

TABLE 5

PROBIT DISTANCES SPANNED BY DIFFERENT BRISTLE CLASSES IN THE OREGON + STOCK AT FOUR TEMPERATURES

Temperature (°C)	Females		Males
	4 Class	5 Class	4 Class
30	(5·02)*	0·35	5·34
25	5·38	—	(5·92)*
20	4·87	0·95	5·40
15	4·62	0·87	5·09

*Indicates incomplete classes.

But this does not happen. At 30°C HL overlaps LL at one end of the scale and at 15°C at the other. Whatever may have been the change in genotype in the two lines since selection started, the genotypes in LL are much less sensitive to temperature change and it seems probable that the bunching of phenotypes due to selection around 2 bristles at 25°C is due to selection for insensitivity of the phenotype to changes in both genotype and environment, which has brought about a situation similar to that found for the 4-bristle phenotype in unselected stocks.

When we attempt to explain the resistance to temperature change in terms of the stability of phenotype at 25°C we find the resistance to temperature change in LL is greater than we expect. The relative variability of the two lines has been assessed by converting the frequencies of the phenotypic classes into a probit scale and comparing the probit distances occupied by the corresponding phenotypic classes. On the assumption that the basic variables responsible for phenotypic differences are distributed similarly in the two lines, the probit distance occupied by a class reflects the extent to which changes in the basic variable will change the phenotype of the class. Thus in LL the 2 class occupies 2·7 probits in females and 2·2 in males whereas in HL it occupies 1·4 and 1·4 respectively. This indicates that to move from 1 to 3 bristles takes one and a half to two times as great a change in the basic variable in LL as it does in HL. So that when an external source of variation, in

this case change in temperature, is imposed on the two lines we expect them both to change the same amount on the probit scale; but that this will result in a much bigger phenotypic change in HL than in LL. In fact, the change on the probit scale is very much greater in HL than in LL; as shown in Figure 3 it is about 2·5 times as great from 30°C to 15°C. In other words our measure of variability indicates that at 25°C, the temperature at which the two lines were selected for different sensitivity, the difference in sensitivity is less than at higher or lower temperatures.

There seem to be two possible lines of explanation for this lack of fit between sensitivity within a restricted temperature range and sensitivity to large changes in temperature. These we shall mention briefly but we have no data adequate to test

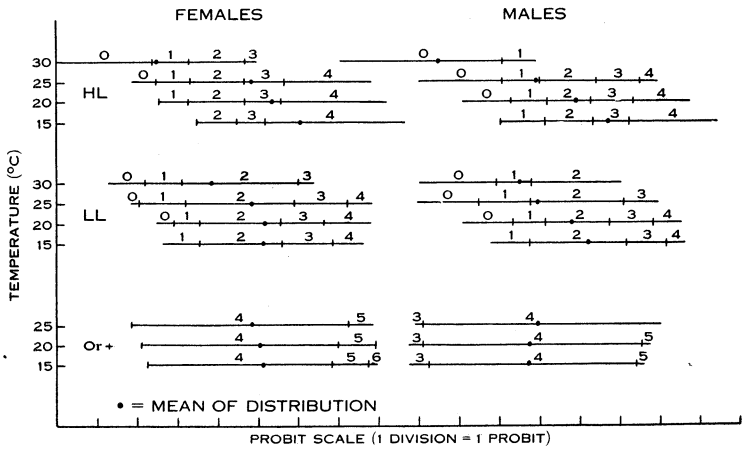

Fig. 3.—Temperature effect on the distribution of the number of scutellar bristles of HL, LL, and Oregon wild-types flies. Distances spanned by the different bristle classes (0, 1, 2, 3, 4, 5, 6) given in probits.

either as yet. The first is that the probit scale of HL cannot be equated to that of LL because the amount of basic variability in the two lines is not the same. It will be remembered that we selected not only for high and low variance but also for intermediate expression and this is expected to lead to some degree of homozygosis (e.g. Robertson 1956). It is possible that in HL the contraction of probit distances in the earlier generations really did reflect an increase in sensitivity but that this has been counteracted by a steady decrease in genetic variance due to selection for intermediates; this selection will have increased in intensity as variability of genetic deviants became phenotypically more obvious. It is possible that in LL selection has also resulted in approach to homozygosis but that selection has decreased in intensity as genetic deviants have come to have a smaller phenotypic effect. The end result might be that the amount of genetic variance in HL has become negligible whereas in LL it is still high. Thus the two probit scales would not be directly comparable as they would measure different things. To account for the results we have found, we have to suppose one scale spans at least twice the variability of the other; to account for this on the ground that one line has lost all its genetic

variance we should have to suppose that at least 60 per cent. of the within-temperature variance was genetic and that HL now has no genetic variance left, but that LL still has much the same as at the start. The second line of explanation would be that the response to temperature was to some extent independent of the factors we have tried to analyse here. It will be noticed that the degree of canalization, measured by the width of the 2-bristle zone, tends to be reduced as temperature falls. It is possible there are other effects which do not correspond to variability of the type taking place within a narrow temperature range.

The effect of temperature differences on a non-scute stock has not added much information which assists the interpretation of the effects on the selection lines. In general, the results can be taken as following the same trends. The ++ line is more strongly canalized and has a smaller temperature effect. It could also be expected to have more genetic variance as it has not been selected in the recent past in a way expected to result in homozygosis. The fact that its response is less than that of LL even on the probit scale could be either associated with the greater strength of canalization or due to the fact that the probit analysis is based on a greater amount of variability.

Whatever the explanation of the discrepancy between the reduction of variation within lines and their sensitivity to temperature, the selection experiment has shown that variation can be reduced by selection and that this reduction of variation is not accounted for by homozygosis. Further, invariability at the temperature at which selection was carried out has been accompanied by a surprisingly large increase in insensitivity to changes in temperature. The invariability produced by selection, therefore, is general and not specific to the variation brought about by a particular set of genes. Finally, invariability at one temperature has resulted in invariability at all temperatures in which the flies were tested.

In a previous paper (Rendel 1959*b*) genetic dosage was measured on a probit scale and plotted against phenotypic change. The relationship between genotypic and phenotypic change was calculated from the curve and it was suggested that heritability measurements and predictions could be corrected by using such a relationship. It was assumed that environmental effects would follow the same relationship. That is to say, an environmental change would have a phenotypic effect at one level of expression of the scute phenotype, which would be related to phenotypic effects at other levels by the same curve as that describing genetic changes. The low line in that study was reared at about 30°C in order to depress the scute phenotype to a level where at least some $sc^{sc}sc^{+}$ flies would have only three bristles. Despite the differences in temperature and the differences in direction of selection the effect of gene substitutions measured in probits was the same in both lines indicating that this method of measuring gene dosage had some generality. Had there been interaction between gene dosage on the probit scale and temperature this would not have been so. On the phenotypic scale there is very considerable gene–temperature interaction due apparently to the fact that temperature, like genotype, is far more effective on some potential phenotypes than on others. However, in the lines reported in this paper, where selection has been for variability and intermediate expression instead of extreme expression only, the two lines are not

comparable. This may indicate a true gene–environment interaction between the genotypes of the two lines and temperature or that the probit scale has a different value in the two lines owing to reduction in the basic variables of one of them. Despite this inconsistency between lines it can be seen that there is some consistency within lines at different temperatures, for differences between estimates of canalization at different temperatures, though perhaps there, are trivial compared to differences between lines.

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