THE EFFECT OF HOMOZYGOSITY ON DEVELOPMENTAL STABILITY

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ALTHOUGH close inbreeding does divide a population up into lines, members of which are in general very similar—WRIGHT claims there was never any difficulty in telling to which of his 23 lines of inbred guinea pigs any individual guinea pig belonged—it has often been observed that some characters in some lines become more variable with inbreeding. The characters for which this has been found to be true are those usually called quantitative. It is common to find that the phenotypic variance of a quantitative character increases with extreme inbreeding (EMMENS 1939; REEVE and ROBERTSON 1953; MCLAREN and MICHIE 1954; BIGGERS and CLARINGBOLD 1954). Since close inbreeding reduces genotypic variance, except as described by ROBERTSON (1952) in the early generations which are not under consideration here, one must suppose that homozygosis has actually increased phenotypic variance by increasing nongenetic variance. This seems to be confirmed by the finding of BIGGERS and CLARINGBOLD (1954) that response to estrogen treatment was most variable in inbred lines, less so in random-bred lines but least variable in F_1 crosses between inbred lines. Members of such F_1 crosses would be genotypically identical but less homozygous even than the random-bred group.

The experimental findings leave no doubt that inbreeding, even to the point of homozygosis at virtually all loci, can result in an increase in total variance which must result from an increase in nongenetic variance. The reason why a highly inbred individual should be more susceptible to nongenetic changes than an outbred one is not immediately obvious. It is well known that mutants are more variable than their corresponding wild-type genotypes and it may be that inbreeding fixes recessive genes at some loci in every line, and that these loci are responsible for increases in variability in the characters they affect.

It must not be assumed that such an increase in variability is a characteristic of the homozygous recessive locus itself. WRIGHT (1934a, b) found abnormalities such as extra toes and otocephaly appearing in some of his lines of inbred guinea pigs but not in others. The frequency of occurrence of these abnormalities was different in the lines in which it occurred; extra toes for example occurred in lines at frequencies of 0.3, 1.7, 2.0, 7.7, 13.5, and 19.0 percent. The average severity of the abnormality was directly related to its frequency in a line. In this example we have two types of variation exhibited, that which distinguishes normal from abnormal individuals and that which renders some abnormal individuals more extreme than others. Detailed work of GOLDSCHMIDT (1937), GOLDSCHMIDT and HÖNER (1937) and others, leaves little doubt that in cases of this sort it is not any par-

ticular locus which determines sensitivity of the developmental process to change, but the sum total of developmental activity due to genetic and nongenetic influences. If this total is greater than a certain threshold amount individuals are normal, but if less they will be abnormal, the degree of abnormality being determined by the amount activity falls below the threshold. The idea is shown graphically in Figure 1. The curves are frequency diagrams of individuals in a population, the base scale being degrees of developmental activity and the vertical scale the frequency of individuals with that level of activity, the threshold shows the level of activity below which abnormality occurs. One curve could represent a population with 0.3 percent abnormal the other one with 19 percent. The population with 19 percent abnormal clearly extends below the threshold to a greater degree than the one with 0.3 percent and will therefore have individuals more extremely affected.

According to this diagram any individual lying above the threshold develops normally; therefore the further the genotype fixes the mean level of activity above the threshold the less likely it is that nongenetic influences will take the level of activity below the threshold. The amount of free play allowed above the threshold would thus be one component of developmental stability. Once the level comes to fall below the threshold every change causes a variation and so a second source of variability is introduced. The second source comes from the fact that levels of developmental activity below the threshold are directly reflected in the phenotype whereas all levels above the threshold are normal. A population will exhibit maximum instability when the mean level of activity is such that all individuals of the population fall below the threshold and this is the case for recessive genes with 100 percent penetrance.

It is clear from Figure 1 that there is no way of distinguishing between genetic changes which move the threshold from those which change the mean level of activity of the population. However, RENDEL (1959a, 1962, 1963) has shown that

FIGURE 1.—Relationship between phenotype and degree of developmental activity (genetic and nongenetic) when a single threshold occurs in the system.

FIGURE 2.—Relationship between phenotype and degree of developmental activity or “make” for the character scutellar bristles. Two thresholds occur at either end of the normal phenotype, four bristles.

the scutellar bristle character in *Drosophila melanogaster* has two thresholds, not one, as in Figure 2. The scute gene (*sc*) lowers the level of activity to such an extent that the whole population falls below Threshold 1. All flies in an unselected scute population have less than four scutellar bristles. But wild-type flies having degrees of activity lying between the thresholds develop four bristles, whereas those with activity above the second threshold all have more than four bristles. It is possible to measure the distance between the two thresholds and distinguish changes in this distance from changes in the amount of mean activity relative to either one of the thresholds.

It is recognised that what has been referred to as developmental activity may be genetic or environmental in origin, and may be in the nature of a rate, an amount, or a timing mechanism and so on. What is referred to is the tendency of the fly to develop scutellar bristles; the tendency is analogous to force in mechanics and is that which gives rise to or tends to give rise to the development of scutellar bristles. The term “make” has been introduced to cover this idea (RENDEL 1962). “Make” is that which gives rise to or tends to give rise to the development of bristles. It can conveniently be measured in standard deviations of the variation in bristle number in a population.

In this paper the effect of inbreeding on mean make is compared with the effect of inbreeding on the distance between Thresholds 1 and 2 or, in other words, the range of make values over which bristle number is canalised.

MATERIALS AND METHODS

RENDEL and SHELDON (1960) have shown that scutellar bristle number in a homozygous scute stock, which is normally uncanalised at the mutant level of expression, can be canalised by artificial selection for low variability. Selection in this line has continued since then with further increase in degree of canalisation. In the experiment to be reported here, isogenic lines of this low variance (L.V.) scute stock, canalised artificially at two bristles, and of a wild-type Oregon-RC

stock canalised naturally, as are all such stocks, at four bristles, have been examined for changes in mean make and in the range of make values over which bristle number is canalised.

Eleven isogenic lines were derived from the low variance (L.V.) scute selection line at about generation 92, by using a *Basc*; *Cy*; *Sb*; T(2;3) *Mé* stock as follows: L.V. females and males were mated to males and females respectively of this marker stock and F_1 females were backcrossed singly to marker stock males, i.e. $\frac{Basc}{L.V.}; \frac{Cy}{L.V.}; \frac{Sb}{L.V.}$ females $\times$ $\frac{Basc\ Cy; Sb}{M\acute{e}}$ males. From each of

these families, five single matings were made of presumed $\frac{Basc}{L.V.}; \frac{Cy}{L.V.}; \frac{Sb}{L.V.}$ females $\times$ presumed

$\frac{L.V.}{.}; \frac{Cy}{L.V.}; \frac{Sb}{L.V.}$ male. However, many of these individuals would have been *Mé*, because of

the difficulty of classifying *Mé* in the presence of $\frac{Basc}{L.V.}$ or $\frac{L.V.}{.}$. The L.V. chromosome 1 is marked

with *w^{bl}* as well as *sc*. Mass matings of presumed $\frac{L.V.}{L.V.}; \frac{L.V.}{L.V.}; \frac{L.V.}{L.V.}$ females $\times$ presumed $\frac{L.V.}{.}$;

$\frac{L.V.}{L.V.}; \frac{L.V.}{L.V.}$ males were then taken off each of these single matings, and at the same time a sample

of males from each was crossed to Or-RC wild females to provide a test for presence of *Mé*. One of the five mass cultures in each family was kept if it threw no *Mé* on test. These were the isogenic lines, each being homozygous, theoretically at least, for a single set of L.V. first, second and

third chromosomes, derived from a single F_1 $\frac{Basc}{L.V.}; \frac{Cy}{L.V.}; \frac{Sb}{L.V.}$ female. The degree to which

homozygosity in each isogenic line fell short of 100 percent for these chromosomes (chromosome four being ignored), depends of course on the efficiency of *Basc*, *Cy* and *Sb* as crossover-suppressors, especially when together in the one individual. In two cases it was possible to obtain

two or three isogenic lines from the same F_1 $\frac{Basc}{L.V.}; \frac{Cy}{L.V.}; \frac{Sb}{L.V.}$ female. Theoretically, these should

have been exact replicates, except for chromosome 4, and the extent of differences between such replicates should reflect to some extent the crossing over in the multiple-inversion heterozygote F_1 female. In fact where replicates were available they proved to be homogeneous, indicating that a very high degree of homozygosity was achieved. The replicates have been pooled in presenting the results.

Thirteen isogenic lines were derived in the same way from an Or-RC wild-type stock. The only difference was that there was no need to do progeny tests for presence of *Mé*, because the only eye colour involved was wild-type. A further five lines, isogenic only for chromosomes 2 and 3, were derived from Or-RC. For this *Cy*; *Sb*; T(2;3) *Mé* was used without *Basc*. Ten inbred lines derived from the Or-RC wild-type stock by brother-sister mating were also made available to us at about the 100th generation by Mr. YU-SHENG PAN of the C.S.I.R.O. McMaster Field Station, Badgery's Creek, New South Wales.

Both the base populations (Or-RC wild-type and low variance scute) and the isogenic and inbred lines were scored in mass cultures each derived from about five pairs of parents, except where otherwise indicated. Cultures were 5 oz cream bottles and culture medium was standard wheatmeal-treacle(molasses)-agar, seeded with live yeast. The temperature was $25^\circ \pm 1^\circ\text{C}$. Cultures were usually scored up till the 18th or 19th day after setting up.

The character scored was scutellar bristle number. The method of calculating the distance between the two thresholds in the wild-type isogenic lines derived from the Or-RC stock was as follows. Sufficient flies were counted so that a number fell in the 3-bristle class, that is below the $3/4$ threshold, and a number in the 5-bristle class, that is above the $4/5$ threshold. By expressing as a percentage the number of flies with less than three bristles, it is possible by reference to a table of probits (FISHER and YATES 1957) to find in standard deviations how far this threshold lies below the mean of the population. Similarly by expressing the number of flies with more

that four bristles as a percentage, it is possible to find out how far the 4/5 cut-off is above the mean of the population. The distance between the cut-off points is then the sum of the distance of each from the mean. This distance is the number of standard deviations of make over which flies develop four bristles, and measures the strength of canalisation at 4. The low variance lines are treated in the same manner except that in these lines canalisation is at two bristles and counts were made to include sufficient flies with three and one bristles to estimate the range of make values which give rise to two bristles.

Counting until a number of flies of a particular kind had emerged might have introduced bias if scoring had stopped with the appearance of the particular fly desired. Such bias is easily avoided; in this experiment all flies in a large number of bottles were scored in each line. If these proved insufficient a further batch of many bottles was set up and all flies scored.

The estimates of probits and probit differences on which measurement of canalisation and mean make are based have standard errors which were calculated according to formulae pointed out to us by DR. S. S. Y. YOUNG, C.S.I.R.O. Division of Animal Genetics, and DR. B. D. H. LATTEr, C.S.I.R.O. Division of Plant Industry. The formulae used are as follows (ref. KENDALL and STUART 1958, Volume 1, p. 239).

$$\text{Variance } D_m = \frac{1}{z_m^2} \cdot \frac{P_m(1-P_m)}{N} + \frac{1}{z_n^2} \cdot \frac{P_{n+1}(1-P_{n+1})}{N} - 2 \cdot \frac{1}{z_m z_n} \cdot \frac{P_m P_{n+1}}{N}$$

where D_m = a distance on the probit scale, representing a certain proportion of the population, P_m = the proportion of the distribution falling below D_m , P_{n+1} = the proportion of the distribution falling above D_m , z_m and z_n = the ordinates of the normal distribution at the lower and upper cut-off points, and N = the total number in the population being considered.

RESULTS

The scutellar bristle scores of the low variance (L.V.) series of lines are given in Table 1 and of the wild-type Or-RC series in Table 2. The distributions in Tables 1 and 2 were converted into percentages from which the distance in probits between 1/2 and the 2/3 cut-off was calculated in the L.V. line and the distance between the 3/4 and the 4/5 cut-off in the Or-RC lines. The level of mean make was related to the cut-offs. In estimating changes of mean make some fixed point must be taken. The fixed point used here is the mid-point of the canalisation zone, since it seems reasonable to suppose that if canalisation increases or decreases it will do so from both ends. If it does not, our estimate of changes in make will be out by that amount. Tables 3 and 4 list the following figures: The "width" column is an estimate of the strength of canalisation, that is the number of standard deviations of make subtended by the 4-bristle class in Or-RC and by the 2-bristle class in L.V. Column (i) shows by how many standard deviations mean make exceeds the lower cut-offs, column (ii) how many it falls short of the higher cut-off. Column (iii) shows how much mean make deviates from the midpoint between the two cut-offs. The last column is the fraction of flies with a bristle number other than that canalised. Figure 3 illustrates Tables 3 and 4.

It can be seen from Table 3 that the isogenic lines of the low variance series are all less strongly canalised at two bristles than the original stock. The 2 class spans about 3.6σ in females in the base population, but only 3.35σ to 2.12σ in the isogenic lines. The lower strength of canalisation however, is not the only cause of increased variation. In the females of the original stock, mean make is about

TABLE 1

Frequency distribution of scutellar bristles in the scute low variance (L.V.) selection line and the isogenic lines derived from it

Line	Number of bristles									
	Females					Males				
	0	1	2	3	4	0	1	2	3	4
L.V. selection line										
Estimate (a)*	1	12	604	49	..	13	169	474	25	2
Estimate (b)†	..	26	1095	58	2	34	227	931	25	..
Estimate (c)‡	..	21	927	49	2	42	274	666	11	..
Estimate (d)§	2	5	381	23	..	21	118	270	5	..
Estimate (e)	7	71	2237	109	2	54	380	2116	45	..
Isogenic lines										
2	..	19	376	20	..	42	142	274	4	..
5	5	69	560	17	..	122	248	455	4	..
3	38	144	786	14	..	240	458	425	3	..
9	213	674	1514	10	..	784	1181	808	2	..
6	14	42	415	21	1	55	155	316	9	..
4	961	1725	1914	12	..	2226	1786	879	2	..
1	36	94	246	8	1	153	180	122	2	..
11	17	82	651	100	8	106	267	582	22	2
20	254	720	1221	30	2	998	792	407	2	..
24	147	296	757	34	1	388	517	430	4	..
19	145	208	391	15	1	282	346	233	3	..

* Generation 92 of selection line; cultures from selected parents set up as single pairs (RENDEL and SHELDON 1960); cultures scored only till 14th day after setting up.

† Generation 106 of selection line.

‡ Parents a random sample from Generation 106; set up as single-pair cultures.

§ Replicate of (c) except that some parents of (c) had died before transferring to new (d) cultures.

|| Parents a random sample from Generation 105; set up six pairs per bottle.

0.2 above the midpoint of canalisation, whereas in the isogenic lines it varies from 0.02 above the midpoint to 1.50 below it, a point below the lower threshold and so outside the range of canalisation altogether. Much the same story is told by the males. Note that the proportion of flies having a number of bristles other than two is higher in Line 4 than in any of the other isogenic lines. Yet the degree of canalisation at 2 is quite strong, being 2.58 in females and 2.44 in males. The reason for the great variability of Line 4 is the low level of mean make, which is so low that most of the population has a make below the level canalised. It is noteworthy that mean make is universally lower in the isogenic lines than in the L.V. parent stock, as is the range of make values canalised at two bristles.

Table 4 shows the Or-RC stock in the same way. In this series of isogenic lines there is increase as well as decrease in canalisation at four bristles. The base stock is canalised over about 5.42σ of make in females and about 5.80σ in males; the isogenic lines range from 6.44σ to 3.34σ in females and from 6.32σ to 4.06σ in males. Changes in mean make are relatively slight. The most variable line, Line 20, owes its variability chiefly to a fall in the range of make canalised from 5.42σ to 3.34σ in females and 5.80σ to 4.06σ in males. Changes in mean make have made a substantial contribution to increased variability only in a couple of lines,

TABLE 2

Frequency distribution of scutellar bristles in the Or-RC stock and the isogenic lines derived from it

Line	Number of bristles									
	Females					Males				
	2	3	4	5	6	2	3	4	5	6
Oregon-RC stock										
(a) RENDEL AND										
SHELDON (1960)	..	1	788	7	..	..	2	823	..	..
(b) Sample (1961)	..	1	3955	106	6	..	5	3769	19	1
(c) Sample (1962)	..	4	3526	45	..	..	7	3749	6	..
(d) Sample (1962)	..	1	3625	36	..	..	5	3529	6	..
Isogenic lines										
5	..	1	8124	24	..	..	6	8808	8	..
102	..	..	7075	61	..	..	5	7539	13	..
15	..	3	7197	25	..	..	22	7293	3	..
17	..	2	6402	30	..	..	7	7030	13	..
104	..	1	5302	65	..	..	5	5217	8	..
103	..	1	2902	26	1	..	6	3207	6	..
9	..	2	3383	23	..	..	5	3010	5	..
3	..	1	6992	147	6	..	3	7020	50	3
24	..	2	8312	213	4	1	9	8922	61	..
1	..	2	7443	193	8	..	7	7912	17	..
7	..	1	1359	21	..	..	4	1474	5	1
105	..	..	4936	212	15	..	11	5087	92	3
11	..	4	10071	322	6	..	6	11042	101	..
19	..	1	5211	350	4	..	18	5590	72	1
10	..	4	2483	70	4	..	3	1991	23	..
8	..	5	4560	191	6	..	39	4919	115	2
101	..	3	4179	258	21	..	18	4096	92	2
20	1	18	1250	180	14	1	10	1376	71	4

chiefly in females of line 19, where it increased to +1.03. In contrast to the isogenic lines derived from the low variance stock, the lines drawn from the wild-type stock have make values little different from that of the parent stock, though on the whole higher. About half of the isogenic lines are more strongly canalised at four bristles than the parent stock, and about half are less strongly canalised.

The scutellar scores of the Or-RC inbred lines derived by brother-sister mating are given in Table 5. The N lines were inbred without any selection but the Y lines were also selected for increased abdominal bristles. It can be shown by taking probits that these lines are spread above and below the parent stock, in the same way as the isogenic lines, with respect to both the strength of canalisation and the level of mean make.

DISCUSSION

There have been two effects of inbreeding on the expression of the scutellar phenotype in this experiment. In both the wild-type and the mutant lines the

TABLE 3
Estimates of the population means and the width of the canalised 2-bristle class in the scute low variance (L.V.) series

Line	Females				Males				Percentage of population with other than 2 bristles
	Width of 2-bristle class (Probits)	Mean			Width of 2-bristle class (Probits)	Mean			
		(i)	(ii)	(iii)		(i)	(ii)	(iii)	
L.V. selection line									
(a)	3.51 ± 0.13	+2.06	1.45	+0.31	2.38 ± 0.10	+0.62	1.76	-0.57	{ 28.1 }
(b)	3.65 ± 0.10	2.01	1.64	0.19	2.83 ± 0.09	0.79	2.04	-0.62	
(c)	3.66 ± 0.11	2.03	1.63	0.20	2.76 ± 0.11	0.47	2.29	-0.91	
(d)	3.71 ± 0.18	2.12	1.59	0.27	2.67 ± 0.18	0.42	2.25	-0.91	
(e)	3.54 ± 0.07	1.85	1.69	0.08	3.08 ± 0.06	0.97	2.11	-0.57	
Isogenic lines									
2	3.35 ± 0.15	1.69	1.66	+0.02	2.64 ± 0.19	+0.26	2.38	-1.06	40.7
5	3.15 ± 0.12	1.21	1.94	-0.36	2.71 ± 0.17	+0.13	2.58	-1.22	45.1
3	3.09 ± 0.11	0.90	2.19	-0.64	2.48 ± 0.18	-0.30	2.78	-1.54	62.3
9	2.98 ± 0.11	0.34	2.64	-1.15	2.64 ± 0.20	-0.55	3.19	-1.87	70.9
6	2.91 ± 0.12	1.21	1.70	-0.24	2.39 ± 0.14	+0.27	2.12	-0.92	40.9
4	2.58 ± 0.09	-0.21	2.79	-1.50	2.44 ± 0.20	-0.91	3.35	-2.13	82.0
1	2.41 ± 0.15	0.42	1.99	-0.79	2.01 ± 0.24	-0.61	2.62	-1.62	73.3
11	2.35 ± 0.07	1.20	1.15	+0.02	2.27 ± 0.09	+0.30	1.97	-0.84	40.6
20	2.35 ± 0.07	0.16	2.19	-1.01	2.23 ± 0.21	-0.89	3.12	-2.00	81.5
24	2.27 ± 0.08	0.36	1.91	-0.77	2.29 ± 0.17	-0.46	2.75	-1.60	67.9
19	2.12 ± 0.11	0.09	2.03	-0.97	2.10 ± 0.19	-0.60	2.70	-1.65	73.0
Mean	2.69	0.67	2.02	-0.67	2.38	-0.31	2.69	-1.49	

(i) Distance above 1,2 cut-off; (ii) Distance below 2,3 cut-off; (iii) Deviation from midpoint of canalised (2-bristle) class; all in probits.

TABLE 4
Estimates of the population means and the width of the canalised 4-bristle class in the Oregon-RC series

Line	Females			Males			Percentage of population with other than 4 bristles
	Width of 4-bristle class (Probits)		Mean	Width of 4-bristle class (Probits)		Mean	
	(i)	(ii)		(i)	(ii)		
Oregon-RC stock							
(a)	5.38 ± 0.32	+3.01	2.37	+0.32	{ 1.5	5.87 ± 0.38	{ 0.4
(b)	5.45 ± 0.32	3.54	1.91	+0.82		5.57 ± 0.16	
(c)	5.30 ± 0.16	3.06	2.25	+0.41		5.84 ± 0.17	
(d)	5.77 ± 0.26	3.43	2.34	+0.55		5.92 ± 0.19	
Isogenic lines							
5	6.44 ± 0.27	3.68	2.76	0.46	0.3	6.32 ± 0.16	0.2
102	6.04 ± 0.28	3.65	2.39	0.63	0.8	6.03 ± 0.15	0.2
15	6.04 ± 0.17	3.34	2.70	0.32	0.4	6.10 ± 0.17	0.3
17	6.02 ± 0.20	3.42	2.60	0.41	0.5	6.00 ± 0.14	0.3
104	5.78 ± 0.24	3.53	2.25	0.64	1.2	6.07 ± 0.17	0.2
103	5.76 ± 0.29	3.40	2.36	0.52	1.0	5.88 ± 0.21	0.4
9	5.71 ± 0.21	3.24	2.47	0.39	0.7	5.88 ± 0.21	0.3
3	5.68 ± 0.27	3.65	2.03	0.81	2.2	5.77 ± 0.17	0.8
24	5.46 ± 0.20	3.51	1.95	0.78	2.6	5.54 ± 0.11	0.8
1	5.38 ± 0.17	3.44	1.94	0.75	2.7	5.99 ± 0.14	0.3
7	5.35 ± 0.30	3.19	2.16	0.52	1.6	5.43 ± 0.21	0.7
105	5.25 ± 0.25	3.54	1.71	0.92	4.4	4.95 ± 0.10	2.0
11	5.22 ± 0.14	3.36	1.86	0.75	3.2	5.61 ± 0.11	1.0
19	5.11 ± 0.28	3.58	1.53	1.03	6.4	4.96 ± 0.09	1.6
10	4.86 ± 0.16	2.96	1.90	0.53	3.0	5.25 ± 0.20	1.3
8	4.82 ± 0.14	3.09	1.73	0.68	4.2	4.42 ± 0.07	3.1
101*	4.74 ± 0.17	3.21	1.53	0.84	6.3	4.64 ± 0.09	2.7
20	3.34 ± 0.10	2.23	1.11	0.56	14.6	4.05 ± 0.12	5.9
Mean	5.39	3.33	2.06	0.64		5.50	0.19

(i) Distance above 3,4 cut-off; (ii) Distance below 4,5 cut-off; (iii) Deviation from midpoint of canalised (4-bristle) class; all in probits.
 * Lines 101-105 not isogenic for chromosome 1.

(a) Low Variance Series

(b) Oregon R.C. Series

FIGURE 3.—The frequency distributions of the lines in Tables 1 and 2 plotted on the probit scale. In each series (a) and (b) the isogenic lines are arranged in order with the most strongly canalised at the top, i.e. with the widest 2-bristle class in (a) and the widest 4-bristle class in (b). Broken lines represent incomplete bristle classes; ● indicates the mean of each distribution.

TABLE 5

Frequency distribution of scutellar bristles in the Oregon-RC inbred lines

Inbred line	Bristle number							
	Females				Males			
	3	4	5	6	3	4	5	6
Y1	.	1246	18	.	3	1453	2	.
Y2	2	2731	36	.	4	2619	3	.
Y3	.	179	50	4	.	240	12	.
Y4	.	1859	44	3	4	1848	11	.
Y5	1	1964	113	1	2	2123	38	.
N1	1	2179	129	5	6	2461	22	1
N2	1	2177	253	10	3	2454	52	.
N3	.	2796	37	.	3	3012	5	.
N4	.	1541	51	2	1	1614	4	.
N5	3	3016	7	.	5	3084	3	.

degree of canalisation has been affected. In the wild-type lines the greatest range of make values canalised is nearly 1σ in females and 0.5σ in males more than the range canalised in the parent stock, whereas the least range is over 2σ less in females and nearly 2σ less in males. The degree of canalisation, measured as the range of make values which have an identical phenotype, is a measure of developmental stability which is clearly and simply defined. Since the degree of canalisation can be increased as well as decreased by a very considerable increase in homozygosis, it seems unlikely that it depends at all on heterozygosis. It would appear that we are dealing with a character controlled by genes, possibly with some degree of dominance, which have been fixed at random in the different lines. The tendency for there to be a bigger decrease than increase in the range of make canalised suggests that the genes heterozygous in the parent population tend to be dominant in the direction of increased canalisation. If dominance in this direction is the rule it will account for the low variance lines all being canalised over a smaller range than the parent low variance stock, which is not so strongly canalised as the wild-type lines, and is of more recent origin.

The second effect is on the value of mean make. Make has been most strongly affected in the low variance lines. It is lower in all the isogenic lines than it is in the original parent stock. The changes are relatively trivial in the wild-type Or-RC lines. The low variance lines show how changes in mean make, which cannot of themselves be considered to have any direct influence on developmental stability, can alter the variability. If chance fixation of genes increases or decreases mean make to a level which is not canalised or which is near one of the thresholds of the canalised range, a considerable fraction of the population comes to have an abnormal number of bristles.

Thus, as far as scutellar bristles are concerned, where inbreeding has increased variability it has done so in part by reducing the range of make values over which development is canalised. To this extent it has affected developmental stability. It has also increased variability by fixing mean make at levels near the canalisa-

tion boundaries or even outside them, and in doing so has increased variability without altering the system controlling developmental stability but by, as it were, by-passing it. The net effect in any particular line is of course dependent on both these components. These two mechanisms by which inbreeding has affected variability are very different. In one variability is affected because developmental stability is affected; in the other because mean expression is changed as a result of a change in mean make. It seems to us necessary to make a distinction therefore between developmental stability and variability of a character in a population.

There is no reason to suppose that heterozygosis has played a significant part in maintaining a low level of variability in either parent stock as far as the degree of canalisation or level of mean make is concerned. Isogenicity has been shown capable of increasing and decreasing canalisation as well as increasing and decreasing mean make. This is typical of the effect of random fixation of genes, and not like the disintegration of a character dependent on heterozygosity. Heterozygosity may be associated with absence of extreme variability since heterozygotes are more likely to have intermediate genotypic values than homozygotes which seems to be important in establishing the optimum level of make. But since isogeny which leads to homozygosis can either increase or decrease both the degree of canalisation and the level of mean make it is an intermediate genotypic value in the case of mean make and an extreme one in case of canalisation that results in minimum variability. In neither case is there the least indication that the optimum genotypic value need be arrived at by heterozygosity. Evidence for supposing that developmental stability may depend on heterozygosity is reviewed by LERNER (1952) and discussed by WADDINGTON (1957). A great deal of the evidence appears to us to be susceptible of the explanation given here for the effect of inbreeding on scutellar bristles.

There is little direct evidence to show how generally the results of this experiment can be applied. Few characters have been examined to see if they have two thresholds in the way scutellars have. DUN and FRASER (1959), FRASER and KINDRED (1960), and KINDRED (1963) have shown that secondary vibrissae in mice are controlled in the same way. A much larger number of genes have been examined at the lower threshold. All recessive mutants worked on in detail have been shown to behave in the same way as scutellar bristles at the lower threshold. It seems reasonable to assume they will prove to be the same when investigated at the upper threshold, until it is proved to the contrary. If this is true, the effects described here as due to changes in mean make will be very widespread and will account for much of the increase in variability of characters on inbreeding and many of the cases of sporadic abnormalities such as extra toes and otocephaly in guinea pigs, crooked toes in chickens, and so on. In so far as only characters which affect fitness, or are associated with those that do, will be canalised, one may expect those effects of inbreeding which increase variability also to lower fitness.

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SUMMARY

Eighteen isogenic lines were derived from an Oregon-RC wild-type laboratory stock, and 11 isogenic lines from a homozygous scute (*sc/sc*) low variance stock which had previously been artificially selected for a degree of canalisation at two scutellar bristles. Large populations of all these lines were scored for number of scutellar bristles. Trends in mean bristle number and the degree of canalisation were analysed by transformation of the data to the probit scale. Ten inbred lines derived from the same Or-RC stock by brother-sister mating were also observed.

In the Or-RC lines the means varied around the base population mean and the strength of canalisation of the 4-bristle phenotype increased in almost as many lines as showed a decrease. It is concluded that heterozygosity is not a significant factor in the wild-type canalisation system for this character.

In the low variance lines both the mean and the width of the canalised 2-bristle class were reduced in nearly all lines, though canalisation was not destroyed in any line. It is concluded that dominance effects are important in this artificially selected canalisation system, but heterozygosity is not a significant component.

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