THE "EXTRA-BRISTLE" COMPLEX IN *DROSOPHILA MELANOGASTER* AND ITS REACTION WITH SCUTE[8]*

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(With Eleven Text-figures)

Introduction

Genes concerned with the production of extra bristles have previously been found to be of frequent occurrence in wild populations of *Drosophila melanogaster* (Sturtevant, 1921; Tschetwerikoff, 1927; Dubinin, 1934). Payne (1918), Reeves (1916) and others have studied extra-bristle characters in *Drosophila* but they confined their studies to a relatively small number of bristle loci. More recently several workers (Goldschmidt, 1931; Sturtevant & Schultz, 1931; Child, 1935*a*, *b*, 1936) have attacked the problem of bristle pattern in *Drosophila*, using the scute allelomorphs, but only one, Rokizky (1930), had attempted to study the problem by combining scute and extra-bristle genes and observing the effect on bristle pattern. From a study of bristle pattern in Dichaete flies Plunkett (1926) concluded that the bristle-reducing action was greatest about a particular "centre" (of diffusion) from which it decreased in all directions. Goldschmidt (1931) and Sturtevant & Schultz (1931) have considered that scute acts by determining the diffusion centres of the bristle-reducing substance. Child (1936) doubts if there is a problem of bristle pattern involved at all, but believes that the appearance of a pattern is an illusion produced by looking at the statistical differences in mean numbers of bristles instead of at the individual fly. However, Goldschmidt (1938) thinks that this argument is not valid. Our paper is an attempt to clarify the problem of bristle pattern and bristle-determining substance or substances in relation to a determination stream by combining plus and minus (extra and scute) bristle-modifying genes in the same fly.

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Materials and methods

The experimental material consisted of a scute^6 stock and a strain of extra-bristled flies derived from a single wild female collected near Montreal by Mr J. M. Henderson. This female had an extra anterior scutellar bristle and some of her progeny also showed the extra-bristle character. Inbreeding and selection of her progeny for extra bristles on the thorax and scutellum have resulted in a stock in which nearly all individuals have one or more extra bristles. The scute^8 stock used was a laboratory strain which has been mass-inbred for several years. Scute^8 is inseparable from the long inversion, In (l) sc^8, and is linked with a marker, apricot, w^a. Furthermore sc^8 has what is known as the "*Hw*" effect, the production of extra bristles at some loci.

The usual *Drosophila* culture methods were employed and cultures were reared at room temperatures which varied a few degrees from 21° C. This temperature fluctuation was not sufficient to affect our conclusions greatly, which are of a general nature.

Statistical methods

In order to determine whether the bristles affected by the extra or scute genes vary independently of each other, association coefficients were calculated separately for males and females of both stocks. The method for finding the association coefficient between any two bristles on the same side of the fly is nicely illustrated by Child (1935*a*). An association coefficient of approximately zero indicates that genetic and environmental factors affecting the two bristles are constant, or nearly so. It was not possible to find coefficients when either bristle had less than 1 % of duplication. If the two bristles in question were not duplicated at all on the same side of the fly the coefficient is -1 ($ab = 0$). As stated by Child, if $\frac{AB}{B} - \frac{Ab}{b}$ is greater than twice its standard error, the coefficient is significant.

The formula for calculating the coefficient of correlation for a particular bristle pair on opposite sides of a fly is

$$r = \frac{(AB)(ab) - (Ab)(aB)}{\sqrt{\{(A)(a)(B)(b)\}}}.$$

Prof. Sewall Wright has kindly supplied the formula for the standard error of the coefficient:

$$\sigma_r = \sqrt{\left\{\left(\frac{b}{N}\right)\left(\frac{B}{N}\right)\left(\frac{1}{a} + \frac{1}{A}\right)\right\}}.$$

If Δ is greater than twice σ_r the coefficient is significant, where

$$\Delta = \frac{ab}{a} - \frac{Ab}{A}.$$

Experimental

The scute[8] *stock*

The frequencies of duplicated and missing bristles in 400 female and 300 male half-flies of the *sc*[8] stock are shown in Fig. 2 and Table VI. It is particularly noteworthy that the expression of scute[8] and of the *Hw* effect in the two sexes is decidedly different. In the figure the normal bristle condition for each bristle on the half-fly is 1·0; the values exceeding 1·0 indicate extra bristles and those below 1·0 indicate missing bristles. The same bristle may be either duplicated or missing, but not of course on the same half-fly. Plotted on such a graph the bristle frequencies of a normal stock would be a horizontal straight line. In Fig. 2, out of the 400 female half-flies, 42 had an extra *pdc* and another 50 had no *pdc* bristle; this leaves 308 half-flies in which the *pdc* was present and normal. The remainder of the bristles (*adc*, *pnp*, etc.) are plotted in a similar fashion, each independently of the others. It will be noted that all the figures of this paper have been plotted according to the seriation of the step-allelomorph advocates; this was merely to maintain uniformity with previous publications by others and does not in itself, indicate either agreement or disagreement with the step-allelomorph theory of gene structure.

The association coefficients for the scute stock are given in Table I. As scute[8] affects only a few bristles of female flies, the number of association coefficients possible for females is reduced to two. Data were from 400 female half-flies and 300 male half-flies.

The left-right correlations for scute[8] are given in Table II.

The extra-bristle stock

One of the first things to attract our attention was the decidedly different response of females to extra-bristle genes as compared with the response of males. Of the first thirty-five flies with extra bristles only two were males (5·7 %). Following one to three generations of inbreeding the percentage of males had risen to 12·8 % in families H, N and O. One or two more generations of inbreeding gave 30 % males with extra scutellar bristles to 70 % females in families GG, HH, KK, SS, UU, VV, AG, and AH in which there were 115 "extra" females to 50 "extra" males as well as the 256 normal flies. Combining "extra" flies and

TABLE I

Associations between missing bristles in the scute^{8} *stock*

	♀♀		♂♂	
Bristles	Assoc. coef.	$\frac{(AB)}{B} - \frac{(Ab)}{b}$ / S.E.	Assoc. coef.	$\frac{(AB)}{B} - \frac{(Ab)}{b}$ / S.E.
ps–asa	—	—	+0·055	0·005 / 0·056
ps–asc	—	—	−0·331	−0·050 / 0·033
ps–psc	—	—	+0·126	0·120 / 0·092
adc–psa	+0·688	0·017 / 0·033	—	—
pdc–psa	+0·472*	0·118 / 0·035	—	—
ppa–asc	—	—	+0·631	0·022 / 0·014
ppa–psc	—	—	+0·843*	0·040 / 0·016
ppa–asa	—	—	+0·851*	0·107 / 0·031
asa–asc	—	—	−0·027	−0·003 / 0·090
asa–psc	—	—	+0·221	0·025 / 0·030
asc–psc	—	—	+0·032	0·014 / 0·065

* Coefficients which are statistically significant.

TABLE II

Left-right correlations for the scute^{8} *stock*

	♀♀		♂♂	
Bristles	Correl. coef.	$\frac{\Delta}{\sigma_r}$	Correl. coef.	$\frac{\Delta}{\sigma_r}$
asa	—	—	+0·073	0·055 / 0·079
psa	+0·391*	0·390 / 0·023	—	—
pdc	+0·224*	0·210 / 0·066	—	—
asc	—	—	+0·162	0·163 / 0·082
psc	—	—	−0·049	0·054 / 0·089

* Coefficients which are statistically significant.

normals, the whole population of these eight families was composed of 206 females and 215 males, practically equality. It is clear that a higher percentage of females than males give a phenotypic response to the same "extra" genotype. So far we have considered only the scutellar bristles. If we include the bristles of the thorax we find that there were 58·8 % females with one or more extra bristles to 41·2 % males with extras in the eight families.

With increased inbreeding the percentage of "extra" males in relation to "extra" females seems to increase almost to equality. A comparable situation was found by Payne (1918) who studied a similar extra-bristle character. Of the flies with extra bristles in his third inbred generation there were 16·1 % males to 83·9 % females but this gradually changed until in the twenty-second generation there were 48·5 % males with extra bristles. Thereafter the fluctuations about equality of the sexes were random. Payne's counts were concerned only with the scutellars.

In Payne's extra-bristle stock, evidence of penetrance or normal overlapping was found. It was unnecessary for us to repeat his experiment as the one similar cross which we made (normal × normal from "extra" × "extra" parents) gave 23 extra-bristled flies out of a total of 224 (10·3 %). A cross between "extra" sibs of the normal × normal animals gave 21 flies with extra bristles out of a total of 163 flies (12·9 %).

The "extra" character shows incomplete dominance, as is the case with many characters which show penetrance. "Extra" females were crossed with males from a highly inbred normal strain (43rd generation of brother × sister matings) and produced 46 progeny with extra scutellars to 679 flies with normal scutellars. This is a partial dominance of 6·8 %.

As inbreeding proceeded the sex difference in extra-bristle frequency tended to become less and at the same time the expression of the character became more severe, as many as four extra bristles appearing at some loci. Also the average number of loci affected increased from 1·03 per female in the first few generations to 2·84 in the last three generations. There was a similar increase in the males from 0·38 to 1·45. The bristle frequencies at two different stages of inbreeding are shown in Figs. 3 and 4 and Table VI. All the bristle loci are affected but some more strongly than others. Those bristle loci most often duplicated (*asc*, *ppa*) are the ones which most frequently show more than one extra bristle. In calculating the extra-bristle frequencies no distinction was made between a locus with one extra bristle and one with two or more. An extra-bristle locus was counted as one extra bristle regardless of the number of bristles present. In 1006 female half-flies the *asc* locus was affected 693 times,

but of these loci 586 had one extra, 98 two extras and 9 had three extras. The *ppa* locus was affected 267 times, of which 15 loci had two extra bristles and one had three.

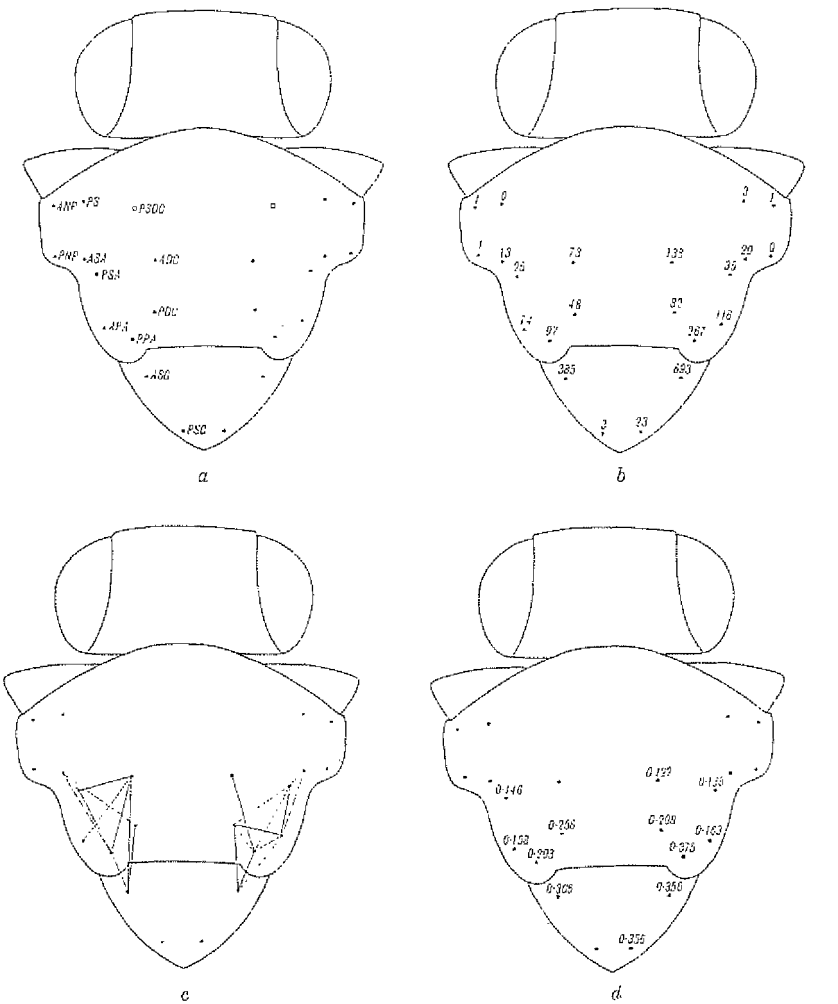
A new macrochaeta appeared on some of the "extra" flies. It has been called the presutural dorsocentral (*psdc*) as it is anterior to the transverse suture on the thorax and in line with the dorsocentrals. It is of interest that certain species of Drosophilinae normally have presutural dorsocentrals, e.g. *Blaesochaetophora* (Sturtevant, 1921). The new bristle reached a frequency of 25% in one of the families (AQ), but occurred less frequently in others. The exact position and the size of the new bristle vary considerably.

In order to clarify the problem of extra-bristle pattern the frequencies of extra bristles for each locus of approximately 1000 half-flies of each sex have been plotted in Fig. 1*b*. The left half of the figure gives the frequencies for males and the right for females. If the frequencies are expressed as percentages of the total number of extra bristles and not as percentage of bristle loci the similarities of the bristle patterns are more apparent, showing that the two sexes have the same basic pattern and differ only in the degree of expression. This is not true of the scute[8] pattern in which the sex difference is very distinct. A glance at Fig. 1*b* shows that *asc* is the most frequently affected locus and that, except for *adc* and *psc*, the frequencies of extra bristles decrease as the distance from *asc* increases.

Association coefficients between bristles on the same side of the fly are given in Table III for approximately 1000 half-flies of each sex of the inbred extra bristle stock. There are 12 significant associations in the females and 10 in the males (Fig. 1*c*). It is of interest that calculations of associations at an earlier stage of inbreeding showed only five which were significant (*ps* with *psa*, *adc*, *asc*, *ppa*, and *psdc* with *asc*) in the females and only one pair in the males (*ps–asc*).

Left-right correlations for bristles duplicated in more than 1% of the cases are given in Table IV. The *adc* does not show a significant correlation in the males. This bristle is also exceptional in being duplicated more often than *pdc* in spite of being further away from the *asc* locus (Fig. 1*b*). As found for the association coefficients, more left-right correlations are significant in the more highly inbred line than at an earlier stage of inbreeding (*ps*, *pdc*, and *adc* in the females and none in the males). Fig. 1*d* illustrates the fact that the correlations tend to decrease anteriorly from *asc*.

A test of asymmetry of expression of extra *asc* bristles failed to show



ig. 1. (*a*) is redrawn from Plunkett (1926); (*b*), (*c*), and (*d*) illustrate data from 974 male half-flies (left sides) and 1006 female half-flies (right sides) of the inbred extra-bristle stock. (*a*) Location and names of bristles of *Drosophila melanogaster*: *anp*, *pnp*, anterior and posterior notopleurals; *ps*, presutural; *asa*, *psa*, anterior and posterior supra-alars; *adc*, *pdc*, anterior and posterior dorsocentrals; *asc*, *psc*, anterior and posterior scutellars; *psdc*, new bristle found in the extra-bristle stock, presutural dorsocentral. (*b*) Gradient of extra-bristle frequencies. (*c*) Diagram of extra-bristle associations, constructed from the data from Table III. The lines joining loci pairs indicate a significant association of extra bristles at those loci. (*d*) Left-right correlations between corresponding loci on opposite sides of extra-bristle flies.

TABLE III

Associations between extra bristles

Bristles	♀♀ Assoc. coef.	♀♀ $\frac{(AB)}{B} - \frac{(Ab)}{b}$ / S.E.	♂♂ Assoc. coef.	♂♂ $\frac{(AB)}{B} - \frac{(Ab)}{b}$ / S.E.
ps–adc	+0·352	$\frac{0·006}{0·004}$	*ab* = 0	—
ps–ppa	−0·040	$\frac{0·0003}{0·0045}$	*ab* = 0	—
adc–psa	+0·232	$\frac{0·065}{0·059}$	+0·600*	$\frac{0·130}{0·053}$
adc–apa	+0·071	$\frac{0·017}{0·035}$	+0·456*	$\frac{0·095}{0·032}$
adc–pdc	+0·145	$\frac{0·041}{0·037}$	−0·312	$-\frac{0·032}{0·039}$
adc–ppa	+0·188*	$\frac{0·050}{0·024}$	+0·356*	$\frac{0·064}{0·028}$
adc–asc	+0·196	$\frac{0·005}{0·024}$	+0·269*	$\frac{0·040}{0·017}$
adc–psc	−0·561	$-\frac{0·095}{0·072}$	*ab* = 0	—
pdc–ppa	+0·490*	$\frac{0·095}{0·020}$	+0·305	$\frac{0·037}{0·023}$
pdc–asc	+0·477*	$\frac{0·060}{0·019}$	+0·385*	$\frac{0·039}{0·014}$
pdc–psc	+0·420	$\frac{0·094}{0·057}$	*ab* = 0	—
pdc–apa	+0·430*	$\frac{0·090}{0·027}$	+0·286	$\frac{0·033}{0·026}$
pdc–psa	+0·568*	$\frac{0·152}{0·046}$	−0·112	$-\frac{0·008}{0·044}$
pdc–asa	+0·339	$\frac{0·070}{0·062}$	+0·718*	$\frac{0·181}{0·061}$
asc–asa	+0·154	$\frac{0·062}{0·104}$	+0·425	$\frac{0·223}{0·136}$
asc–psa	+0·157	$\frac{0·056}{0·080}$	+0·094	$\frac{0·045}{0·099}$
asc–apa	+0·497*	$\frac{0·189}{0·045}$	−0·101	$-\frac{0·047}{0·059}$
asc–ppa	+0·487*	$\frac{0·200}{0·033}$	+0·286*	$\frac{0·144}{0·052}$
asc–psc	+0·016	$\frac{0·008}{0·031}$	*ab* = 0	—
psc–asa	+0·395	$\frac{0·028}{0·106}$	*ab* = 0	—
psc–psa	*ab* = 0	—	*ab* = 0	—

* Coefficients which are statistically significant.

TABLE III (*continued*)

Bristles	♀♀ Assoc. coef.	♀♀ $\frac{(AB)}{B} - \frac{(Ab)}{b}$ / S.E.	♂♂ Assoc. coef.	♂♂ $\frac{(AB)}{B} - \frac{(Ab)}{b}$ / S.E.
psc–apa	+0·550	$\frac{0\cdot042}{0\cdot047}$	$ab=0$	—
psc–ppa	+0·198	$\frac{0\cdot010}{0\cdot034}$	$ab=0$	—
ppa–apa	+0·532*	$\frac{0\cdot147}{0\cdot043}$	+0·050	$\frac{0\cdot010}{0\cdot036}$
ppa–psa	+0·466*	$\frac{0\cdot228}{0\cdot076}$	+0·575*	$\frac{0\cdot185}{0\cdot060}$
ppa–asa	+0·302	$\frac{0\cdot138}{0\cdot100}$	+0·610*	$\frac{0\cdot212}{0\cdot083}$
apa–psa	+0·695*	$\frac{0\cdot293}{0\cdot055}$	+0·695	$\frac{0\cdot086}{0\cdot053}$
apa–asa	+0·682*	$\frac{0\cdot287}{0\cdot073}$	+0·006	$\frac{0\cdot001}{0\cdot073}$
asa–psa	+0·769*	$\frac{0\cdot097}{0\cdot024}$	+0·768*	$\frac{0\cdot070}{0\cdot023}$

* Coefficients which are statistically significant.

TABLE IV

Left-right correlations for extra bristles

Bristles	♀♀ Correl. coef.	♀♀ $\frac{\Delta}{\sigma_r}$	♂♂ Correl. coef.	♂♂ $\frac{\Delta}{\sigma_r}$
anp	$ab=0$	—	$ab=0$	—
pnp	$ab=0$	—	$ab=0$	—
ps	$ab=0$	—	$ab=0$	—
asa	$ab=0$	—	$ab=0$	—
psa	+0·138*	$\frac{0\cdot163}{0\cdot053}$	+0·146*	$\frac{0\cdot217}{0\cdot067}$
apa	+0·163*	$\frac{0\cdot173}{0\cdot047}$	+0·158*	$\frac{0\cdot171}{0\cdot051}$
ppa	+0·375*	$\frac{0\cdot377}{0\cdot045}$	+0·203*	$\frac{0\cdot205}{0\cdot047}$
adc	+0·122*	$\frac{0\cdot109}{0\cdot040}$	+0·073	$\frac{0\cdot073}{0\cdot045}$
pdc	+0·208*	$\frac{0\cdot208}{0\cdot043}$	+0·258*	$\frac{0\cdot229}{0\cdot040}$
asc	+0·350*	$\frac{0\cdot354}{0\cdot046}$	+0·306*	$\frac{0\cdot304}{0\cdot045}$
psc	+0·356*	$\frac{0\cdot427}{0\cdot053}$	$ab=0$	—

* Coefficients which are statistically significant.

a significant difference between the right and left sides. Considering the families which were being inbred for "extra" and the animals in which only one of the *asc* bristles was duplicated, there were 140 flies with an extra left *asc* to 136 flies with an extra right *asc*. When flies with a single right *asc* were crossed, 21 offspring with a single left *asc* appeared and 33 with a single right *asc*. This is a large difference but it is not statistically significant (Dev. = 2·42 times its P.E.) and therefore could have arisen by chance. Flies, each with a single left *asc*, were mated and produced 18 offspring with a single left *asc* to 15 with a single right *asc*. A fly with a single left *asc* mated with flies of a normal stock produced among their offspring 6 flies with a left *asc* to 8 flies with an extra *asc* on the right side.

In addition to the extra-bristle complex the original wild female carried several other mutant genes which appeared during inbreeding. The first mutant found was a sex-linked bristle-affecting gene which we identified as a singed allelomorph by crossing with singed^3 (sn^3, 1–21). However, it differs from sn^3 in that the females are sterile. A second recessive mutant which we call "droop" and which affects texture and conformation of the wing appeared, but we have not yet identified its locus. The wings varied from normal to very much crumpled, waved or unfolded and were usually slightly drooped or arched downward.

In later generations many flies appeared with peculiarly formed thorax, wings or legs or with any combination of these. The thorax frequently showed abnormal evaginations from one or both dorso-lateral surfaces just behind the wing stub. In such cases the wing or wings were reduced to a "vestigial" condition or completely lacking. The legs were variable in form with twisted or bent femurs, knotty joints, vestigial, partly or completely missing or partly duplicated legs. One or more legs might be affected. The anterior pair of legs were less often affected than the others. This character is somewhat similar to the mutant, "crippled", described by Komai (1926). It seems possible that the thoracic, wing and leg abnormalities may be merely manifold effects of the same gene or gene complex, as they were frequently all present in the same individual, while more rarely abnormalities in development of the eyes and antennae were observed also.

The cross of the scute^8 and extra-bristle stocks

Homozygous apricot females ($w^a w^a$) of the scute^8 stock were crossed with males from the moderately inbred "extra" stock. Only the males were useful for our study of the F_1, as the females were heterozygous for scute and consequently had no bristles missing. A small percentage

(4·4 %) had one or more extra bristles but as the dominance of the extra complex is weak and the *Hw* effect is recessive the F_1 females were practically normal. The F_1 males, however, were far from normal. Each son expressed the sex-linked scute gene, the *Hw* effect and the dominance effect of "extra" which is therefore located on the autosomes, because the only *X*-chromosome of these males comes from the scute stock. These sons were all genetically scute and all had apricot eyes. In later generations the apricot-eye animals should always be scute also, because the inversion should prevent cross-overs between scute and apricot which might occur naturally. In 5·3 % of the males of the scute^8 stock there was no observable bristle abnormality, an indication that scute occasionally overlaps with the normal. In the "extra" stock from which the F_1 males were secured, 76·8 % of the males had normal bristles. In the F_1 generation of 645 males, 9·2 % had no observable bristle abnormality of the thorax and scutellum. Fig. 5 shows the frequencies of extra and missing bristles at each point on the F_1 male half-flies.

The reader may compare these F_1 males with the males of the "extra" stock shown in Fig. 3, and with the males of the scute^8 stock of Fig. 2. It should be borne in mind that comparisons must always be made between males and males or between females and females of succeeding generations and never between males and females because the scute character is expressed differently in the two sexes.

A comparison of bristle frequencies of the parental stocks with the F_1 (Table V) which is genetically scute and at the same time exhibits the dominance of the "extra" stock gives some interesting results. The *pdc* locus is rather strangely affected (see also Fig. 5), being normal in the scute stock but duplicated in 13 % of the F_1. A similar disproportionate increase in the frequency of extra bristles of the F_1 over either parental stock has occurred at loci *adc*, *ps* and *anp*. The *adc* bristle shows a further interesting situation. It is not only duplicated more often in the F_1 than in either parental stock (or their summation) but it is also removed more often than in the scute parent. In the extra and normal stocks *adc* is always present. Since the *sc*^{8} chromosome of the F_1 male is precisely the same as the *sc*^{8} chromosome of the parental scute stock we may conclude that the increase in the frequency of scute expression is due to autosomal genes. This is demonstrated more clearly in the case of *psc* and *asc* bristles, but from another angle. In the case of the *psc* bristle, the "extra" condition appears in about 1 % of the male half-flies in the F_1 and in both parental stocks. In the *sc*^{8} stock males the *psc* is present in 73·7 % of the half-flies but in the F_1 males it was present in 89·9 % of the

cases. This is a difference in phenotypic expression of 16·2 %, which, if genetic, must be due to a different set of genes in the autosomes of the F_1 from that of the sc^8 stock. The same situation is true for the *asc* bristle which is present on 59·0 % of the half-flies of the sc^8 stock and shows an increase to 90·8 % in the F_1 males, a difference of 31·8 %.

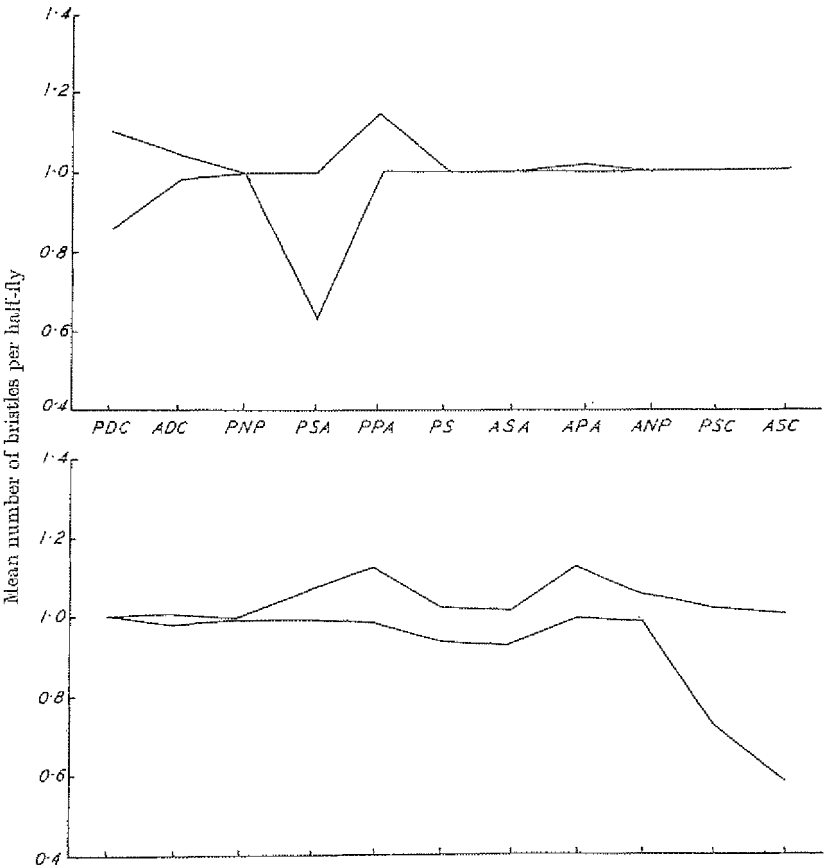


Fig. 2. The scute^8 stock, showing *Hw* effect as well. (Upper graph females, lower males.)

Before we consider the F_2 generation it may be well to observe the results of the F_1 in a cross of sc^8 females to a strain of flies which had been brother-sister inbred for fifty generations and showed no extra or missing bristles. This F_1 is plotted in the usual way in Fig. 6. The number of half-flies is too small to give a very reliable curve, but it will be seen that except for the *ps* and *asa* bristles the F_1 males are very similar to the scute^8 males.

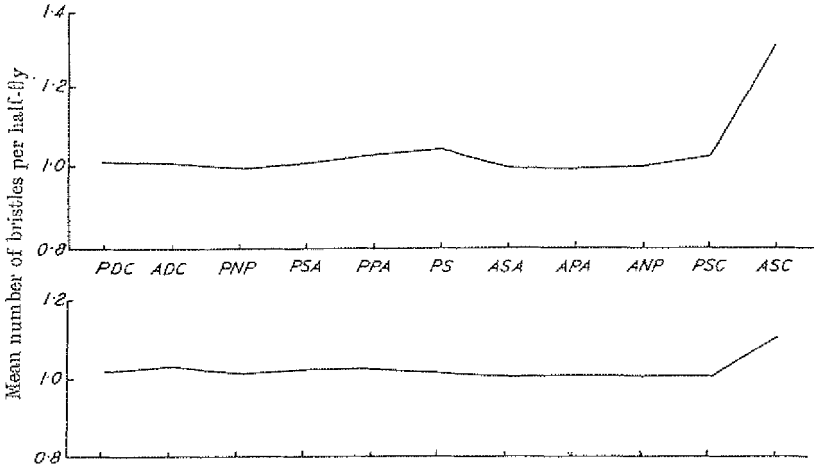


Fig. 3. The "extra" stock at an early stage of inbreeding. (Upper graph females, lower males.)

TABLE V

Frequencies in percentage of extra (E) and missing (M) bristles for males in "extra" and scute[8] stocks and the F_1 of scute[8] × "extra"

		pdc	*adc*	*pnp*	*psa*	*ppa*	*ps*	*asa*	*apa*	*anp*	*psc*	*asc*
"Extra"	E	1·0	1·1	0·8	1·1	1·0	1·0	0·2	0·6	0·2	0·8	12·0
Scute	E	0·0	0·7	0·0	8·0	12·0	2·0	1·3	12·0	6·0	1·7	0·3
F_1	E	13·0	12·0	1·2	1·0	8·0	8·0	1·6	1·5	19·0	1·0	6·0
Scute	M	0·0	1·4	0·7	0·3	1·7	5·0	5·7	0·0	0·7	26·0	41·0
F_1	M	0·6	6·6	0·2	3·5	0·3	4·3	3·0	0·0	0·2	10·0	9·0

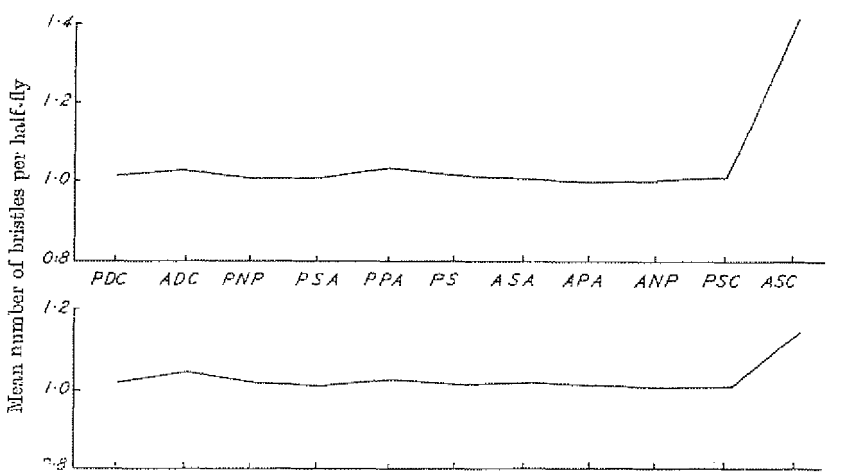


Fig. 4. The "extra" stock. Four moderately inbred families. (Upper graph females, lower males.)

Fig. 7 is the graphic representation of the scute flies of the F_2 generation. It may be seen that there is a remarkable agreement between the F_1 and F_2 males in the expression of extra and of missing bristles. The graphs for the F_1 and F_2 males are nearly identical in shape and correspond, point for point, though the frequency of expression of both

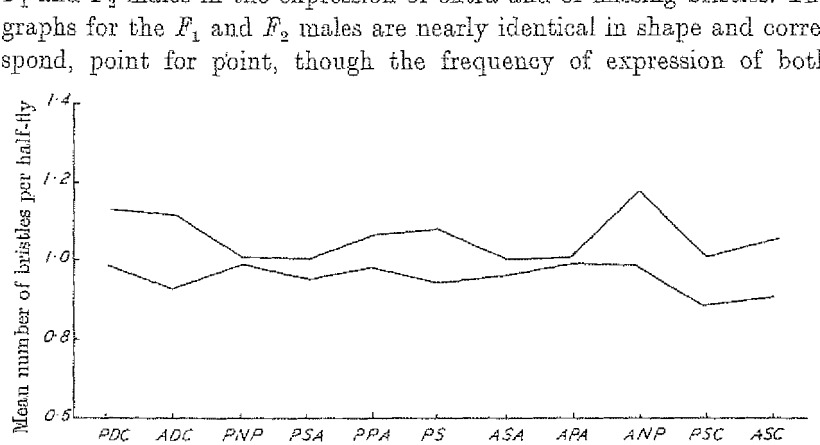


Fig. 5. F_1 males from scute^8 × "extra".

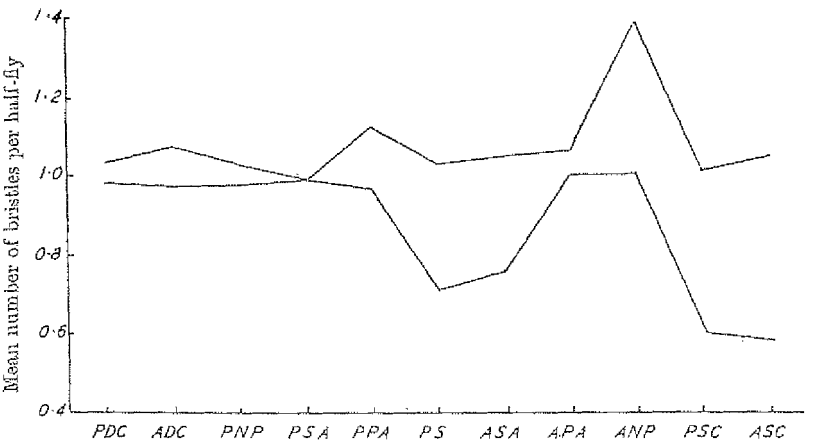


Fig. 6. F_1 males from scute^8 × wild type.

"extra" and scute has been slightly increased in the F_2, possibly because of recombinations of recessive modifiers on the autosomes. The same phenomena observed for the *pdc* and *asc* bristles of the F_1 are seen in the F_2 and to a slightly greater degree. The F_2 females show the effects of modifiers on the autosomes less than the F_1 and F_2 males though it may be seen that the *psa* of the F_2 females was present in only 45·1 % of the cases while in the parental *sc*^{8} stock the *psa* was present 63·8 % of the time—a difference in expression of 18·7 %.

A backcross of F_1 females heterozygous for scute and the extra factors to the scute^8 stock produced the apricot-eyed progeny shown in Fig. 8, and normal red-eyed females. The red-eyed progeny are never shown in the graphs as they are not homozygous for scute. We have the records for the red-eyed flies which have occasional extra bristles but never missing ones. The progeny of the back-cross generation are very much like the scute^8 population which is what one might expect, as the scute^8 genotype

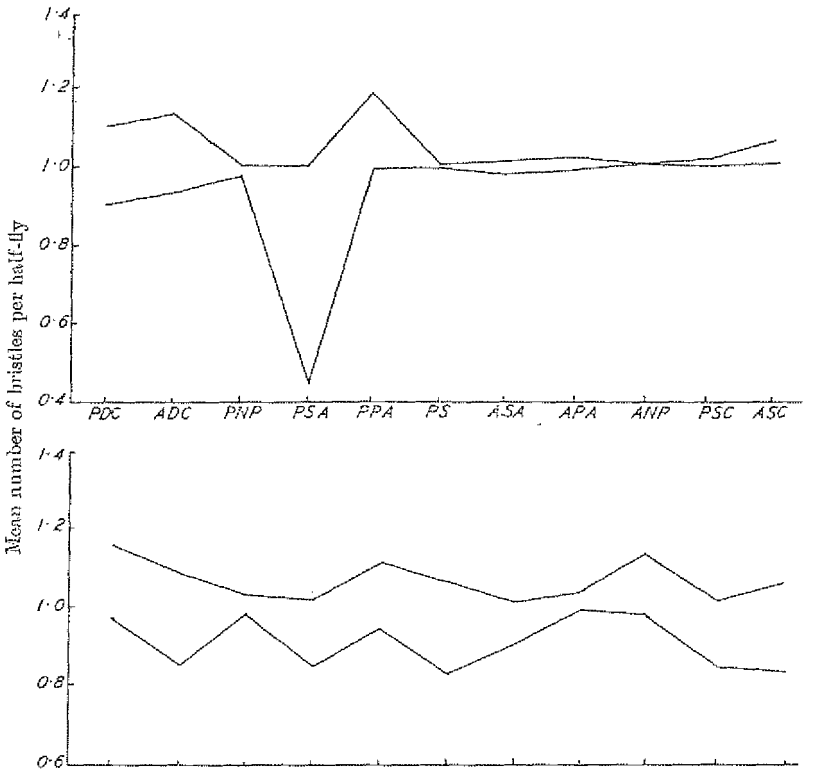


Fig. 7. F_2 of scute^8 × "extra". (Upper graph females, lower males.)

will have been largely regained and the genes from the "extra" stock mostly lost.

The most important generation bred from the original scute-extra cross should be the F_3 and succeeding ones, because in these selection becomes effective. F_2 flies showing a considerable number of extra and missing bristles on the same fly were mated and produced the F_3 shown in Fig. 9. The most striking change in the F_3, when compared with F_2, concerns scute in the F_3 males. The males show a curve for extra bristles

which is nearly identical in shape to that of the F_2 males but the frequencies of missing bristles are greatly, but uniformly, increased. In these F_3 males scute is much more strongly expressed than in the parental sc^8. By way of illustration, the *psa* bristle is present 99·7 % of the time in the scute^8 stock but only present in 68·8 % of the cases in the F_3; *ps* in sc^8 males = 94·6 %, in F_3 males = 48·5 %; *asa* in sc^8 males = 94·3 %; in F_3 males = 54·7 %; *psc* in sc^8 males = 73·7 %, in F_3 males = 43·7 %. At the

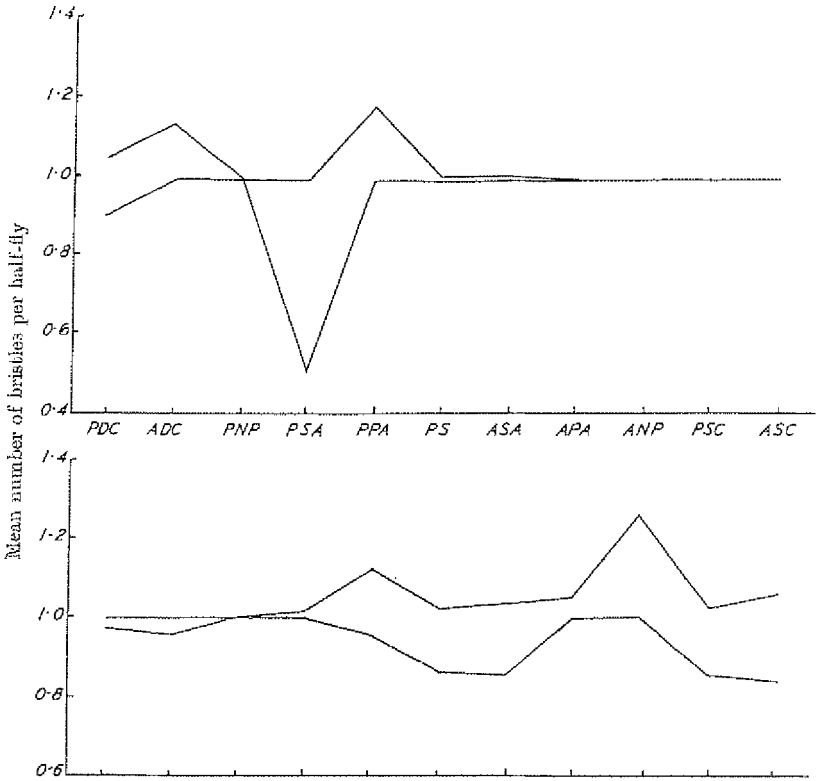


Fig. 8. Backcross, F_1 females × scute^8 males. (Upper graph females, lower males.)

same time that scute is more strongly expressed in the F_3 males than in the original scute stock, the F_3 males also support slightly higher frequencies of extra bristles. Two points should be noted here. The great increase in the frequency of expression of scute as a result of selection in one generation shows that there are important modifiers of scute on the autosomes. The whole pattern of missing bristles shows greater expression, not merely individual bristles. The second point is that the processes which cause scute and "extra" must be distinct and not dependent

merely upon the deficiency or excess of one substance to cause missing or extra bristles. This is illustrated even better by the F_4 generation, shown in Fig. 10, than in the F_3.

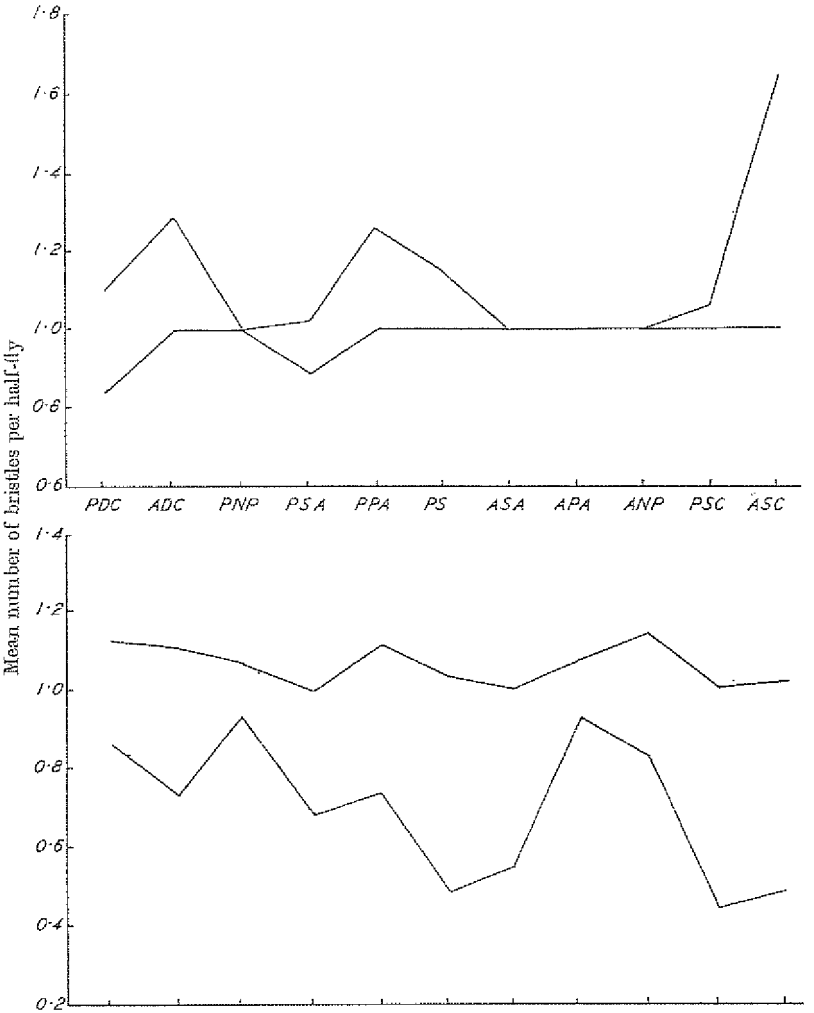


Fig. 9. F_3 of scute^3 × "extra". (Upper graph females, lower males.)

We know from a glance at the males of Fig. 9 that we have been highly successful in selecting for greater expression of scute even though females of the F_4 and F_5 generations show little response to the increase in scute modifiers. We also know that we have been highly successful in

selecting for greater expression of the extra character if we observe the *asc* in the females which increased from 6·0 % in F_2 to 65·5 % in the F_3 to

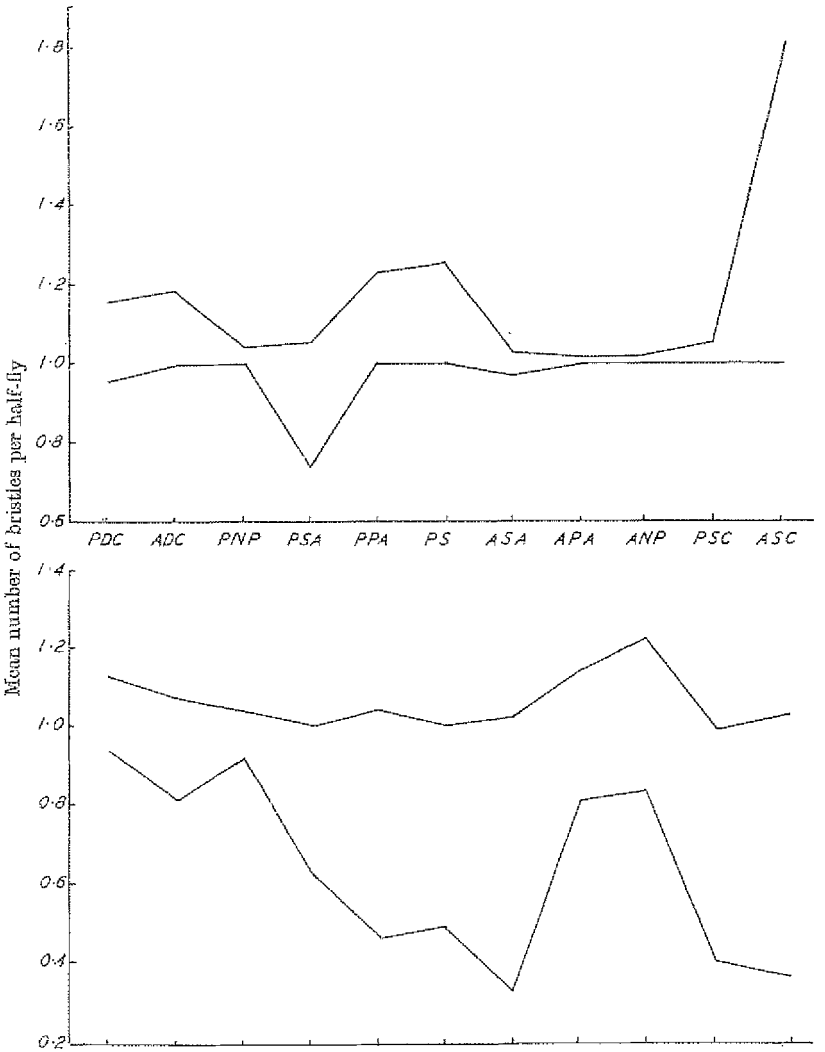


Fig. 10. F_4 of scute^3 × "extra". (Upper graph females, lower males.)

81·1 % in the F_4. This high expression of "extra" is possible because the scute^3 gene never removes the *asc* in females; thus there is no destruction or inhibition of extra bristles at this point. It should be noted, however, that the extra *asc* bristles were characteristically very small in these

females, often being reduced to the size of microchaetae. In the F_3 and F_4 males, however, the scute^8 gene does remove the *asc*. In the F_4 males the *asc* is present only 37·7 % of the time. When any *asc* was present it was duplicated 8·5 % of the time. Had the scute gene not inhibited or

TABLE VI

Bristle counts of the stocks and generations studied

Row designated E gives number of extra bristles.
Row designated M gives number of missing bristles.

		No. of half-flies		*pdc*	*adc*	*pnp*	*psa*	*ppa*	*ps*	*asa*	*apa*	*anp*	*psc*	*asc*
Fig. 1 (*b*)	♀♀	1006	E	82	138	0	35	267	4	20	118	1	23	693
	♂♂	974	E	48	73	1	25	97	0	13	74	0	3	385
Fig. 2	♀♀	400	E	42	20	0	0	65	0	0	7	0	0	1
			M	50	4	0	145	0	0	0	0	0	0	0
	♂♂	300	E	0	2	0	24	37	6	4	37	18	5	1
			M	0	4	2	1	5	16	17	0	2	79	123
Fig. 3	♀♀	538	E	16	13	2	14	21	28	1	0	1	11	172
	♂♂	528	E	5	6	4	6	7	5	1	3	1	4	63
Fig. 4	♀♀	504	E	7	12	1	3	12	8	4	1	1	6	211
	♂♂	496	E	1	12	2	0	8	2	2	1	0	1	71
Fig. 5	♂♂	1290	E	168	153	16	13	109	113	21	20	243	14	83
			M	10	85	3	45	4	55	38	0	3	130	119
Fig. 6	♂♂	86	E	5	8	4	0	11	2	4	6	33	0	2
			M	0	1	1	0	2	15	20	0	0	35	33
Fig. 7	♀♀	282	E	31	41	2	1	54	2	3	4	0	1	17
			M	25	15	3	155	0	0	3	1	0	1	2
	♂♂	262	E	46	24	8	7	30	19	3	10	34	3	17
			M	5	37	2	38	13	45	25	2	4	40	42
Fig. 8	♀♀	102	E	6	15	0	0	20	1	1	0	0	1	2
			M	10	0	0	50	0	0	0	0	0	0	0
	♂♂	74	E	0	0	0	1	9	2	3	4	20	1	5
			M	1	2	0	0	3	10	11	0	0	10	12
Fig. 9	♀♀	58	E	6	17	0	1	16	9	0	0	0	4	38
			M	9	0	0	6	0	0	0	0	0	0	0
	♂♂	64	E	8	7	5	0	7	2	0	5	9	0	1
			M	8	17	4	20	17	33	29	4	11	36	33
Fig. 10	♀♀	74	E	12	14	3	4	17	19	2	1	1	4	60
			M	3	0	0	19	0	0	2	0	0	0	0
	♂♂	64	E	9	6	4	1	4	1	2	10	15	0	2
			M	3	11	4	23	33	32	42	11	10	38	40
Fig. 11	♀♀	70	E	5	13	0	2	7	13	0	3	0	4	59
			M	26	0	0	26	1	0	0	1	0	0	0
	♂♂	44	E	6	4	2	0	2	2	0	6	3	0	4
			M	2	10	3	10	20	23	26	6	17	30	28

removed the potentially extra *asc* bristles of the F_3 and F_4 males there most certainly should have been a much higher percentage of extra *asc* bristles, as we have seen from the *asc* of the females that selection had been effective. It appears, then, that extra bristles are destroyed or inhibited by the scute^8 gene as well as ordinary bristles. This seems true because in regions of the fly where scute is unable to come to phenotypic

expression and where ordinarily the extra complex has expression we find extra bristles in the F_3 and F_4 generations. Were there a single

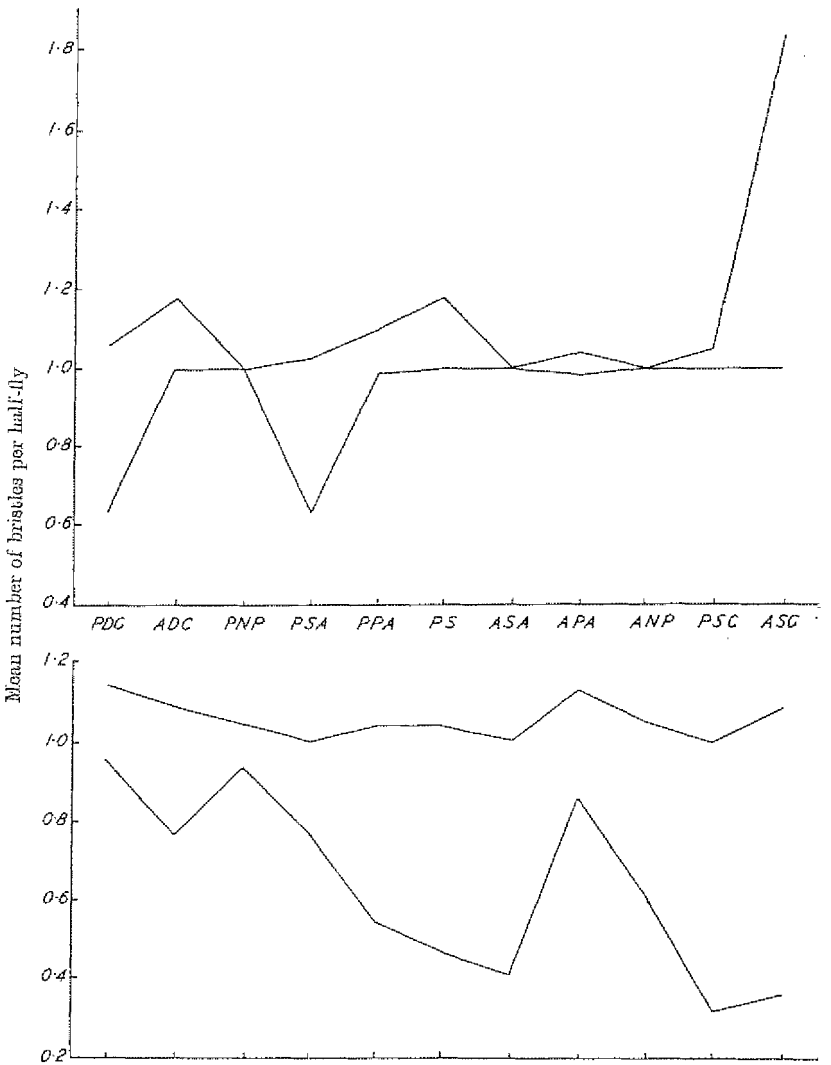


Fig. 11. F_5 of scute^8 × "extra". (Upper graph females, lower males.)

substance of which a deficiency caused scute and an excess the "extra" character, selection could work in one direction at a time, either for more or for less of the substance; thus we should expect the flies of the F_3, F_4 and F_5 to show either many bristles missing and none extra or to show

many extra and none missing. Such is not the case, however, as individual flies showing as many as eight missing out of 22 possible bristles had two or more of the remaining bristles duplicated (family AM).

Embryological observations as to whether normal and duplicated bristles first form and are then destroyed by action of the scute gene or whether the trichogenic cells, which give rise to the bristles, are inhibited from differentiating normally so that no bristles can be produced would be of very great value. Such observations, as far as we are aware, have not yet been made.

DISCUSSION

The small number of significant associations between bristles on the same side of sc^8 flies (Table I) is hardly compatible with the idea of a determination stream and certainly two of the three associated bristle pairs do not agree with Goldschmidt's (1931) morphogenetic fields, as *pdc*, *psa* and *psc* are all in different fields. Further the left-right correlations for sc^8 flies (Table II) show only one significant correlation (*pdc*) in the females and one of doubtful significance in the males (*asc*) for the two fields (numbers 2 and 3 of Goldschmidt) including both members of the four bristle pairs *adc*, *pdc*, *asc* and *psc*. The fact that, in the females, the two loci which have significant left-right correlations are the only two bristles which are significantly associated would seem to indicate that there was a residual heterozygosity in the $scute^8$ stock affecting these bristles. This conclusion is supported by the fact that before these correlations had been calculated it had been noticed that some cultures showed a very high phenotypic expression of the *psa* and *pdc* loci while other bottles gave low expression. However, we cannot be certain that temperature fluctuations, though small, did not have some effect on these two bristles.

It seems very probable that several genes affect the expression of the extra character. Many of these are of the nature of modifiers and selection for these proceeds very rapidly. As shown in the crosses with scute which produced F_1 males having a single set of autosomes but no X-chromosome from the "extra" stock, many of these "extra" genes and modifiers are located on the three pairs of autosomes. We have no evidence, however, of the proportionate distribution of the "extra" genes on the autosomes.

In the extra-bristle stock inbreeding and selection of positive modifiers for "extra" is accompanied by a shift toward equality of males and females which have extra bristles. As a result of inbreeding, the number of flies with extra bristles, compared with normal sibs, was increased

from 0·98 % in the first generation of inbreeding to 100 % in the twenty-second generation of Payne's study. It is clear from Payne's Table I that as modifiers were accumulated the phenotypic response is quicker in females than in males in the production of extra bristles. In other words, with a given "extra" genotype, the threshold which must be exceeded to produce extra bristles is more readily exceeded in the female as a result of a difference in physiological responses in the two sexes. After sufficient positive modifiers have been accumulated by artificial selection so that all flies of the generation have extra bristles, the physiological differences in threshold value still exist, no doubt, but there are now present sufficient positive genes for "extra" so that even the threshold of the males is always exceeded. Our data, in so far as they go, agree with those of Payne in regard to the difference in the physiological response of males and females to any "extra" gene complex. It is possible, though not as probable, that there is no difference in physiological threshold between the sexes, but that the greater proportion of females with extra bristles merely results from the quantitative effect of the single dose of modifiers on the *X*-chromosome of the male as compared with the double dose of the female.

Goldschmidt (1931) has assumed the existence of a determination stream to explain the bristle pattern in scute flies. In our extra bristle stock we could assume a determination stream which originates at *asc* and diffuses in all directions, but more rapidly anteriorly. There is no evidence in the "extra" stock of four different fields of the thorax and scutellum which Goldschmidt assumed for the scute alleles. However, if there is a distinct substance causing extra bristles and another substance causing scute there is no reason to assume that their respective determination streams have identical fields.

The evidence for a determination stream in the extra-bristle stock is (1) the positive associations of bristles on the same side of the fly (Table III); (2) the gradient of bristle frequencies (Fig. 1 *b*); (3) the gradient of left-right correlations (Fig. 1 *d*); and (4) that (1) and (3) are not due to heterozygosity is indicated by the fact that inbreeding *increased* the number of significant bristle associations and left-right correlations.

There are two facts which are not in accord with the theory: (1) the occurrence of normal bristles nearer to the *asc* than a duplicated bristle, or in other words the determination stream skips certain loci; (2) if the determination stream spread by diffusion in all directions we should expect *psc* to be affected more often and *adc* not so frequently. Our explanation for (1) is that chance fluctuations in the stage or rate of

development might make some loci unable to respond to the determining substance. A plausible explanation for the large number of *adc* "extras" (i.e. larger than *pdc* in spite of being further away from *asc*) is the fact that extra bristles in front of *adc* were scored as extra *adc* bristles when they may really have been *psdc* bristles whose threshold value is exceeded in extra-bristle flies more readily than in normal flies.

The cross of the scute^8 and "extra" stocks has given information pertinent to the step-allelomorph hypothesis. The scute^8 gene was plotted according to the seriation of Sturtevant & Schultz (1931) in all cases, but in no case did the males or females give a smooth unimodal curve, as the hypothesis would seem to require. A glance at the F_4 males (Fig. 9) shows every bristle from the left end of the seriation (*pdc*) to the right end (*asc*) to be missing at least 5% of the time and most of them missing much oftener than that. We think that if the linear series does exist at all that it results from observations of a developmental pattern which is not the result of a particular arrangement of subgenes at the scute locus. Child's (1935*a* and *b*) and Ives's (1939) evidence that the seriation may be affected with temperature changes does not support the step-allelomorphism hypothesis.

Rokizky (1930) made a somewhat similar cross using Dichaete and Hairy-wing, the first character being a bristle-reducer and the second giving extra bristles. He raised only fifty flies or less of each sex and presented his results in terms of whole flies instead of half-flies. His data for the F_1 appear to give an intermediate result. Whether or not this is the case cannot be determined as his method was to average extra bristles on one side with missing on the other side. This diminishes the value of his findings considerably. He did not study the effects of selection on later generations and, as he states, further study would be desirable if conclusions are to be drawn from his work.

The data on bristle frequencies in F_1 males compared with parental stocks show that there is a disproportionately large increase in bristle frequencies at some loci (*pdc*, *adc*, *ps* and *anp*). It cannot be assumed that this increase in expression of the extra character is due solely to the simple summation of modifiers. It may be that, as the genotype of the F_1 is different from that of males of either parent stock, the physiological condition of the F_1 males causes an entirely new frequency of expression of these bristles. The expression might be widely different for similar genotypes and, as Child (1935*a*) and Ives (1939) have shown, may be greatly altered by environmental changes such as temperature.

Our interpretation of the disproportionate effects is that the genotype

as a whole produces a certain physiological condition and hence we may expect different physiological responses for each new genotype. Hence different physiological responses may give widely different phenotypic expressions to similar sets of extra-bristle genes which may be present in otherwise different genotypes. This means simply that we cannot say that the disproportionate effects of the combined extra and scute genes are due merely to these two genes but that other genes present in one or both of the parental stocks act as modifiers in the new F_1 genotype. Probably the gene interactions are geometric in nature.

We have shown that selection for scute and "extra" together has been successful. It seems logical to conclude, therefore, that scute and extra bristles are not determined by a quantitative difference of a single substance. This conclusion is supported by Ives's (1939) work on the effects of temperature on bristle frequencies in scute and wild-type flies. He found that a temperature of 36° C. decreased the bristle frequencies in *sc* flies but increased it in wild-type males which he believed carried a group of bristle-modifying genes. This would indicate that there must be two different reactions concerned, one resulting in scute and the other tending to increase the bristle frequencies. Presumably there are different bristle-determining or inhibiting substances concerned in the two reactions.

Summary

1. A complex of genes causing extra-bristles on the thorax and scutellum of *Drosophila melanogaster* was stabilized in one stock following preliminary selection and inbreeding.

2. Bristle frequency studies show that the *asc* bristles are the most strongly affected and expression of the character is decidedly more severe in females than in males. The expression increases markedly during inbreeding accompanied by selection.

3. A cross was made between the extra stock and a scute^8 stock. *Sc*^{8} causes the destruction or inhibition of bristles according to a pattern which is quite different in the two sexes.

4. *Sc*^{8} does not fit the seriation essential to the hypothesis of step-allelomorphism, and thus provides some contrary evidence.

5. Significant left-right correlations and associations between certain bristle pairs in the extra-bristled flies indicate that there is a determination stream or diffusion centre concerned, with origin near *asc* from whence it spreads anteriorly.

6. The disproportionate effect of combining extra and scute genes

cannot be attributed solely to the interactions of these two genes, for apparently, other genes present in one or both of the parent stocks act as modifying genes in the new F_1 genotype.

7. The results of crossing sc^8 with extra-bristle flies indicate that the difference between scute and "extra" flies is not due to a quantitative difference of a single bristle-determining substance. There must be at least one substance causing "extra" and another substance causing scute. The former seems to spread by diffusion or in a determination stream differing from any so far postulated for scute. No evidence of a similar determination stream was found for sc^8.

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