

Differences in bristle-making abilities in scute and wild-type *Drosophila melanogaster*

BY S. S. Y. YOUNG AND R. C. LEWONTIN

C.S.I.R.O., Division of Animal Genetics, Delhi Road, North Ryde, N.S.W., Australia
and
Department of Zoology, The University of Chicago, Illinois, U.S.A.

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1. INTRODUCTION

Scutellar bristles as well as the ocelli and the associated bristles on the head of *Drosophila* are highly canalized (Rendel, 1959; Smith & Sondhi, 1960) in wild-type flies. That is, underlying genetic variation for scutellar bristles is not manifested phenotypically in wild stocks. In scute mutants (*sc*¹) the phenotypic variations of both the bristle systems are increased and there is also a marked reduction of the total number of bristles on the body surface of the fly. Of the head bristles, the ocelli are most frequently absent, while some of the post-verticals and the orbitals are also often missing (Dubinin, 1933). The numbers of sternital abdominal bristles in scute flies are reduced to about one-third of the numbers in wild-type flies. The scutellars are most strongly affected with a mean of about one bristle in *sc*¹ stocks as opposed to the normal four bristles (Rendel, 1959).

It was postulated by Rendel (1959, 1962) that the force tending to form bristles in flies might be a continuous variable which he called *make*. Variation in *make* is expressed only when an upper or a lower bristle threshold is crossed. He deduced that the primary action of the scute gene was to reduce the total bristle *make* of an individual below that threshold.

It would be reasonable to think that bristle *make* is a result of interaction between some bristle-making substances, tissue competence and the pre-pattern of bristle systems. The event of the differentiation of a bristle, then, is a result of the utilization of bristle-making substances by the competent tissues according to pre-patterns. The fact that the scute flies have lower numbers of bristles, and hence lower *make*, might be due to a lower amount of bristle-making substances, or to lower tissue competence or to different pre-patterns. The difference of opinion between Rendel (1965) and Robertson (1965) on the extent to which control of scutellar bristle number extends over the whole scutellum is really a difference of opinion about the weight to be given to control of total amount of bristle-forming substance as opposed to control of tissue competence.

It is of interest to investigate the properties of *make*, especially to investigate its

relationship to factors such as pre-pattern, tissue competence and gene-controlled product (Stern, 1954; Waddington, 1962). One well-known method of throwing light on this question is by the use of mosaic flies where tissues of different gene content exist side by side in an individual. In this communication, data are presented from flies mosaic for scute and wild-type tissues.

Mosaic flies for scute and wild-type genotypes were discussed by Sturtevant (1932) who indicated that in large areas scute tissue was autonomous, but no detailed information was given. It seemed worthwhile to obtain mosaic flies with large areas of wild-type and scute tissues in order to provide some detailed information and to speculate on the properties of make in the light of such information.

2. MATERIALS AND METHODS

It was shown by Brown & Hannah (1952) that matings of male flies carrying the ring X chromosome with aged females usually resulted in a relatively high proportion of gynandromorphs, due to the elimination of the ring chromosomes in early embryonic divisions. In the present experiment a stock carrying a ring X chromosome (X^{c2}) was kindly supplied to us by Professor Stern. Matings were carried out between males carrying the ring X chromosome and a Y chromosome with attachment of sc^8 and y^+ , and 12–15-day-old virgin females homozygous for the *scute* and the *yellow* genes. Six bottles, each containing two males and five females, were set up and the offspring examined for mosaic patches on the body. In matings of this sort, three types of progeny were distinguishable. These consisted of males without the Y chromosome (XY^-), which were yellow scute in appearance, XY males and $X^{c2}X$ females, which were wild-type in phenotype. However, about 95% of the progeny were males of either the XY^- type or of the XY type, the remaining flies being gynanders and females. The high proportion of males suggested a severe loss of X^{c2} gametes during spermatogenesis. From about 2000 flies examined fifty-six 'useful' gynanders were found. 'Useful' mosaic flies were those gynanders which exhibited a patch or patches of wild-type tissue large enough to be detected under $40\times$ magnification.

In the gynanders, scute areas were easily distinguishable by the yellow bristles as well as the slightly lighter colour of the patches. Bilateral mosaic flies were particularly easy to detect as the female sides were always slightly larger than the male sides.

Three bristle systems were examined; these were the head, the scutellar and the sternital abdominal bristles. Observations on the head bristles consisted of counting the number of ocellar, orbital, vertical and the post-vertical bristles. The numbers of scutellar bristles were counted and the positions of the bristles noted. Observation on the abdominal bristles consisted of counting the number of bristles on the 5th segment only, the positions not being noted. Finally, the positions of the head and scutellar bristles, as well as the positions and sizes of the mosaic patches of each gynander, were drawn on a prepared sheet showing dorsal and ventral views of the fly.

3. RESULTS AND DISCUSSION

Initial measurements were made of the relative sizes of the dorsal surfaces of the heads, thoraces and the abdomens on a number of flies. It was found that if the area of the head was taken as 1 unit, the area of the thorax was on the average about 2.5 units and that of the abdomen was about 4.5 units. Hence the total area of a fly, summing over both the dorsal and ventral surfaces, can be considered to be 16 units.

From the above approximations, together with the individual drawings of the gynanders, it was possible to divide the flies into four classes in terms of the sizes of mosaic regions. In the present material all but one gynander had areas of scute tissues larger than those of wild-type. This was probably due to frequent losses of the ring chromosomes during the first mitotic divisions of the zygotes, as later losses would have led to flies with more wild-type tissue. Hence in the present work a 'mosaic region' refers to an area where a patch of wild-type tissue is situated in a yellow scute background, so that a $\frac{1}{4}$ mosaic is a fly with $\frac{3}{4}$ yellow scute and $\frac{1}{4}$ wild-type tissue on the body surfaces. The classification of the flies according to mosaic area gave the following numbers:

<i>Mosaic area</i>	<i>Number of flies</i>	<i>Sites</i>
$\frac{1}{2} \geq \text{area}$	10	mainly on head; 2 flies with abdominal patches
$\frac{1}{2} < \text{area} \leq \frac{1}{8}$	12	mainly on head; one fly with abdominal patches
$\frac{1}{16} < \text{area} \leq \frac{1}{8}$	11	mainly on head and thorax; one fly with abdominal patches
$\frac{1}{8} < \text{area} \leq \frac{3}{4}$	23	All three regions

Complete bilateral mosaics were not found in this experiment; some flies had only their heads equally divided into two regions, while others had their abdomens or thoraces equally divided, or various combinations of this sort. The dorsal and ventral surfaces were also frequently different in appearance, a fly which was mosaic on the dorsal surface of the head perhaps being completely yellow scute on the ventral surface and so on.

Ignoring for the moment the observations from flies with small and irregular patches, the data can be divided into observations on completely wild-type or scute parts (head, thorax or abdomen) and observations on mosaic parts divided equally into two regions. The mean numbers of bristles in the various classifications are shown in Table 1.

The number of bristles on wild-type heads showed very little variation. All flies, with one exception, had fourteen bristles on the head; the remaining fly had one median orbital bristle missing. In heads consisting entirely of scute tissues the ocellar bristles were almost always missing; of nineteen such flies, only one differentiated an ocellar bristle on the scute tissue. In general the number of head bristles in the scute tissue was slightly less than half the number found in the wild-type tissue. The lack of ocellar bristles was general in the remaining twenty-three flies with smaller patches of scute tissues on the area where ocellar bristles are

usually situated; this is so even when the scute tissue occupied less than one-quarter of the total dorsal area of the head.

Mosaic patches in the present material on the scutellum were always bilaterally divided into a scute and a wild-type side. Twenty-two out of twenty-three such flies had no bristle on the scute side and two normal bristles on the wild-type side. The exceptions were one fly with no scutellar bristle on either region of the scutellum and one fly with one yellow bristle on the scute side, in addition to the two normal bristles on the wild-type region. It should be pointed out that the mean number of scutellar bristles in the original scute stock used here was extremely low, the mean in the XY⁻ sibs of the gynanders being only 0.06 ± 0.28 bristles. A low mean number of scutellar bristles on the scute regions on the gynanders would therefore be expected.

Table 1. *Mean number of bristles on scute and on wild-type tissues over the body in Drosophila gynandromorphs*

	Head				Abdomen (5th segment)				Scutellum			
	Complete		Bilateral mosaic		Complete		Bilateral mosaic		Complete		Bilateral mosaic	
	+	sc	+	sc	+	sc	+	sc	+	sc	+	sc
Number of observations	12	19	11	11	3	37	13	13	—	33	23	23
Mean number of bristles	13.92	6.79	7.00	3.36	15.33	6.51	9.69	3.62	—	0.03	1.91	0.04
Standard error	0.08	0.25	0	0.20	1.33	0.18	0.65	0.29	—	0.03	0.09	0.04

Professor M. M. Green and Mr W. Scowcroft have done a similar experiment using the *claret* nondisjunctional stock and they have kindly allowed us to report their result. Among forty bilateral scutellar mosaics they found the mean bristle number of wild-type tissue to be 2.00 and the mean bristle number on the scute half to be 0.15 bristles. They also found that in mosaics involving the head region, the scute half always lacked the post-vertical while the wild-type half was normal. We are most grateful to Green and Scowcroft for allowing us to quote these unpublished results, which confirm ours completely.

The mean number of bristles on the 5th abdominal segment in the scute areas was less than half the mean number in the wild-type areas. This was so when the comparison was based either on whole segments between flies, or on adjacent mosaic regions within flies.

Small scute regions on the abdomen were always sparsely populated by abdominal bristles. When small scute regions were found on the head these were always accompanied by a reduction of major head bristles; as mentioned earlier, if such a patch was situated near the centre of the head, the ocellar bristles were often missing. On the other hand, small patches of wild-type tissue on the head were always accompanied by the normal complement of bristles, irrespective of the sizes of scute tissues surrounding them.

The above evidence confirms the statement of Sturtevant (1932) that large patches of scute tissue are autonomous, and in addition points to the autonomy of the wild-type tissues situated in a scute background, at least with respect to the differentiation of the three bristle systems under discussion. The last statement is probably true if the mosaic fly which failed to grow any scutellar bristles on the wild-type side mentioned earlier was not due to interference by the neighbouring scute tissues. Since no evidence of interference in bristle formation was found in the small wild-type patches on the head, the failure to grow any wild-type scutellars in this particular gynander might be due to developmental accidents. The evidence suggests that bristle make is not diffusible from a high region to a low region, or we would expect to find distributions of bristle numbers in the gynanders different from those observed. For example, we would expect to find one bristle on each side of a scutellum which is bilaterally mosaic for the two types of tissue. In addition, if make is diffusible, we would not expect to find a large difference in mean abdominal bristle number between the adjacent mosaic regions of approximately equal size.

From the present data, flies with $\frac{15}{16}$ scute and $\frac{1}{16}$ wild-type tissues would presumably have a lower general supply of bristle-making substances than the wild-type flies. Even in such flies the wild-type areas grew a normal wild-type quota of bristles, which suggests that diffusible bristle-making substances are not limiting and are not responsible for the differences in make between the wild-type and scute flies.

In unselected populations of scute flies, the mean numbers of both the scutellar bristles and the head bristles are greatly reduced. But scute flies can grow bristles on any of the four normal sites on the scutellum (Rendel, 1965) as well as on any of the fourteen sites on the head (Young, unpublished). It seems reasonable to conclude, therefore, that the pre-patterns for bristle systems are similar in both the unselected scute and wild-type flies. Differences in make between the two genotypes therefore may be due to difference in competence between the scute and the wild-type tissues.

How are these results to be squared with those of Rendel (1965) who showed very clear lack of independence between various bristle sites on the thorax of scute flies? For example, in scute flies with one anterior and one posterior scutellar these are nearly always on opposite sides of the fly. Yet our mosaic results suggest complete autonomy of bristle-forming sites. Reconciliation lies in the difference in the level of expression of the scute gene in the two experiments. In our case scute tissue, whether or not mosaic, was nearly always *completely* lacking in scutellar bristles. At this extreme level of expression, tissue competence is the limiting factor so that no amount of bristle-forming substance provided by the wild-type tissue could induce a bristle in scute tissue. In Rendel's data all the scute stocks had a rather large number of scutellar bristles, the lowest being a mean of 0.5 bristles with a significant number of flies with two bristles. In such lines tissue competence is presumably high enough so that differences in some diffusible substance come into play. In such situations an increase in diffusible substance may be accompanied by an increase in bristle number. Alternatively, an increase in the ability of the tissue

to react with the substance will also increase the bristle number. In flies with a mean scutellar bristle number close to 4 any increase in number may require an increase in the number of active bristle sites on the scutellum as well as an increase in tissue competence.

An alternative model that explains both the present results and those of Rendel is one that rests on the assumption that diffusible substance is limited in supply and is used up in the formation of a scutellar bristle. If this were true then on a mosaic scutellum the normal tissue, because of its higher competence, would form bristles and in the process use up all of the diffusible substance available. The scute portions of the scutellum would then be unable to form bristles. Thus, the differences in competence between normal and scute tissue are reflexions of the sensitivity of the cells to induction by diffusible substance. We may imagine scute tissue requires a very much higher concentration of diffusible substance to induce the formation of a bristle. Since in a mosaic there is normal tissue competing for diffusible substance, and very much more sensitive to induction by this substance, the observation that all bristles are formed in wild-type tissue is perfectly reasonable. Yet this apparent autonomy is an autonomy only of tissue competence and not of inducing substance. In flies that are entirely scute but have variable amounts of bristle-making substance the correlations observed by Rendel (1965) would be expected.

If the above speculation is reasonably correct, then selection for higher bristle number in wild-type flies would tend to select for higher tissue competence as well as for a change in pre-patterns. On the other hand, similar selection in the scute flies could be mainly directed to increasing tissue competence. If this is so then it may account for the fact that selection for more scutellar bristle in scute flies could only increase the number of scutellar bristles in the wild-type sibs after many generations of selection (Rendel, 1959, and unpublished). The genetic gain in actual scutellar bristle number in wild-type sibs when selection is practised on scute flies is also smaller than when selection is applied directly to the wild-type flies under similar selection pressure (Sheldon, personal communication). Further experimental evidence on both the changes in number and patterns in selection lines of different genotypes are required to clarify this speculation.

SUMMARY

Difference in bristle-making abilities in extreme scute and in wild-type *Drosophila* were investigated using flies mosaic for the two kinds of tissues. It was found that both wild-type and scute tissues were autonomous with respect to bristle differentiation. From the experimental evidence it was postulated that the differences in bristle-making abilities between the two genotypes might be due to differences in tissue competence rather than differences in pre-pattern or in level of bristle-making substances.

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