THE EFFECTS OF SEXCOMBLESS ON THE FORELEGS OF DROSOPHILA MELANOGASTER[1]

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THE role of genes in differentiation has long been recognized. That there must be a discipline that guides the nonrandom differentiation of patterns is obvious from one simple fact: that the pattern is reproducible in every generation. It is evident that at certain developmental stages specific cells or cell areas are singled out from the rest to differentiate and thus form a pattern of differentiation. An underlying cause of this specific pattern was seen in existence, previous to the differentiation, of a nonuniform distribution of properties termed "prepatterns" (STERN 1954, a, b). No specific definition of the nature of prepattern was given, leaving it open whether they have a morphodynamic identity or provide simple stimuli such as might result from specific physical or chemical properties. One may then ask: is there any way to conceive a mechanism by which a prepattern lays a foundation for the realized pattern? This question must ultimately be answered in biochemical terms. Already tissue specific patterns have been measured at the protein level as for instance in respect to isozyme patterns in the lactate dehydrogenase (LDH) system of mice (MARKERT and URSPRUNG 1962). It has been suggested the steps preceding formation of LDH may serve as examples of prepattern for the final pattern of the enzyme system (see (URSPRUNG 1963).

Presumably, a consecutive series of prepatterns may be formed in the course of development until a final pattern is realized. In the course of evolution of a species a specific terminal morphogenetic pattern might be lost by a single mutation but the prepatterned site might still be there. The interalar bristle, for example, is present on the thorax of some Dipterans normally, but not present in *Drosophila melanogaster*. STERN (1956) has shown that the prepattern for this particular bristle is present in both normal and the mutant form, Theta, but they differ from each other in having different cellular competence to respond to the prepattern. A second example concerns the development of the wing in the chick embryo. Here, the fibulare, a bony structure normally absent in modern birds can be caused to appear by simple genetic or morphogenetic alterations (HAMPÉ 1959; see WADDINGTON 1962).

The present work involves a study of the effects of the gene sexcombless (*sx*)

and its interaction with several other genes affecting the basitarsus of the pro-thoracic legs of *D. melanogaster*. It furnishes data to test for differential levels of action of genes and to find a means of distinguishing between pattern and pre-pattern genes. Earlier work on the effect of genes in various combinations has shown that the effect of one gene in producing a certain phenotype may be limited to subthreshold action, yet two or more genes combined together may have an additive or a superadditive effect (NEEL 1941, 1943; HOUSE 1953 a, b).

MATERIALS AND METHODS

Sexcombless (*sx*) is sexlinked and causes absence or a reduction in the number of teeth of the sex comb. Sexcombless males are poorly viable and are sterile because of abnormal differentiation of the genital disc usually resulting in lack of attachment between the testis and vas deferens (STERN 1941). The females carrying *sx*, therefore, are always heterozygous. The stocks contain-ing *sx* were of the following types. *sx vb² sy*/FM4 females × FM4/Y males (strain 1) and *y ac sn³ sx vb² sy/y sc^{S1} In dl-49 B v w^a sc^8* females × *y sc^{S1} In dl-49 B v w^a sc^8*/Y males (strain 2). For studies of the basitarsi of *sx* individuals the symbols *sx*-(1) and *sx*-(2) will desig-nate strains 1 and 2, respectively. For interaction studies *sx* will imply the strain 2, unless other-wise specified.

sx is associated with a compound inversion and is inseparable from two other mutant genes: vibrissae² (*vb²*) and small eye (*sy*). Salivary gland chromosome analysis shows that it involves an overlapping double inversion covering the region between 11D and 15E (MUKHERJEE 1963).

Three mutants were used for studies on interaction effects with *sx*: engrailed (*en*), recessive, second chromosome; transformer (*tra*), recessive, third chromosome; and eyeless-dominant (*ey^D*), dominant, fourth chromosome. A description of these mutants will be given later.

Samarkand (Sam +) and Canton-S wild-type individuals and combinations of mutants not including *sx* were used as controls. In view of the complex nature of the *sx* carrying X chro-mosome, no attempt was made to make the controls isogenic with *sx* stocks. Stock cultures were kept at 23 ± 1°C, as this was found to give a maximum yield of adult *sx* males. Also, *sx* males were obtained in much higher frequency when the heterozygous females were outcrossed than when inbred.

The forelegs were dissected out in 70 percent alcohol and mounted immediately in a drop of ANDRÉ medium (GOTTLIEB 1963), either on a slide or between two coverglasses. After mounting, the legs were observed under magnifications of approximately 500 to 900× and drawings were made with a camera lucida.

RESULTS

1. *Morphology and chaetotaxy of basitarsi of* sx *male forelegs*

General: During the development of the foreleg of a wild-type fly tissue located in the distal and ventral area of the female basitarsus remains more or less un-changed in its topographic arrangement and differentiates the terminal transverse rows of bristles. In the wild-type male this tissue undergoes a shift so as to place the potential transverse row or rows in a more or less longitudinal position and, in addition, leads to the differentiation of sex-comb teeth (TOKUNAGA 1962).

A comparison of basitarsi of sexcombless males with those of normal males and females shows that apart from the reduction of the sex comb the gene *sx* causes a change in the ultimate pattern (Figure 1). In *sx* males (1) the number of transverse rows is more like that in the normal female than in the normal male, (2) the arrangement of bristles in the sex-comb area, although greatly distorted, approaches often that in normal females, (3) the distal bractless
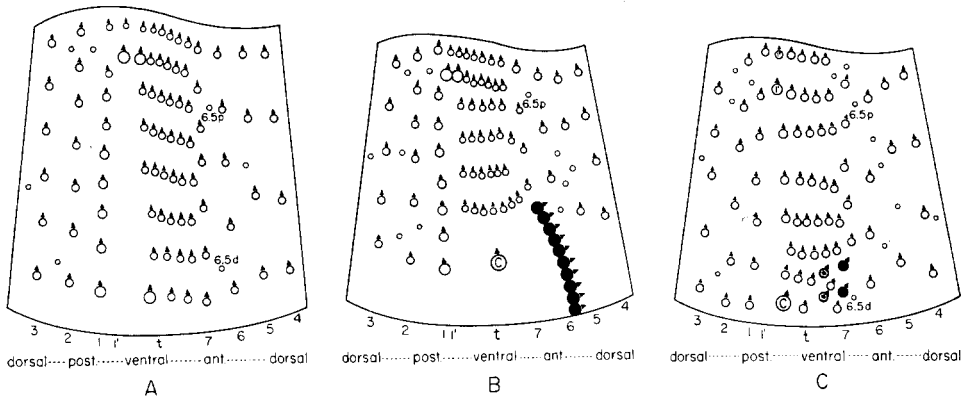
FIGURE 1.—A. Diagram of the basitarsus of a normal female foreleg. B. Basitarsus of a normal male foreleg. C. Basitarsus of a *sx* male foreleg. The basitarsi have been represented as cut lengthwise between longitudinal rows 3 and 4, and laid out flat. t = region of transverse rows; numbers 1' and 1 to 7 denote the various longitudinal rows; open circles with triangles = normal bristles with bracts; open circles = bractless bristles; dot-centered circles with triangles = intermediate bristles with bracts; 6.5p, 6.5d = proximal and distal bractless bristles respectively, between longitudinal rows 6 and 7; solid circles and with triangles = primary sex-comb teeth; C = central bristle. (A: TOKUNAGA, unpublished data; B: after TOKUNAGA 1962. The number of bristles indicated in the various rows varies in different strains. The diagrams for the normal female and male basitarsus are based on average bristle numbers in the wild-type Oregon-RC stock).

bristle, 6.5d, is almost always present in a position similar to that in normal females, and (4) the bristle pattern in the last transverse row, often, is indistinguishable from that in the normal female. This transverse row consists usually of three bristles in normal females but is absent in normal males. These facts suggest that *sx* tends to feminize the basitarsi of the male. However, as will be described later, certain peculiarities of the *sx* basitarsi are not simply derivable from feminization.

In females heterozygous for *sx*, the basitarsal bristle pattern is not different from that in homozygous normals.

The sex comb: A typical tooth is a bracted bristle which is highly pigmented, thick and blunt at the free end (Figure 2). In normal males, as stated above, the bases of the teeth form a longitudinal row but the teeth themselves point in an approximately transverse direction, in contrast to the proximal-distal orientation of normal bristles. However, isolated teeth may be oriented proximal distally or at an intermediate angle (see TOKUNAGA 1962).

The shape of teeth in *sx* males varies considerably. Five types are shown in the right part of Figure 3. The "subtypical" teeth in 1, 2 and 3 are dark like typical teeth but less thick and less blunt than those in 4 and 5, which are typical teeth. Figure 3, left, shows the various morphological forms of intermediate bristles which range from nearly normal but thickened macrochaetae to an almost typical tooth, which however, is pointed and less dark (see also Figure 4). The existence of various types of intermediate bristles shows that two different processes are concerned, involving (a) shape and size, and (b) concentration of pig-
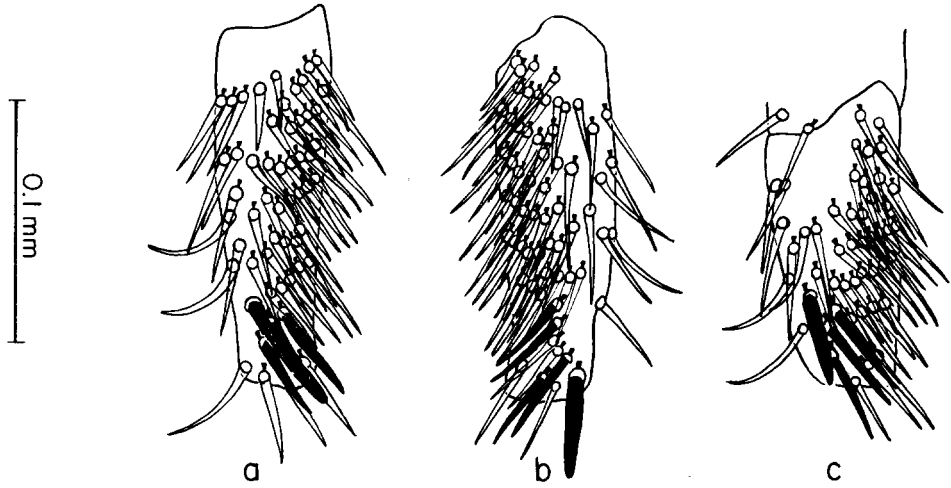
FIGURE 2.—Basitarsi of *sx* male forelegs showing orientation of normal bristles, teeth and intermediate bristles on the ventral side. Solid blunt and solid pointed bristles are teeth and intermediate bristles, respectively. Note extra bristles in the proximal region of all three basitarsi. (a) Two teeth and two intermediate bristles oriented at an intermediate angle. (b) three intermediate bristles, two ordinary bristles and one tooth forming more or less a single row. (c) a short basitarsus, with a tooth proximal to the three intermediate bristles.
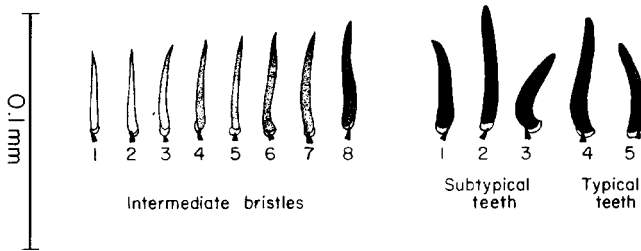
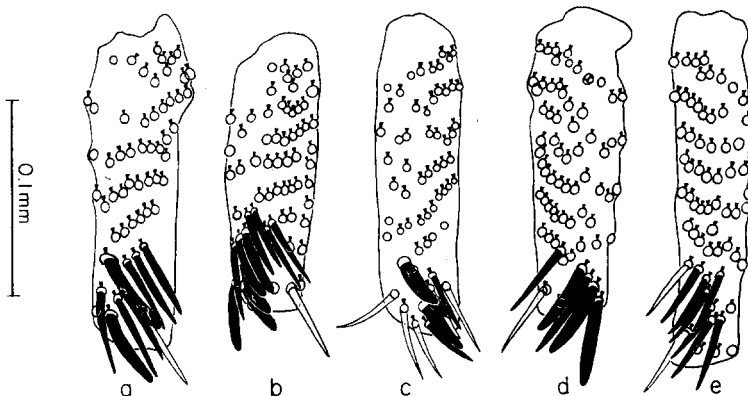FIGURE 3.—Various forms of intermediate bristles and teeth found in *sx* male basitarsi

FIGURE 4.—(a) to (d) Basitarsi of *sx* male forelegs showing teeth and intermediate bristles of different forms and in different positions. (e) Basitarsus showing seven intermediate bristles and no teeth: an extreme effect of *sx*.

TABLE 1

Distribution of teeth in primary sexcombs of sexcombless and nonsexcombless males

Genotype	Number of legs	Number of teeth							Mean±SE
		In sx type							
		0	1	2	3	4	5	6	
(a) sx-(1)	151	31	49	49	18	3	1	0	1.44±0.09
(b) sx-(2)(Y)	20	4	11	5	0	0	0	0	1.05±0.15
(c) sx-(2)(sc^8·Y)	42	10	25	7	0	0	0	0	0.93±0.1
(d) sx-(2)(0)	116	60	42	13	1	0	0	0	0.61±0.07
		In sx^+ type							
		7	8	9	10	11	12	13	
(e) Samarkand +*									
(Sam +)	30	0	3	11	11	4	1	0	9.63±0.18
(f) Canton-S*	30	1	7	9	11	2	0	0	9.20±0.18
(g) y ac sn^3 v/Y	30	0	1	1	5	9	7	7	11.37±0.23
(h) +/0	30	0	8	18	3	1	0	0	8.90±0.13

* Original data from DR. P. E. HILDRETH.

ment. The shape and size of intermediate bristles vary from short and slender to thick and long. The pigmentation of these bristles varies to some degree independently. A long intermediate bristle, although usually dark, may have lighter pigmentation than a short one. The arrangement of teeth on the sx basitarsus is variable: teeth may be relatively far apart or adjacent to one another (Figure 4).

Four genotypically different strains carrying sx were examined for number of teeth (Table 1). The X chromosomes of b, c and d are derived from strain 2, but the flies differ in respect to the Y chromosome and the autosomes. Table 1 (lower half) also presents the number of teeth in four different sx^+ strains.

The mean average of the numbers of teeth in sx was 1.14 (range: 0–5). Some of the differences in the mean number of the four strains are highly significant, but no detailed analysis of the variability was made.

Forty teeth in 27 sx-(1) and four Sam + males have been measured by means of an ocular micrometer, corrected on the basis of estimates made for those teeth which are bent, or not in horizontal plane. The length varies from 16 to 27 units in sx males as compared to from 21 to 26 units in Sam + males (1 unit = 1.75 microns). However, the mean lengths, 23.33 and 24.2, respectively, are not significantly different from each other. The differences in lengths of teeth in sx do not seem to be a function of the position of the teeth along the length of the sex-comb area (Figure 4 c, d).

Altogether, 333 legs have been analysed for intermediate bristles (Table 2). Their overall mean number is 2.3. Again, two intermediate bristles may lie adjacent to each other or in different regions of the basitarsus (Figure 4 a to e). In some cases two intermediate bristles may remain separated by a gap with or without a macrochaeta in between (Figures 2a and 4a). Figure 4e presents an extreme case having seven intermediate bristles oriented more or less like a sex comb, but there is no tooth.

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TABLE 2

Distribution of intermediate bristles in sexcombless strains

| Genotype | Number of legs | Number of intermediate bristles | | | | | | | | Mean ± SE |
		0	1	2	3	4	5	6	7	
(a) sx-(1)	151	12	29	33	34	27	8	6	2	2.62 ± 0.13
(b) sx-(2)(Y)	20	6	3	6	4	1	0	0	0	1.55 ± 0.36
(c) sx-(2)(sc⁸·Y)	42	0	8	13	10	6	4	1	0	2.72 ± 0.2
(d) sx-(2)(0)	120	11	34	43	21	10	1	0	0	1.93 ± 0.1

Transverse rows of bristles: Table 3 presents the mean numbers of transverse rows, of bristles per row and of bristles in the second transverse row, in normal and *sx* male basitarsi. The reason for the presentation of the mean number of a specific, the second transverse row, is to convey an impression of the degree of constancy of bristle number in terms of individual rows. The second transverse row is characteristically distinguishable from all others by its close association with the second 1′ bristle. Although, sometimes, an additional row is intercalated between the first and the row under consideration, the mean numbers presented in column 3 of Table 3 refer to the typical "second" row which is in association with the second 1′ bristle.

A minimum of three bristles in a row has been considered as a criterion for a true transverse row. However, in cases where two bristles appear in transverse arrangement, between two typical rows, a value of 0.5 is added to the number of complete transverse rows. This designation was not applied to the most distally present bristles—unless there were at least three bristles, present in a distinct row, they were not considered as constituting a transverse row. This classification is based on the fact that, in case of two bristles only, the posterior one corresponds presumably to the central bristle of the normal male leg while the anterior one

TABLE 3

Comparison among males of various genotypes of bracted bristles in transverse rows and of bractless bristles between longitudinal rows 4 and 7

| | Transverse rows | | | Bractless bristles | |
| | | Mean average No. of bristles | | Mean number | |
Genotype	Mean No. of rows (1)	per row (2)	in 2nd row (3)	6.5*	between long. rows 4—7
(a) Canton-S†	5.52 ± 0.08	6.12 ± 0.03	6.7 ± 0.23	1.0	5.0
(b) Sam +†	5.85 ± 0.06	5.87 ± 0.07	6.03 ± 0.23	1.0	5.0
(c) γ ac sn³ v	6.32 ± 0.1	5.88 ± 0.1	6.3 ± 0.17	0.9	4.5
(d) sx-(1)	7.03 ± 0.1	5.12 ± 0.08	5.23 ± 0.1	2.6	5.8
(e) sx-(2)(Y)	6.75 ± 0.2	4.41 ± 0.1	5.0 ± 0.1	1.7	5.3
(f) sx-(2)(sc⁸·Y)	7.2 ± 0.16	5.3 ± 0.14	5.4 ± 1.2	2.6	6.6
(g) sx-(2)(0)	6.94 ± 0.12	4.7 ± 0.1	5.0 ± 0.5	1.5	5.3

* The mean number of bristles 6.5 in Sam + females equals 2.
† Data from DR. P. E. HILDRETH.
Number of legs studied: a, b, c: 30; d-(1): 147; d-(2), (3): 30; e: 20; f-(1): 35; f-(2), (3): 16; g: 23.

is presumably homologous to the distal bristle of the seventh longitudinal row (TOKUNAGA 1962).

The number of transverse rows in sx males varies from five to nine. Their mean number in the four sx strains is approximately seven, as compared to only about six in the three sx^+ strains (Table 3). The difference is significant at the 1 percent level. In contrast, the mean average of bristles per row and specifically of bristles on the second row is reduced by about one in sx males as compared to those in normal males.

The difference between sx and + types in the morphology and chaetotaxy of the distal-ventral part of the male basitarsus is striking. The distal transverse rows are often greatly distorted or lie at an angle intermediate between the angle formed by the other transverse rows and that formed by sex-comb teeth (Figures 2 and 4). Sometimes, the last transverse row is oriented in such a way that some of the bristles are arranged like the bristles in other transverse rows, and others at an angle. The former bristles are normal macrochaeta-like; the latter are of intermediate forms (Figures 2b and 4b). In most cases the intermediate bristles of such partially "rotated" transverse rows are present in the region close to the sex-comb forming area (see also Figure 7b). However, occurrence of ordinary bristles in that area is not inhibited by such an arrangement.

Longitudinal rows of bristles: The bristle pattern in the areas of the longitudinal rows in sx males differs from that in the sx^+ types by a constant feature: the appearance of the 6.5d bractless bristles in sx males, distally, as in normal females, between longitudinal rows 6 and 7, as compared to the existence of about four bractless bristles at somewhat proximal and anterior positions in normal males (Figure 1). Occasionally, in sx other bractless bristles appear between longitudinal rows 6 and 7 and also between the longitudinal row 7 and the region of the transverse rows (Figure 2). The mean numbers of all 6.5 bractless bristles, which vary in different strains of sx studied, and those of all bractless bristles in the area between longitudinal rows 4 and 7 are higher by about one in sx males than those in the corresponding non-sexcombless strains (Table 3, compare numbers in the last two columns).

Central bristle: In normal male basitarsi a large bristle, the central bristle, is found regularly in the area posterior to the sex comb which is otherwise bare. In sx basitarsi, although the central bristle often remains distinct, its location varies considerably. It has been repeatedly observed both in sx males as well as in mosaic patches with sx (MUKHERJEE 1964) that whenever the distal-most region is hemizygous for sx the posterior bristle of what corresponds to the last transverse row of the female remains unchanged in shape, while the two anterior bristles may be transformed into intermediate bristles. Also, sx basitarsi have been found having one or two teeth at the distal end lying close to the unchanged bristle in the posterior region, or having a tooth and an intermediate bristle in the distal-most region with the posterior bristle slightly shifted (Figure 4 a, d). These findings support the interpretations earlier made by TOKUNAGA (1962) that the posterior bristle in the distal transverse row is the potential central bristle of the male basitarsus.

Correlation tests: The facts reported suggest that *sx* interferes with the differentiation of seta-forming cells into teeth. The results obtained from correlation tests of different pairs of characters are compatible with this hypothesis.

Three genotypes were studied: typical *sx* males, *sx*; *tra/tra* males and *sx/+*; *tra/tra* individuals transformed into phenotypic males by their *tra/tra* genotype. (The gene transformer (*tra*) changes the female into male phenotype and thus causes the production of male external genitalia and sex combs. It has no effect on the male [STURTEVANT 1945]). No significant correlations were found for (a) number and (b) length of teeth *vs.* length of basitarsus. In all three genotypes the correlation coefficients for the number of transverse rows *vs.* the sums of numbers of teeth and intermediate bristles are positive, those in *sx* and *sx/+*; *tra/tra* not being significant at the 5 percent level, and that in *sx*; *tra/tra* being significant at the 1 percent level. Number of teeth *vs.* number of intermediate bristles showed negative correlations, significant at the 5 percent level for *sx* and at the 1 percent level for the other genotypes. These negative correlations suggest a trend toward constancy of total number of chaetae with potential differentiation into bristles or teeth. Such a trend for the bristles of the whole region of transverse rows is also indicated by negative correlations for all three genotypes between the numbers of transverse rows and bristles per row. It is highly significant for *sx* and *sx/+*; *tra/tra* but does not reach significance at the 5 percent level for *sx*; *tra/tra*.

II. *Effects of interaction in different genotypes*

Eyeless-dominant (*ey^D*) causes the formation of abnormal eyes, and of a large number of teeth in the primary sex comb (BRIDGES and BREHME 1944). Engrailed (*en*) apart from effects on the wing and the scutellum, causes the appearance of a secondary sex comb arranged in a mirror image fashion to the primary (BRASTED 1941). The effect on the scutellum served as primary marker for the identification of engrailed phenotypes.

Basitarsi of male forelegs from the following combination genotypes were examined: *sx;ey^D/+*, *sx;en/en*, *en/en;ey^D/+*, *sx;en/en;ey^D/+*, *sx;en/+*, *sx; en/+;ey^D/+*, *sx;tra/tra* and *sx/+;tra/tra*. These will be referred to as *sx;ey^D*, *sx;en*, *en;ey^D*, *sx;en;ey^D*, *sx;en/+*, *sx;en/+;ey^D*, *sx;tra/tra* and *sx/+;tra/tra*, respectively.

A. sx-en-ey^D *interactions. General:* In both *ey^D* males and females the distal region of the basitarsus which includes the sex-comb area is greatly enlarged (Figure 5), and in many specimens continues without separation into the second tarsal segment. Often, the *ey^D* basitarsus extends lobes of tissue into the second segment with one or two bristles in each lobe (similar to those in Figures 6e and 7b). The number of teeth in the sex-comb area is greatly increased and they are arranged in two or more rows. Sometimes the rows extend into the distal end of the second tarsal segment (STERN, unpublished; see also STERN and MUKHERJEE 1964). The swelling of the sex-comb area, the fusion of the basitarsal and second tarsal segments in a telescoping fashion and the formation of sex-comb teeth in
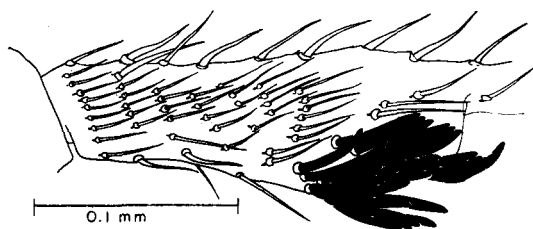
FIGURE 5.—Basitarsus of ey^D male foreleg. (From STERN and MUKHERJEE 1964).

two or more rows are associated with an increase in the number of bristles corresponding closely, or lying adjacent, to the central bristle. These peculiarities suggest a multiplication of tissue in that area.

As in ey^D, so also in $sx;ey^D$, there is a complete or partial failure of separation between the basitarsus and the second tarsal segment. In addition, in $sx;ey^D$ there are rows of "rotated" or "shifted" bristles in the sex-comb area, sometimes extending into the second segment.

Different degrees of epistatic effects of sx over ey^D are shown in Figure 6: (a), (b) represent two extreme specimens with more or less ey^D-like manifestation, (e) the opposite extreme resembling a sx basitarsus, and (c), (d) representing intermediate forms.

Thus a comparison of the sx, ey^D and $sx;ey^D$ male basitarsi shows that in the combination the independent effects of sx and ey^D are superimposed on each other to a variable extent. This will further be established by analysis of the basitarsal chaetotaxy.

In $sx;en$ and $sx;en;ey^D$ combinations the forelegs are abnormally developed. The first and second tarsal segments are affected invariably. Frequently, other
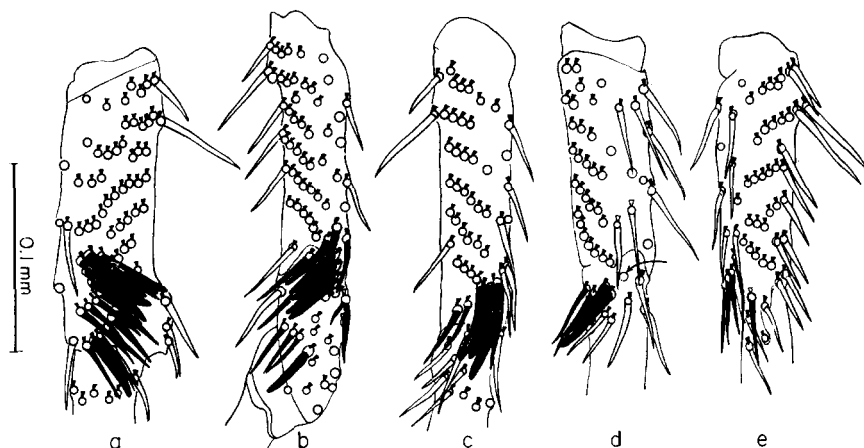

FIGURE 6.—Basitarsi and second tarsal segments of $sx;ey^D$ male forelegs showing expressions from extreme ey^D type to extreme sx type. Note the lobe-like structure at the junction of the basitarsus and second tarsus in (e) and the 6.5d bractless bristle (arrow) located in the second tarsus in (d).

TABLE 4

Mean numbers of teeth and intermediate bristles (I.B.) in primary and secondary sexcombs of sx, en, ey^D *and their combinations, and those of secondary transverse rows in* sx-en *combinations*

Genotype	Number of legs	Primary sexcomb		Secondary sexcomb		Secondary transverse rows	
		Teeth	I.B.	Teeth	I.B.	Number	Brsl./row
sx	20	1.05	1.55	. . .	. . .	. . .	. . .
ey^D	30	±28	. . .	. . .	. . .	. . .	. . .
en	15	10.4	. . .	6.53	. . .	. . .	. . .
en;ey^D	25	±31	. . .	25.3	. . .	. . .	. . .
sx;ey^D	100	1.96	3.63	. . .	. . .	. . .	. . .
sx;en	45	1.53	1.7	0.31*	2.3	6.0	2.35†
sn;en;ey^D	40	2.9	1.5	2.55	2.75	6.93	2.91†

* In *sx*-(1);*en* the mean number is about 0.6.
† Based on 15 legs.

segments tend to fuse and look shortened and telescoped. The overall bristle pattern is greatly distorted, such that the bristles particularly on the dorsal side are very slender, bent and pointing in all directions; sometimes, whorls similar to those on the thorax of dumpy-vortex mutants, appear on the dorsal side (Figures 8b, 9b); diverging wrinkle-like lines come out from whorls. Also bristles, mostly long, a few short and sharp-pointed, appear in or around the whorl. The long bristles are bent and oriented in such a way that they form a "spiral."

Analysis of the chaetotaxy in *sx;en*/+ and *sx;en*/+;*ey^D* basitarsi shows that *en* has no subthreshold effect for the baritarsal bristle pattern in heterozygote *en*/+.

Sex-comb teeth and intermediate bristles: Table 4 summarizes the mean numbers of teeth and intermediate bristles in the primary and secondary sex combs. The mean number of teeth in the primary sex comb in *sx;ey^D* is significantly higher by about one than that in *sx* males (P < 0.02). The ranges are 15–40 in *ey^D*, 0–2 in *sx* and 0–6 in *sx;ey^D*. The average numbers of primary sex-comb teeth in *sx;en* and *en;ey^D* are higher than those in *sx* and *ey^D*, respectively. Therefore, as expected, the mean number of teeth in the combined genotype *sx;en;ey^D* is further increased: 2.9 in this genotype as compared to 1.96 in *sx;ey^D*. Such increases in the number of teeth in the combined genotypes are also seen for the secondary sex comb. While the mean number of secondary sex-comb teeth in *en;ey^D* (range 6–35) is increased by a factor of about four over that in *en* (range 4–8) the mean number of secondary sex-comb teeth in *sx;en;ey^D* (range 0–7) is increased by a factor of about eight over that in *sx;en* (range 0–2). This increase may correspond more or less to simple additive effects of these genes. This is, however, not true with the intermediate bristles in the primary sex-comb area. While their mean numbers are similar in *sx*, *sx;en* and *sx;en;ey^D* types, the number is greater by about two in *sx;ey^D*.

Two cases of *sx;ey^D* males are worthy of special discussion. In these several teeth or intermediate bristles, or both, are arranged more or less in two rows (Figure 7). The relatively proximal and anterior row contains teeth and very
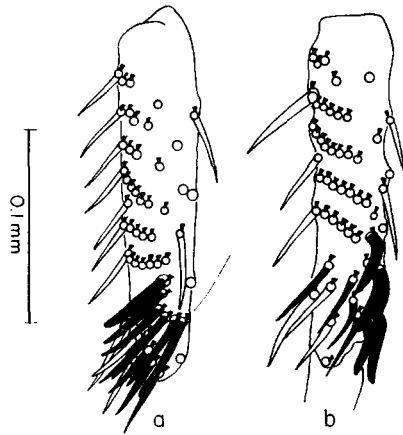
FIGURE 7.—*sx;ey^D* basitarsi showing two rows of teeth and intermediate bristles oriented at various angles. In (a) five intermediate bristles and one tooth form a semicircular pattern; in (b) a bracted bristle within a lobe-like structure in the second tarsal segment.

heavy intermediate bristles, while the distal and posterior row contains light intermediate bristles and no teeth. Further, in the specimen in Figure 7a, the bases of bristles in the proximal-anterior row form a semicircular pattern. It appears that there is a specific region in the sex-comb area which can react with a relatively lower threshold for the formation of teeth. This is the peak area of the effective prepattern gradient. The semicircular row presents a "still picture" of the bristle-cell to tooth formation process, which involves shifting of strips of tissue from transverse rows (and longitudinal rows 6 and 7) into the potential sex-comb area.

In neither specimen is there any striking enlargement of the sex-comb area, as compared to *sx* basitarsi, but that there is an increase in the underlying organization is evident from the appearance of two or three bristles, in addition to, and in the vicinity of, the central bristle.

Transverse and longitudinal rows of bristles: The means and range of numbers of transverse rows in the various combinations of *en* and *ey^D* with *sx* are not consistently different from the corresponding genotypes without *sx*. The numbers of bristles per transverse row is lower in *sx* than in *sx^+* genotypes. The total bristle numbers on transverse rows show no significant differences.

The most interesting fact about *sx;en* and *sx;en;ey^D* genotypes is that there are large numbers of extra bristles on the ventral side between longitudinal rows 1 and 3. Some of these extra bristles are arranged in secondary transverse rows, which average about six to seven in number (Table 4). Other extra bristles are located at random on the basitarsus and the incompletely separated second tarsal segment. Owing to crowding of bristles in the secondary transverse row area, the identity of individual bristles in longitudinal row 1 is difficult to establish (see Figures 8a and 9a). The mean number of bristles per secondary transverse row is, however, lower by about two to three, on the average, than that in the primary transverse rows (where it varies from 5.0 to 5.7). The mean numbers of secon-
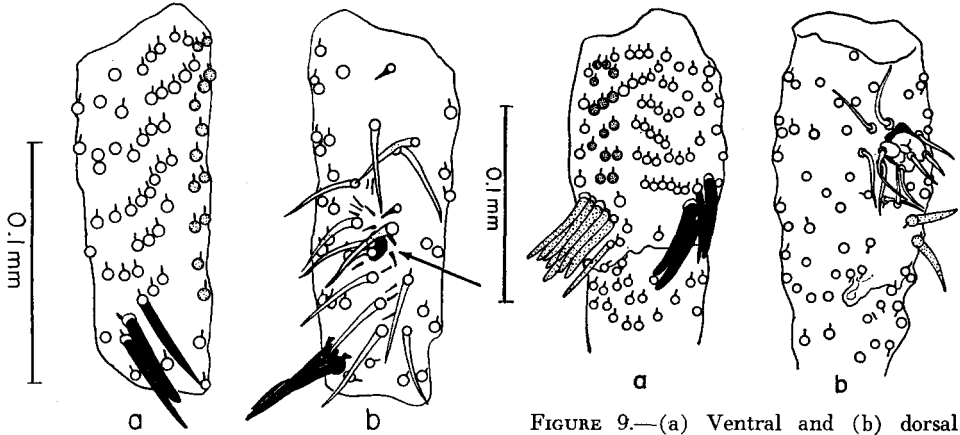
FIGURE 8.—(a) Ventral and (b) dorsal views of a basitarsus of *sx; en* male foreleg. Filled-in bristles = teeth and intermediate bristles. Stippled circles = secondary transverse row bristles; solid area indicated by arrow in (b) shows the whorl (see text).

FIGURE 9.—(a) Ventral and (b) dorsal views of a basitarsus of a *sx; en;ey^D* male foreleg. Here, solid bristles represent primary sex comb teeth and intermediate bristles; stippled bristles = secondary sex comb teeth and intermediate bristles; stippled circles = secondary transverse row bristles. Note the whorl and bent bristles surrounding it in (b).

dary transverse rows and bristles per (secondary) row in *sx;en;ey^D* are significantly higher than those in *sx;en* (P < 0.01).

The bristles in the secondary transverse rows are stronger than those in the primary. The secondary rows are continuous with the primary ones for the two most proximal rows. Beginning with the third row the two series of transverse rows gradually separate from each other, forming a widening gap between them.

The dorsal part of the basitarsi of different combination genotypes appears to be more sensitive to the genic interaction than the ventral side. The result is the production of a large number of bristles, many of which are bractless, in highly random arrangement and orientation (Figures 8b and 9b). So far as the bristle patterns on the various longitudinal rows are concerned no other interaction effect was observed.

B. sx-tra *interaction*. A comparative study of various aspects of bristle pattern of *sx*, *sx/+;tra/tra*, *+/+;tra/tra*, and *sx;tra/tra* basitarsi suggests that *sx* has a subthreshold action in heterozygous condition. This will now be shown.

The orientation of teeth in *sx/+;tra/tra* is almost as normal as in the wild-type sex comb (Figure 10). The number of teeth, however, in these *sx/+*heterozygotes is reduced to about one third that in normal males (Table 5). The range in *sx/+;tra/tra* and *sx;tra/tra* is 1–6 and 0–2, respectively, as compared to 10–14 in *+/+;tra/tra*. (Since the X chromosomes of *+/+;tra/tra* were either homozygous for *y ac sn^3 v* or heterozygous *y sc^{S1} In dl-49 B v w^a sc^s/y ac sn^3 v*, the higher range in case of *+/+;tra/tra*, as compared to *+/Y*—see Sam + and Canton-S in Table 1—is probably due to the genetic composition of the X chromosomes used in the former. The range and mean number of teeth in *y ac sn^3 v* males are 8–13 and 11.37 and those in *y sc^{S1} In dl-49 B v w^a sc^s* males are 10–14 and 11.5,
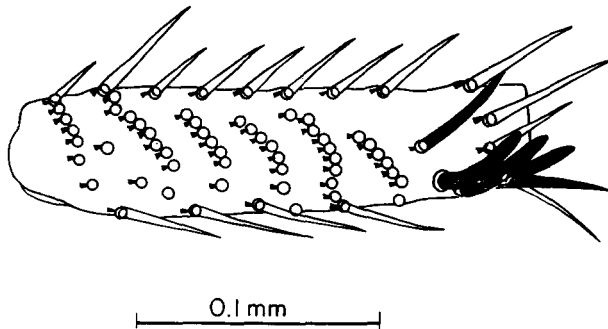
FIGURE 10.—Ventral view of a *sx/+;tra/tra* female basitarsus. Solid bristles = teeth and intermediate bristles.

respectively.) Further, in *sx/+;tra/tra* heterozygotes, intermediate bristles, similar to those in *sx* males, appear often in the distal region of the sex-comb area, sometimes in other parts but never between teeth. The mean number of intermediate bristles in *sx/+;tra/tra* is reduced to about half that in *sx;tra/tra* or *sx* males.

The mean number of teeth in *sx/+;tra/tra* is about four times that in *sx* males and about twice that in *sx;ey^D* males. It appears that the mechanisms underlying the increases in teeth numbers in *sx/+;tra/tra* transformed females and *sx;ey^D* males over that in *sx* males are different from each other. While the increase in the former is due to a subthreshold action of *sx* in heterozygotes, that in the latter is an effect of the increase in the available prospective sex comb area due to the gene *ey^D*.

No significant effect of *sx* was noted on the bristle pattern in various transverse and longitudinal rows of *sx/+;tra/tra* basitarsi. The mean number of bractless bristles in positions 1.5 and 2.5 is slightly higher in *sx/+;tra/tra* as compared to those in *+/+;tra/tra*.

DISCUSSION

The prepattern concept assumes that localized stimuli are present at the time of determination of specific morphogenetic processes and that gene-dependent responses are necessary for the production of the subsequent pattern. It implies

TABLE 5

Mean numbers of teeth and intermediate bristles in tra, sx *and their combinations*

Genotype	Number of legs	Teeth	Intermediate bristles
+/+;tra/tra*	60	11.37	. . .
sx	20	1.05	1.55
sx;tra/tra	25	0.96	2.12
sx/+;tra/tra	50	3.7	1.08

* Thirty of these were *y ac sn³ v/y ac sn³ v* (*sx⁺/sx⁺*) and the rest were heterozygous *y ac sn³ v/y sc^S¹ In dl-49 B v w^a sc⁸* (*sx⁺/sx⁺*); in both cases the mean numbers were identical and the ranges were similar.

that variation in stimulus and response are under independent genetic control. Earlier work has clearly shown the genetic control of response. Less evidence is available for genetic control of stimulus. The present work has bearing on the means of distinguishing between the two processes. In the development of bristle organs a number of different stages have been recognized in order of their being controlled by different genes (LEES and WADDINGTON 1942). Thus, the relationship between a stimulus-affecting gene (prepattern gene) and a response-affecting gene (terminal-pattern gene) may be analogous to two genes with early and late points of attack, respectively.

Sexcombless as a terminal pattern mutant: The studies on the basitarsus of *sx* males suggest that the action of *sx* is markedly different from the effect on the sex comb by other genotypes affecting sexual traits, e.g. Hermaphrodite (*Hr*, FUNG and GOWEN 1957; GOWEN and FUNG 1957), 2X3A intersexes (HANNAH-ALAVA and STERN 1957) and various hyper-intersexes (PIPKIN 1961). While the normal allele, *sx+*, produces the response resulting in a normal sized sex comb, the mutant, *sx*, fails to elicit the response necessary to reach the threshold for normal sex comb formation. However, the production of intermediate bristles and occasional teeth shows that *sx* permits a variable partial response. A true threshold level of response for formation of typical sex comb teeth has been earlier suggested for triploid intersexes and indicated in studies on hyper-intersexes. In these genotypes either a typical tooth or an ordinary bristle are formed. The production of intermediate bristles in *sx* males shows that here the production of teeth or bristles is not an all-or-none phenomenon.

Various facts have led to the assumption that the stimulus for sex comb differentiation is distributed around a peak of effectiveness rising from a gradient of lower potency. The data on *sx* agree with this picture since (a) whenever the various morphological forms of intermediate bristles are present the heavier intermediate bristles are usually located close to the sex comb forming region, (b) the production in combinations of *sx* and *en* genotypes of chaetae in secondary transverse rows outside of the secondary sex-comb area leads to normal type bristles only, (c) in *sx/+;tra/tra* females intermediate bristles frequently appear in the distal region of the sex-comb area, sometimes in other regions but never between teeth, and (d) in genetic mosaics a tooth or an intermediate bristle forms more often when a *sx* patch on a *sx/+* background is located in the region of transverse rows (in addition to that in the sex-comb area) than when it is in longitudinal row 6 or 7 (MUKHERJEE 1964). It seems that the response provided by *sx* leads to teeth in the peak area, to intermediate bristles close by and, further away is unable to produce teeth or intermediate bristles. The difference between the all-or-none effects in normal males and females, triploid intersexes and hyper-intersexes and the graded effect of *sx* suggests that the different genotypes are decisive at different levels of action.

The negative correlation between numbers of sex-comb teeth and intermediate bristles and between numbers of transverse rows and bristles per row, and the lack of correlation between number and size of teeth and length of basitarsus suggest that there is an approach to constancy in the overall number of bristles

on the ventral side of the basitarsi. Actual counts confirm this. Thus it appears that sx only reorganizes whatever bristle pattern is available. Assuming that the realization of the final pattern is a product of interaction between the prepattern and the response, the interpretation can be rephrased as: the gene sx does not change the prepattern but causes a specific response to an invariant prepattern. A comparison of normal male and female basitarsi yields a strong negative correlation between the number of transverse rows and the presence of a sex comb. The absence of correlation in sx males between the number of transverse rows and the sums of teeth and intermediate bristles is a result of the fact that in the mutant the differentiation of teeth and intermediate bristles proceeds usually without full shifting of the sex comb producing transverse row. Moreover, while in normal males the formation of a sex comb from one of the last two transverse rows is accompanied by the nonformation of the other two, in sx males the incomplete formation of a sex comb is correlated with the formation of all transverse rows. as in females.

The mean number of transverse rows in sx males is intermediate between those in normal males and normal females. Also, in extreme cases of inhibition of tooth formation, the basitarsal chaetotaxy in sx males is not much different from that of normal females. Thus the mutant sx produces a feminizing tendency which is intermediate between complete inhibition as in normal females and full expression as in normal males. This interpretation agrees with the fact that male-like $sx/+;tra/tra$ transformed 2X individuals possess sex combs of only about one third the size of that in $+/+;tra/tra$. This is analogous to the reduction of sex-comb size in 3X2A tra/tra metafemales, where the sex comb contains about half the number of teeth in 2X2A tra/tra (STURTEVANT 1945). Obviously tra while able to transform a 2X2A fly into a phenotypic male, leads to only an intersexual condition in 3X2A. It must be stressed, however, that sx and tra have opposite effects. sx leading to some degree toward female differentiation of the basitarsus without obvious feminization elsewhere and tra toward far-reaching male differentiation of the whole individual.

An sx basitarsus may have various types of bristles, each of which may be oriented in a slightly different way from the other. It appears that in sx the prepatterned site for the sex comb, due to deficient response, fails to complete the morphogenetic as well as morphodynamic changes usually accompanying teeth differentiation; thus in most cases intermediate bristles in intermediate orientations differentiate instead. This would imply that a prospective potency of cells to produce either a bristle or a tooth precedes the final determination of its prospective fate. This fate may depend on the kind of response provided by terminal pattern genes.

The results of the present work may be summarized in the form of a scheme which implies differential levels of action of the four genes sx, en, ey^D and tra (Table 6). This scheme involves primarily the patterns of the two sex combs. It is assumed that both male and female legs possess the prepatterns for the primary (P_p) and secondary (P_s) sex combs. A normal male foreleg possesses the proper response (R_p) while the normal female foreleg lacks it (compare 2 $vs.$ 1, Table 6).

TABLE 6

Schematic representation of modes of action of the genes sx, en, ey^D *and* tra.
and their various combinations

Genotype	Mode of Action	Effective pattern		Corresponding approx. number of teeth	
		Primary s.c. area	Secondary s.c. area	Pri. s.c.	Sec. s.c.
1 + ♀	P_p —⊢→ (P_p) ; P_s —⊢→ (P_s)	−	−	−	−
2 + ♂	P_p —R_p→ $R_p \cdot P_p$; P_s —⊢→ (P_s)	++	−	10	−
3 ey^D ♂	$n(P_p)$ —R_p→ $n(R_p \cdot P_p)$; $n(P_s)$ —⊢→ (nP_s)	+++(+)	−	28	−
4 tra(2x) ♂	P_p —R_p→ $R_p \cdot P_p$; P_s —⊢→ (P_s)	++	−	10	−
5 en· ♂	P_p —R_p→ $R_p \cdot P_p$; P_s —R_s→ $R_s \cdot P_s$	++	+	10	7
6 en;ey^D ♂	$n(P_p)$ —R_p→ $n(R_p \cdot P_p)$; $n(P_s)$ —R_s→ $n(R_s \cdot P_s)$	+++(+)	++++	31	25
7 sx ♂	P_p —R_i→ $R_i \cdot P_p$; P_s —⊢→ (P_s)	±−	−	1	−
8 sx;ey^D ♂	$n(P_p)$ —R_i→ $n(R_i \cdot P_p)$; $n(P_s)$ —⊢→ (nP_s)	$^+_{(-)}$	−	2	−
9 sx/sx+;tra(2x) ♂	P_p —$R_i + R_p$→ $(R_i \cdot P_p);(R_p \cdot P_p)$; P_s —⊢→ (P_s)	+	−	4	−
10 sx ;en ♂	P_p —R_i→ $R_i \cdot P_p$; P_s —R_{is}→ $R_{is} \cdot P_s$	±−	±−	1.5	0.5
11 sx ;en;ey^D ♂	$n(P_p)$ —R_i→ $n(R_i \cdot P_p)$; $n(P_s)$ —R_{is}→ $n(R_{is} \cdot P_s)$	±	±	3	2.5

P_p = prepattern for primary sexcomb; P_s = prepattern for secondary sexcomb; R_p = response to primary sexcomb stimulus; R_s = response to secondary sexcomb stimulus; R_i = reduced response to primary sexcomb stimulus due to *sx*; R_{is} = reduced response to secondary sexcomb stimulus; + and — = presence or absence of (terminal) pattern of primary and secondary sexcombs; a bar across an arrow represents a block due to absence of response. For other symbols see text.

Accordingly, only a normal male manifests the pattern ("++") by production of about ten teeth in the primary sex comb. For purposes of discussion one may think of the effective pattern as resulting from a "complex" between gene product and prepattern singularity ($R_p \cdot P_p$). It is expected, then, that if the 2X female foreleg is converted by *tra* into a male foreleg, the male response can occur resulting in $R_p \cdot P_p$ and leading to a normal sex comb. This expectation is fulfilled (4 *vs.* 2). The sex comb in *tra* (2X) is similar in size to that in normal males. (The finding of a smaller size of the sex comb in 2X *tra/tra* as compared to 1X by

BROWN and KING (1961) cannot be generalized. Neither in the present nor in their experiments was the comparison based on essentially isogenic flies).

If the prepattern is quantitatively altered, it is expected that the amount of complex, $R_p \cdot P_p$, will also be altered, assuming that a normal response, R_p, occurs. Evidence for a quantitative change in the prepattern comes from ey^D male forelegs in which the area available for the sex comb (or sex combs) is increased. This would result in a greater amount of the prepattern-response complex, $n(R_p \cdot P_p)$ (3 $vs.$ 2) as expressed by the greatly increased number of teeth, arranged in two or more sex comb rows, and a corresponding increase in the numbers of central bristles and other bristles in the sex comb area or outside. In $sx;ey^D$ the action of sx is superimposed on that of ey^D leading to a reduced number of teeth (8 $vs.$ 3).

The prepattern for a secondary sex comb is present in en females (STERN 1954a). It is also present in phenotypically normal en/en^+ forelegs (TOKUNAGA 1961). However, the response provided by en, R_s, is necessary to form the complex, $R_s \cdot P_s$. An en male, therefore, has both R_p and R_s, is able to form two complexes, $R_p \cdot P_p$ and $R_s \cdot P_s$, and produce a normal sized primary sex comb of about ten teeth and a normal sized secondary sex comb of about seven teeth. In $en;ey^D$ males reactions between $n(P_p)$ and R_p, and between $n(P_s)$ and R_s should result in two multiple complexes. Since both available areas are increased the number of teeth of both sex combs is large (5, 6 $vs.$ 2).

As compared to sx^+ in sx and $sx;en$ the size of the primary sex comb is similarly reduced and in $sx;en$ that of the secondary sex comb is reduced as compared to en (7, 10 $vs.$ 2, 5). Thus sx reduces the response for both primary (R_i) and secondary (R_{is}) sex combs. Whenever sx is introduced into the genotype it will result in the formation of a reduced complex ($R_i \cdot P_p$ or $R_{is} \cdot P_s$, or both). If the available sex comb area is enlarged, i.e. the prepattern singularity is enlarged, in the presence of sx a multiplied $n(R_i \cdot P_p)$ complex will be formed and consequently the primary sex comb size will be increased over that in sx (and also secondary, when en is present). This is actually found in $sx;ey^D$ and $sx;en;ey^D$ males (8, 11 $vs.$ 7, 10).

Finally, if $sx/+$ females are transformed into phenotypic males by tra two types of complexes result: one, the $R_i \cdot P_p$ formed by the interaction between the normal prepattern and the reduced response due to sx; the other, $R_p \cdot P_p$, formed by the normal response evoked by sx^+ on the other X chromosome and the normal prepattern (P_p), the presence of both complexes resulting in a sex comb intermediate in size between sx^+ and sx (9 $vs.$ 2, 7) or between $sx;tra/tra$ and sx^+; tra/tra. One may assume that the responses (R_p and R_i) produced by a sx^+ and sx allele act additively with different efficiency, or competitively such that the net complex that is formed is reduced in comparison to that produced by two normal alleles. The formation of intermediate bristles in the distal part of the sex-comb area is compatible with either additive effect of, or competitive action between, the responses for P_p, the available amount of stimulus remaining constant.

The scheme is incomplete in more than one way. Thus it does not tell anything

about the nature of the sx-initiated R_i. Is it qualitatively or quantitatively differ-
ent from the normal response, R_p? It may be possible to distinguish between these
alternatives if the primary sex comb could be studied in transformed females
with different doses of sx. If one could obtain a small deletion for the sx locus one
could compare $sx/+;tra/tra$ with the "def. sx"$/+;tra/tra$. If the sex combs in the
two genotypes were different a qualitative difference between R_i and R_p within
the limit of the threshold could be assumed.

On the basis of these findings and from the knowledge of the autonomous
action of sx (MUKHERJEE 1964) and en (TOKUNAGA 1961) it may be concluded
that the effect of ey^D precedes those of sx and en, and may be as early as the
division stage of bristle development in the scheme of LEES and WADDINGTON
(1942). The action of tra occurs perhaps earlier than that of sx, since for the
expression of sx the female has to be first converted into a male by tra.

The action of en and sx may be temporally parallel or overlapping. These
similar assumptions are supported by the fact that in combination with en, sx
which is usually only involved in the seta-cell-to-tooth determination process,
shows the new property of leading toward secondary transverse rows.

The results of the present work thus lead one step further toward the under-
standing of the genetic basis of prepattern-pattern relationships. It remains for
further investigation to find out how the gene regulatory systems can determine
the singularities of prepatterns in the process of differentiation of an ultimate
pattern. A search for such a gene control system has been made by FALK (1963).
It is important that more genes affecting a common pathway of gene action be
studied in order to answer this question fully. Perhaps, a discovery of a mutant
with a sex comb, or at least a tooth-like structure in that area, in species of Dro-
sophila which normally do not have it, will be a starting point for the understand-
ing of the evolution of prepattern genes.

SUMMARY

The mutant sx reduces the mean number of teeth in the primary sex comb
to about one, as compared to about ten in normal males. In addition to typical
teeth, intermediate bristles appear in the sex-comb area. They vary in number,
size and pigmentation between normal macrochaetae and typical teeth. Their
mean number was about 2.3. The existence of intermediate bristles in sx is differ-
ent from the "all-or-none" mechanism for either typical teeth or typical macro-
chaetae in 2X3A and hyper-intersexes.—There is an approach to constancy in
the overall bristle number on the ventral side of the basitarsus. This results in a
negative correlation between numbers of teeth and intermediate bristles and be-
tween numbers of transverse rows and bristles per row.—The mean number of
transverse rows in sx males is significantly higher than in normal males and may

closely approach that in normal females. The bractless bristle, 6.5d, is present in similar position on the basitarsus of normal females and sx males, as compared to the existence of four bractless bristles at somewhat proximal and anterior positions, in normal males. These results suggest an incomplete action of sx toward the preservation of the feminine character of the basitarsus.—New observations support the view that the posterior-most bristle of the last transverse row of the female basitarsus corresponds to the central bristle of the male basitarsus.—A comparison of the bristle patterns in sx, en, ey^D and their combinations shows that (1) sx reduces the number of teeth in both primary and secondary sex combs, (2) the ey^D-initiated enlargement of the sex-comb area is seen in both ey^D and $sx;ey^D$, (3) intermediate bristles appear in all combinations with sx, and (4) in combinations of sx and en a series of secondary transverse rows are intercalated between longitudinal rows 1 and 3.—Combinations between sx and tra show that sx has a subthreshold effect in the $sx/+$ heterozygote. In $sx/+;tra/tra$ the mean number of teeth which are arranged normally is about one third that in $+/+;tra/tra$. Also, intermediate bristles appear in $sx/+; tra/tra$. They are located usually at the distal end, sometimes at other places but never between teeth.—The mutant sx seems to act late in development, causes a low response for the formation of sex-comb teeth and is only able to reorganize whatever bristle pattern is available.

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