

DEVELOPMENTAL GENETICS OF THE POSTERIOR LEGS IN *DROSOPHILA MELANOGASTER*

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Received January 22, 1958

ONE of the characteristic morphological features of certain of the species of the Drosophilidae is the sex comb (ctenidium) on the first and in some species also the second tarsal joint of the prothoracic leg of the male but never the female. The males of *Drosophila melanogaster* have a single comb of 10–12 morphologically distinct bristles (teeth) on the distal end of the first tarsal segment, but occasionally a single tooth or several teeth occur as a developmental anomaly on the other tarsal segments of the first leg or on the other legs.

Five mutants are known in *D. melanogaster* which induce the formation of typical sex combs on the metatarsi of the posterior legs. If sex comb teeth are developed on the metatarsi of the posterior legs, it may be because the genetic factor causes differentiation of a sex comb only, and this quite independent of differentiation in the rest of the tarsus or the rest of the leg. Conversely an extra sex comb mutant may have a more profound morphogenetic effect. It may redirect the development pattern so that the posterior legs become first legs with the appropriate region of the male metatarsus differentiating a sex comb.

The legs of *D. melanogaster* are admirably suited for morphogenetic studies for the three legs are differentiated on the basis of size, structure and chaetotaxy (HANNAH-ALAVA, 1958). In addition the metatarsus of the first leg is sexually dimorphic in the total chaetotaxal pattern as well as gross anatomy and certain easily distinguishable bristle groups. A preliminary study by HANNAH (STERN 1954b) showed that some of the extra sex comb mutants did indeed induce profound changes in the metatarsi of the posterior legs of which the sexcombs were only the most obvious morphological change. A detailed analysis, presented in this paper, substantiates the hypotheses that the extra sex comb mutants do not induce sex combs *per se*, but that they are primarily concerned with changing the prepatter in the posterior legs, and after the prepatter is changed development of the legs continues in an orderly fashion but in a new direction. The results also show that the changes in the posterior legs of the female are just as profound as in the male, but always in the direction of the female first leg, as the changes in the posterior legs of the male are in the direction of a male first leg.

MATERIALS AND METHODS

Morphogenetic studies were made of a hybrid between two wild type strains and various combinations of three extra sex comb mutants: extra sex comb

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(*esc*), Polycomb (*Pc*) and Extra sex comb (*Scx*). Consequently it was possible to compare the three mutants not only in simplex but their interactions in compounds.

Genotypes

Wild type: For comparison with the extra sex comb genotypes, offspring from Samarkand females (from a strain inbred 269 generations) and Oregon-R males (from a strain inbred 171 generations) were used as the normal wild type. The Samarkand-Oregon hybrid will be referred to as Sam-Ore or wild type in the text.

extra sex comb: The first extra sex comb mutant, *esc*, found and described by SLIFER (1942) is a second chromosome recessive factor characterized by sex combs on each of the six legs of the male. It is homozygous sterile in both sexes. SLIFER noted especially the variable penetrance, but also reported that it could be increased by a liberal diet of fresh yeast. More recent studies (HANNAH and STRÖMNAES 1955, HANNAH-ALAVA, unpublished) have shown that in normal cultural conditions at least one extra sex comb is present in the majority of *esc* males, and that both penetrance and expressivity are affected by different cultural media and particularly by crowding during development.

Polycomb: *Pc*, a third chromosome dominant mutant (lethal in homozygotes) was found and described by P. LEWIS (1947). It is also characterized by sex combs on all three pairs of legs in the male as well as by other phenotypic effects such as elevated, divergent or crinkled wings and bent humeral and anterior notopleural bristles (LEWIS 1947, HANNAH and STRÖMNAES 1955). As a heterozygote with wild type (*Pc/+*), it has a much lower penetrance and expressivity than homozygous *esc*. Under normal conditions from 50–75 percent of the males have a comb (seldom of more than four teeth) on one or both of the second legs and very rarely even a single tooth on the third legs. With poor cultural condition, crowding or on certain media the extra sex comb characters may not be expressed at all.

Extra sex comb: *Scx* is also a third chromosome dominant and like *Pc* is lethal in homozygotes. As a heterozygote, (*Scx/+*), penetrance and expressivity is between *Pc* and *esc* (HANNAH and STRÖMNAES 1955). Like *Pc* it is very sensitive to environmental conditions, but the third leg is more sensitive than the second. In normal conditions penetrance is between 75–90 percent with both legs being affected in over half of the flies; in a less favorable environment the penetrance drops to below 50 percent with the third legs rarely having sex combs. The pleiotropic effects on other parts of the body, characteristic of *Pc*, are less extreme or do not occur in *Scx* flies.

The *Scx/Pc* compound is neither lethal nor sterile, but the flies have somewhat lower viability than either *Pc/+* or *Scx/+* flies. The sex comb character is completely penetrant except in very poor environmental conditions and most of the males have "good" sex combs on all six legs (HANNAH and STRÖMNAES 1955). In addition to the leg effects, the other pleiotropic effects characteristic of *Pc* are the same or even accentuated in *Scx/Pc* flies; the sternopleural bristles are frequently missing, the humerus is depressed, and the wings are spread or drooping.

The *esc/esc; Scx/+* compound is completely penetrant for the extra sex comb characters and has almost normal viability. The other pleiotropic effects, characteristic of *Pc* and *Pc* compounds, are very slight, if present at all.

The *esc/esc; Scx/Pc* compound is almost lethal, particularly in the female. The surviving flies are smaller in size, weak, highly sterile, and frequently have legs so abnormal that an analysis of the chaetotaxy is impossible. All of the pleiotropic effects are very extreme and of the type described for *Pc/+* and *Scx/Pc* flies.

The extra sex comb strains were maintained as heterozygotes: *esc/Bl*, *Pc/T-(2;3)Mé*, and *Scx/In(3) T M, Mé* (for a description of the markers see LEWIS and GRELL 1953; BRIDGES and BREHME 1944). Compounds were made in several ways: *Pc/+* and *Scx/+* flies were obtained from matings of Samarkand females and *Pc/Mé* or *Scx/Mé* males; *esc/esc* flies from crosses of *esc/Bl* females and males; *Scx/Pc* from *Pc/Mé* × *Scx/Mé* matings, *esc/esc; Scx/+* flies from crosses of *esc/Bl* × *Cy/Pm*; *Scx Sb/+* and the F_1 *esc/Cy; Scx Sb/+* males backcrossed to *esc/Bl* females; and the *esc/esc; Scx Sb/Pc* flies from crosses of *esc/Bl; Scx Sb/Pc* females and males which had been synthesized by appropriate crosses. (Comparison of *Scx Sb/+* to *Scx/+* flies showed that *Sb* had little or no effect upon the expression of the extra sex comb phenotype. This is in agreement with DOBZHANSKY'S (1930) observation that *Sb* flies did not differ from wild type flies in size or shape of the legs, and with HANNAH-ALAVA and STERN'S (1957) finding that *Sb* did not affect either the size or number of sex comb teeth. Consequently *Sb* will not be included in subsequent designations of the genotypes having this marker.)

A simplified form of the genotypes will be used throughout this paper: *esc = esc/esc*, *Scx = Scx/+*, *Pc = Pc/+*, *esc-Scx = esc/esc; Scx/+*, *Scx-Pc = Scx/Pc* and *esc-Scx-Pc = esc/esc; Scx/Pc*.

Techniques

Method of culture: The standard culture medium was employed: cornmeal, agar, molasses and brewer's yeast with the addition of live yeast just before using the culture bottle. Four to eight pairs of flies, of the appropriate genotypes, were permitted to lay eggs for about four days in one bottle then transferred to a fresh bottle for about four to five days before discarding. All cultures were incubated at $26 \pm 1^\circ$ C. Offspring were checked within one day after emergence, then either preserved in alcohol or mounted.

Mounting and recording: In all genotypes, except *esc-Scx-Pc* in which only 16 males and four females were available for mounting, at least 30 mounts were made of each sex for each genotype. To include a representative sample of the various phenotypes larger samples were made for the genotypes with a low penetrance of the sex comb character. The three pairs of legs were cut from the body of the fly as a unit (or if the pairs were separated they were placed in the same relative position) and mounted in euparal. More recently a technique described by STRÖMNAES and HANNAH (1955) which facilitated mounting was used, after confirmatory tests to show that legs so treated were not distorted in size or shape.

Every leg which was in a position suitable for a chaetotaxal study was recorded. The legs were studied with a compound binocular microscope at magnifications ranging from 100–600 \times . The length of each tarsus was measured on an arbitrary scale (using a mm rule) from a camera lucida drawing. At the magnification used for such measurements 1 unit = 3.8 μ .

In addition to the general morphological observations, more detailed studies were made of the number and position of the sex comb teeth, the number and position of the chaetae in the distal part of the tibia, all of the metatarsus (first tarsus) and selected portions of the femur and coxa; in other words a study of the chaetotaxal pattern. In describing the position of the bristles a modification of GRIMSHAW'S system (1905) was used. Thus the surfaces of the segments are described as if the leg were stretched horizontally from the body, with the outer surface (and the preapical bristle) being dorsal and the inner surface (and the apical bristle) being ventral (see HANNAH-ALAVA 1958 for details). To facilitate recording, the chaetotaxal pattern was charted for each leg in a table and upon diagrams similar to those given in Figure 1. In addition camera lucida drawings were made of many of the legs, and of these representative ones were selected for the figures in this paper.

OBSERVATIONS

Morphology and chaetotaxy of the legs of Sam-Ore flies

A detailed morphological analysis of the legs of wild type flies (HANNAH-ALAVA, 1958) showed that there are extensive differences between the three legs in size, shape and chaetotaxy, and that the chaetotaxal pattern, among the different normal genotypes is relatively stable. Secondly, the chaetotaxy of the metatarsus of the first leg is sexually dimorphic both in respect to the pattern and the number of bristles per row in the proximal-distal rows. The chaetotaxal patterns in the second and third legs are not sexually dimorphic but the female consistently has a higher mean number of bristles in each of the proximal distal rows. The number of bristles per row in either sex was not covariant with the tarsus length, thus the variation in bristle number is not proportional to, or dependent upon tarsal length. Thirdly, although the mean number of bristles per row in rows 1, 3–5 and 8 in the metatarsi of the three legs is not the same, because of the position of the rows and the presence of the small bractless bristles in the same relative position between the appropriate rows, there is little doubt that these rows are equivalent and probably homologous.

Diagrams of the typical chaetotaxal pattern of the metatarsus and the distal end of the tibia for the three legs of Sam-Ore males are given in Figure 1, A, C and D and the female first leg in Figure 1B. These diagrams show the 13 "characters" studied: the proximal-distal rows 1 to 8; row 5.5, a group of bractless bristles between rows 5 and 6; the tarsal transverse rows; the tibial transverse rows; the number of sex comb teeth; and the length of the metatarsus. The mean value for each character for the legs of the male is given in Tables 1 and 4 and of the female in Tables 7 and 8.

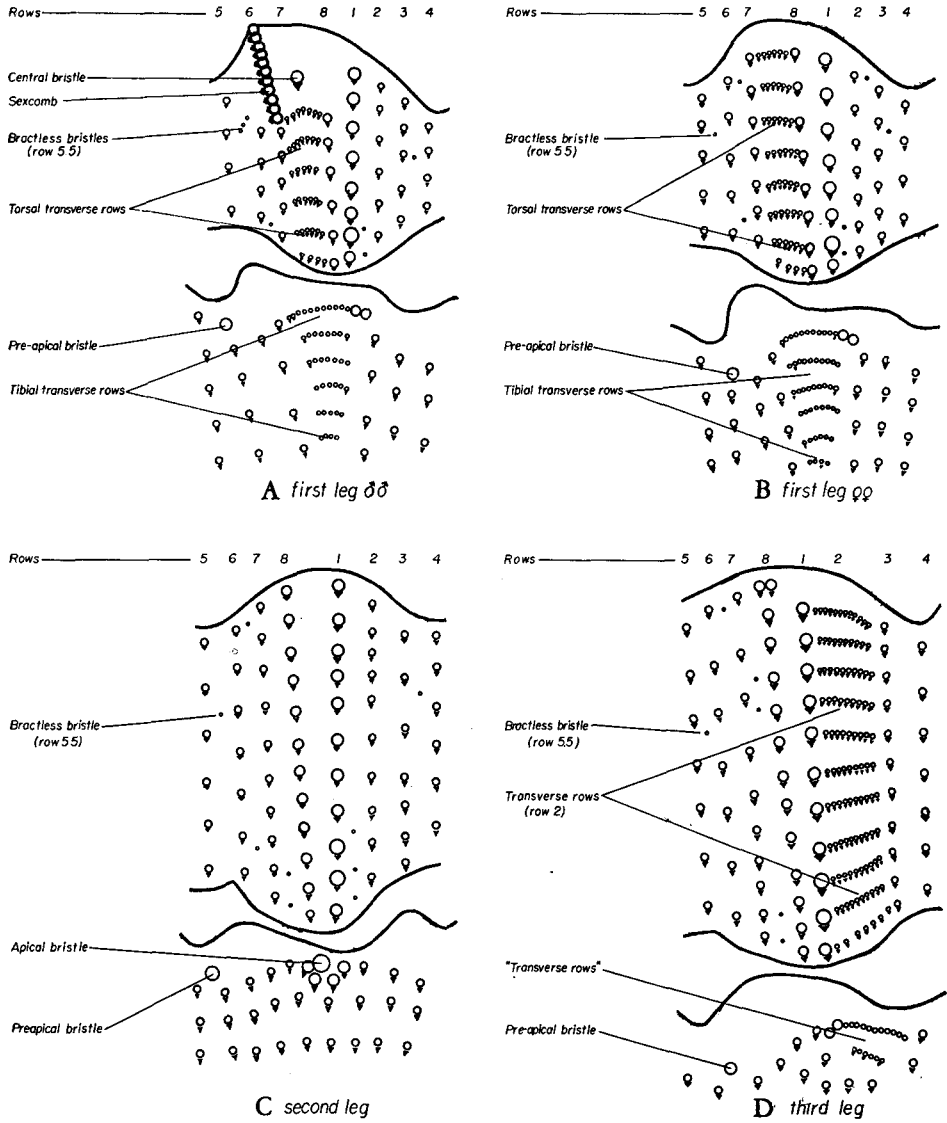


FIGURE 1.—Typical chaetotaxal pattern of the legs of Sam-Ore hybrids (For details see HANNAH-ALAVA 1958). The numbered rows (above each figure) indicate the proximal-distal rows on the tarsus. The circles indicate bristles and the triangles below some of the circles indicate that these bristles have associated bracts. The other characters are labeled in each figure. A.—Diagram of the chaetotaxy of the metatarsus and distal end of the tibia of the first leg of a male. B.—Same for the first leg of a female. C.—Same for the second leg of a male. D.—Same for the third leg of a male.

Effect of extra sex comb mutants upon differentiation of the meso- and metathoracic legs

On the assumption that the second and third legs are transformed by the extra sex comb mutants into first legs, the following morphological or chaetotaxal changes would be expected to occur in the metatarsi of flies with one or more extra sex comb mutants: a decrease in the length of the tarsus accompanied by a decrease in the number of bristles per row in rows 1 to 8; an increase in the male of the number of bractless bristles between rows 5 and 6; formation of transverse rows of bristles between rows 7 and 8 in both male and female legs; formation of a sex comb and a central bristle in the male tarsus. In the tibia the changes expected are: formation of the transverse rows at the distal end; and reduction in the size of the apical bristle of the second leg and its incorporation into the most distal transverse row of bristles. Changes in the more proximal segments would be: development of a complete row of larger bristles along the length of the posterior surface and several preapical bristles on the anterio-dorsal surface of the femur; and changes in the shape, size and chaetotaxy of the coxa.

The evidence, to be presented, shows that all of these changes occur and that they occur increasingly with an increasing number of extra sex comb factors.

A. Changes in the metatarsus and tibia of the male legs

Mesothoracic legs: Comparisons of the chaetotaxal pattern and length of the mesothoracic metatarsus of Sam-Ore males with males having various combinations of the extra sex comb factors are presented in Table 1, and representative types in Figure 2. The mean number of teeth in the sex comb increased from none in Sam-Ore males to 12.3 in *esc-Scx-Pc* flies. The tarsal and tibial transverse rows, which are not present in the wild type males, occurred in flies with one extra sex comb factor and increased in number with an increase in number of factors. Thus, all of the chaetotaxal characters normally present only in the first leg occur in the second leg of flies with one or more extra sex comb factors. Concurrently with the induction of the first-leg characters, there was a decrease in tarsal length and a decrease in the number of bristles per row in all eight proximal-distal rows. Although individually the mutants do not cause a complete transformation of the second leg into a first leg, in the *esc-Scx-Pc* compound the second legs have virtually become first legs for all 13 characters (Table 1). These results are in support of the hypothesis that the primary function of an extra sex comb mutant is to transform a posterior leg into a first leg, not just to induce the formation of a sex comb. The data also suggest that there is a systematic variance for all of the characters in a single genotype or among the genotypes.

The mean expression for each genotype cannot be obtained directly from the means of the characters for they are based upon diverse criteria. In order to evaluate them, each genotype was ranked from one to seven—among the genotypes—for each of the 13 characters. (In ranking, the standard error was taken into consideration; it was assumed that means that overlapped up to 2 S.E. were

TABLE 1
Comparison of the chaetotaxal pattern in the first leg of Sam-Ore males with the pattern in the second legs of males from Sam-Ore and the six extra sex comb genotypes

Genotype Sample	Sam-Ore 20/50	1st leg	2nd leg	Pc/+ 35/104	Scx/+ 88	esc/esc 20/81	Scx/Pc 43	esc/esc; Scx/+ 20/54	esc/esc; Scx/Pc 20/32
Row 1*	7.8±0.1	11.2±0.2	11.9±0.1	11.3±0.1	11.3±0.2	9.7±0.2	9.0±0.2	7.8±0.1	
Row 2	6.3±0.1	10.0±0.1	10.1±0.1	9.7±0.1	9.6±0.2	7.6±0.2	7.7±0.2	6.5±0.2	
Row 3	4.7±0.1	6.6±0.2	7.1±0.1	6.7±0.1	6.9±0.1	5.8±0.1	5.9±0.1	5.6±0.1	
Row 4	4.0±0.1	5.8±0.1	6.0±0.1	5.5±0.1	5.8±0.1	4.7±0.1	4.8±0.1	4.2±0.1	
Row 5	4.2±0.1	6.1±0.1	6.2±0.1	5.6±0.1	5.7±0.2	3.9±0.1	4.6±0.2	3.8±0.1	
Row 5.5	3.7±0.1	1.0±0.0	1.7±0.1	2.5±0.1	2.1±0.2	4.0±0.1	3.9±0.2	4.4±0.2	
Row 6	3.9±0.1	7.0±0.2	6.3±0.1	5.8±0.1	6.0±0.1	3.6±0.1	3.7±0.1	3.3±0.1	
Row 7	5.2±0.2	9.5±0.2	9.1±0.2	7.9±0.2	8.4±0.2	5.2±0.1	5.1±0.1	5.0±0.2	
Row 8	6.4±0.1	11.9±0.2	12.1±0.1	11.3±0.2	11.1±0.3	7.0±0.2	6.5±0.2	6.2±0.1	
Tarsus length	47.8±0.2	76.7±0.3	76.3±0.2	66.9±0.3	68.0±0.4	53.7±0.4	51.6±0.5	44.7±0.5	
Sex comb teeth	11.0±0.1	...	0.6±0.1	4.7±0.4	5.2±0.3	11.2±0.2	11.3±0.2	12.3±0.2	
Ta. tr. rows†	5.8±0.1	...	0.0±0.0+	1.4±0.2	1.1±0.1	6.2±0.2	5.8±0.1	5.9±0.1	
Ti. tr. rows‡	6.1±0.1	...	0.1±0.1	1.6±0.2	1.9±0.2	5.3±0.1	5.6±0.1	5.5±0.1	

The entries in each column include the mean ± 1 S.E. for 13 characters; the number of bristles per row in each of the proximal-distal rows, the number of sex comb teeth, the number of tibial and tarsal transverse rows and the length of the tarsus in units (1 unit = 3.8μ). The mean (and standard error) is based upon a sample of 20 legs for the rows and a sample of 50 legs for the length, sex comb teeth and transverse rows in Sam-Ore, 35 legs and 104 legs respectively for Pc, etc.; Scx and Scx/Pc were completely sampled, 88 legs and 43 legs respectively.

* Row-1—Row-8. Proximal-distal rows of the tarsus.

† Tarsal transverse rows.

‡ Tibial transverse rows.

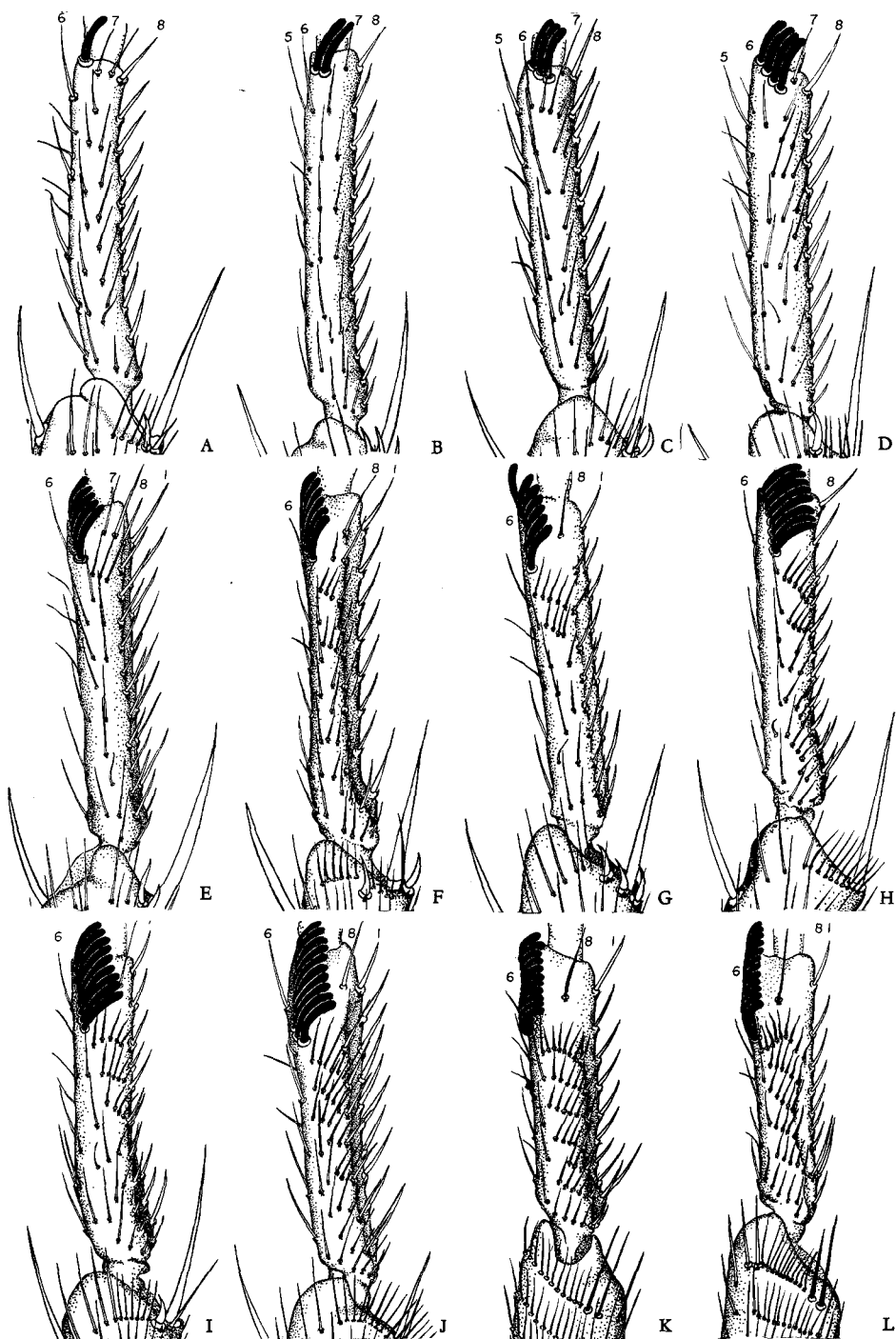


FIGURE 2.—Stages in the transformation of a male second leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-L—Examples of the metatarsus and distal end of the tibia from second legs of males with one or more extra sex comb factors (A-J from Scx and K-L from Scx-Pc). The newly induced or transformed bristles of the transverse rows are in solid black; the bristles characteristic of the second leg are in outline. For details see Figures 1A and C.

of the same rank.) The mean rank for each genotype was derived by averaging the 13 rank values. On the basis of the mean rank, the genotypes form a graduated series in the following order:

Genotype: Sam-Ore > Pc > esc > Scx > Scx-Pc > esc-Scx > esc-Scx-Pc

Mean rank: 1.69 2.09 2.88 3.35 5.65 5.77 6.58

Hence according to their effects, the genotypes can be arranged in a linear series, and a series correlated with the increase in number of extra sex comb factors in the genotypes (Figure 3). The mean rank values also show that the genotypes

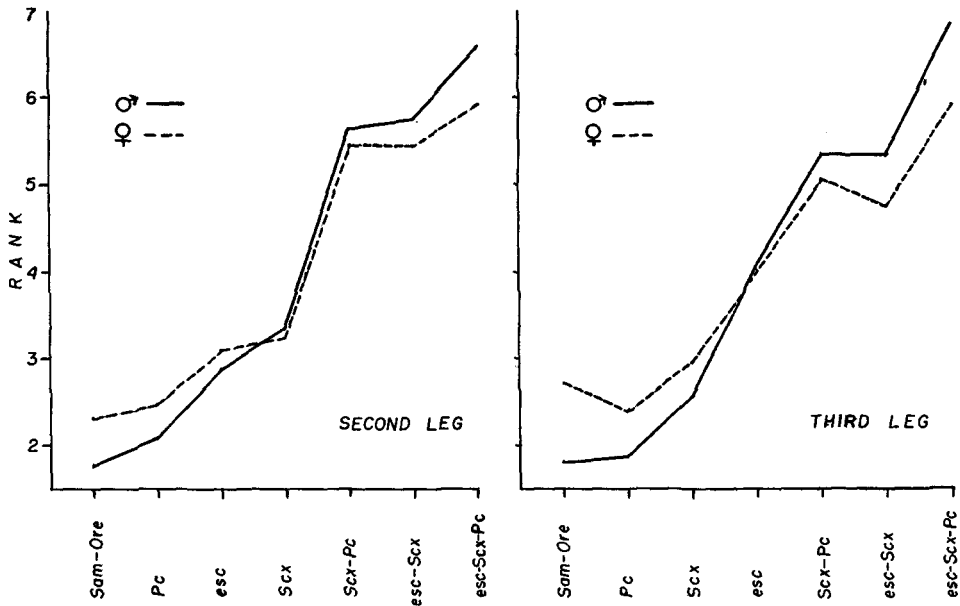


FIGURE 3.—Comparison of the mean rank for each genotype after ranking the seven genotypes for each of the 13 characters in the male and the 12 characters in the female.

overlap in expression of the characters: Sam-Ore has a mean rank of 1.69 instead of 1.00 and esc-Scx-Pc 6.58 instead of 7.00.

These results also point to the probability that the expression of the various characters is correlated. This was tested in two ways: (1) the flies of a specific genotype were divided into classes on the basis of one of the characters (such as number of sex comb teeth) and the means of the other characters tabulated as they were for the genotypes in Table 1; and (2) any two characters were evaluated by means of a correlation test. The first method permits a comparison of all of the characters simultaneously, but only the means without taking into consideration the variances. The second method permits comparison of variance between two characters only but has the advantage that it is simultaneously a measure of covariance.

(1) *Systematic variation within a genotype*: A complete chaetotaxal study of a large sample of flies was made for only two genotypes, Scx and Scx-Pc. Of the

Scx flies, only four had no sex combs on either second leg, the remaining 40 flies had from none to 11 teeth per comb. In the Scx-Pc flies the number of teeth per comb ranged from 8 to 13. As this is the most conspicuous character the legs were separated into classes on the basis of the number of sex comb teeth per leg.

In the five classes of Scx (that is flies with none, 0-2, 3-5, 6-8, and 9-11 teeth per leg) the other characters, with minor exceptions, showed the expected increase or decrease consistent with the increase in number of sex comb teeth (Table 2).

TABLE 2

Evidence for a systematic change in the expression of 13 characters in the second leg and in one- and two-factor genotypes

Genotype No. of teeth Sample	Scx/+						Scx/Pc	
	0 8	0-2 19	3-5 25	6-8 17	9-11 19	8-11 24	12-13 19	
Row 1	12.0	11.3	11.3	11.1	10.9	9.7	9.8	
Row 2	10.5	9.8	9.7	9.7	9.3	7.5	7.8	
Row 3	7.0	6.9	6.7	6.7	6.4	5.9	5.6	
Row 4	5.5	5.4	5.4	5.2	5.5	4.7	4.7	
Row 5	6.0	5.7	5.5	5.4	5.6	3.8	3.9	
Row 5.5	1.0	1.6	2.4	2.8	3.8	3.9	4.1	
Row 6	6.9	7.0	5.8	5.4	4.6	3.3	3.8	
Row 7	9.6	9.4	7.8	7.1	6.4	5.2	5.1	
Row 8	13.3	11.9	11.4	11.1	9.8	7.1	6.9	
Tarsus length	69.6	69.5	66.6	65.8	64.5	53.2	54.3	
Sex comb teeth	0.0	0.8	3.9	7.1	9.7	10.5	12.2	
Ta. tr. rows	0.0	0.1	1.2	2.2	3.2	6.0	6.4	
Ti. tr. rows	0.0	0.3	1.7	2.0	3.1	5.2	5.4	

The 88 legs from Scx males were divided into five groups: flies with no sex comb teeth on either leg, and (of the flies having at least one comb on a posterior leg) second legs with 0-2, 3-5, 6-8 and 9-11 teeth per comb. The 43 legs of Scx-Pc males were separated into two groups, those having 8-11 and those having 12-13 teeth per comb in the second leg. Abbreviations are the same as in Table 1.

Thus the progressive seriation for the 13 characters within the Scx genotype completely paralleled the progressive seriation found among the genotypes. It seems probable that in the Scx genotype a single factor—the extra sex comb factor—is inducing all of the changes and that modifying factors (unless they affect the characters as a unit) are not of significance.

In the two factor compound, Scx-Pc, the flies were divided into two classes, those with 8-11 and those with 12-13 sex comb teeth. The differences between the classes is not great, in fact in some of the characters the legs with the larger number of sex comb teeth actually deviate in the opposite direction from that expected, that is more bristles per row instead of fewer, longer length of the metatarsus instead of shorter. Consequently, in Scx-Pc the seriation is not evident. This probably does not mean that the expressions of the characters are not correlated in this genotype but only that the variance may be great enough to mask a systematic difference.

(2) *Covariance*: Sufficient data were not available for complete correlation tests for Pc. Of the 104 second legs studied only 43 expressed the extra sex comb pheno-

type: four had only tibial or tarsal rows, 27 had only a sex comb (of one to four teeth), and 12 had a sex comb and tibial and/or tarsal rows. The number of sex comb teeth and the metatarsal length were correlated ($r = 0.29^*$). With a larger sample, undoubtedly covariance would be found between more of the characters.

In the other genotypes a high degree of correlation existed between the number of sex comb teeth, the length of the tarsus, and the number of tibial or tarsal rows for most of the genotypes (Table 3). Furthermore, three variates (tibial to tarsal

TABLE 3

Correlation in expression of the extra sex comb characters in the second leg of males with one or more extra sex comb mutants

	<i>esc/esc</i> n=81	<i>Scx/+</i> n=88	<i>esc/esc;</i> <i>Scx/+</i> n=54	<i>Scx/Pc</i> n=50	<i>esc/esc;</i> <i>Scx/Pc</i> n=32
Sex comb teeth to length	0.40†	0.70†	0.48†	0.09	0.18
Sex comb teeth to tarsal rows	0.71†	0.63†	0.50†	0.32†	0.08
Sex comb teeth to tibial rows	0.71†	0.53†	0.22	0.22	0.22
Length to tarsal rows	0.39†	0.62†	0.39†	0.26	0.01
Length to tibial rows	0.17	0.43†	0.28†	0.39†	0.14
Tibial to tarsal rows	0.55†	0.61†	0.42†	0.41†	0.37*
Sex comb teeth to tarsal rows to tibial rows	0.71†	0.64†	0.41†	0.43†	0.42*

* Correlation coefficient (r) is significant at the five percent level.

† Correlation coefficient (r) is significant at the one percent level.

transverse rows to sex comb teeth) were correlated, even in the genotypes which had little correlation for two variates. Thus there is undoubtedly a mutual relationship in the expression of the characters in the extra sex comb genotypes.

The data also show that the expression of any two characters is not perfectly correlated. (In perfect correlation $r = 1.00$ and no correlation $r = 0.00$). In these genotypes (with significant r values between 0.32 and 0.71) there is considerable variation in expression of the characters in each leg. For example in the second legs of *Scx* males, the range in length of the tarsus in flies with no sex comb teeth was 68–72 units; 0–2 teeth, 65–72 units; 3–5 teeth, 62–73 units; 6–8 teeth, 61–69 units and 9–11 teeth 62–69 units (see Table 2 for mean values). In the same genotype 23 of the legs had no tarsal transverse rows yet had from one to five sex comb teeth, 25 legs with no tibial transverse rows had from one to nine sex comb teeth, and 12 legs had tibial but no tarsal rows and 13 legs had tarsal but no tibial rows. Thus there is considerable individual variation in expression of the characters (see also Figure 2, particularly E–H). A similar range in expression of the characters occurred in all single-factor genotypes. Among the 273 legs from *Pc*, *esc* and *Scx*, 66 legs had from one to six sex comb teeth but no tarsal transverse rows, or 60 legs had from one to seven teeth but no tibial row. On the other hand only five legs had tarsal or tibial rows without sex comb teeth. In spite of this great difference in expression, there was no evidence that the deviations are any greater than would be expected by chance. In the two- and three-factor genotypes the

range in expression of the bivariate was not as great: all flies had every character expressed to some degree, the variance for each character was less than in the single-factor genotypes and the means were close to the expected means characteristic of the first leg.

The reason for lack of correlation in five of the six tests for covariance between two characters in the *esc-Scx-Pc* genotypes (Table 3) becomes evident if the means for the characters in the second leg of the extra sex comb genotypes are compared to the means for the same characters in the first leg of *Sam-Ore* males (Table 1). The difference in means was significant (at 2 S.E.) for all 13 characters in the genotypes with a single extra sex comb factor; in each genotype the mean was closer to the mean of a second leg than a first leg. In the flies with either two or three extra sex comb factors the difference was significant for six of the 13 characters. However, in the flies with two factors the mean expression was intermediate between a second and a first leg, but in flies with three factors the mean expression for all characters was closer to a first leg than a second. Furthermore, the mean expression for four of the six characters with a significant difference (as compared to a first leg) made the second leg of *esc-Scx-Pc* flies more first leg than the first leg of *Sam-Ore* males: the metatarsus was shorter, there were fewer bristles in row 6, more bractless bristles in row 5.5 and more sex comb teeth. For all characters combined, the second leg of *esc-Scx-Pc* had become a first leg.

It is obvious from the data in Table 1 that a single extra sex comb factor is, on the average, not capable of transforming a second leg into a first leg. A portion of the flies, however, have changes which approach the mean for each character in the first leg (i.e., *Scx* in Table 2) and individual flies may show complete transformation of a second leg into a first leg. This range in expression of the extra sex comb factors in simplex may be because in each genotype a number of modifying factors are interacting with the primary factor to produce a wide range in the expression or it may mean that the genotypes are very sensitive to external or internal environmental conditions. Transformation of a second leg into a first leg in compounds of two factors is far greater than would be expected from their action singly, even if modifying factors are taken into consideration. For example, the mean incidence of sex comb teeth in *Pc* was 0.6 and *Scx* was 4.7, yet in the compound (*Scx-Pc*) the mean number of sex comb teeth was 11.2; or the number of tarsal transverse rows was none and 1.4 for *Pc* and *Scx* singly and 6.2 in the *Scx-Pc* compound (Table 1). The fact that the action of the extra sex comb factors is augmented in compounds as compared to their action singly is indicative of subthreshold effects of the extra sex comb factors, rather than modifying factors. If this is the case the factors singly contribute to variance in expression of the phenotype, but in compounds they interact to produce full expression of the extra sex comb characters.

The data also indicate that there is an upper threshold in expression of each character. For example, if the number of teeth per sex comb would be augmented in a three-factor compound to the same extent as it was in a two-factor compound, more than 12 sex comb teeth would be expected in *esc-Scx-Pc* males. The same is

true for the tibial and tarsal transverse rows (Table 1). Thus it seems likely that the extra sex comb factors, even when compounded, cannot transform a posterior leg beyond a certain point, that is to more than a first leg.

Metathoracic leg: Comparisons of the length of the metatarsus and the chaetotaxy of the metatarsus and distal end of the tibia of the male third leg for the seven genotypes are presented in Table 4 and examples of the various types in Figure 4. The data show that the extra sex comb factors cause the third leg to be

TABLE 4

Comparisons of the chaetotaxal pattern of the metatarsus and distal end of the tibia and the length of the metatarsus of the third leg of males from seven different genotypes

Genotype Sample	Sam-Ore 20/50	Pc/+ 27/102	Scx/+ 86	esc/esc 20/82	Scx/Pc 46/50	esc/esc; Scx/+ 20/53	esc/esc; Scx/Pc 16/32
Row 1	11.4±0.1	12.6±0.1	11.5±0.1	10.8±0.1	11.1±0.1	10.5±0.1	9.3±0.3
Row 2*	11.7±0.1	12.4±0.1	11.7±0.1	10.6±0.2	11.3±0.1	10.1±0.1	8.8±0.2
Row 3	10.9±0.2	10.6±0.1	9.7±0.1	9.6±0.1	9.2±0.1	9.0±0.2	8.9±0.3
Row 4	7.1±0.1	7.7±0.1	7.2±0.1	6.5±0.2	7.0±0.1	6.6±0.2	5.7±0.2
Row 5	6.6±0.1	7.3±0.1	6.9±0.1	6.2±0.2	5.9±0.2	5.8±0.2	4.9±0.3
Row 5.5	1.0±0	1.9±0.1	1.3±0.1	1.9±0.2	3.6±0.1	2.7±0.2	3.2±0.3
Row 6	7.1±0.1	7.2±0.1	6.9±0.1	6.2±0.2	5.2±0.2	5.6±0.3	4.2±0.3
Row 7	10.3±0.1	9.9±0.2	10.0±0.1	8.5±0.3	7.1±0.2	7.3±0.2	6.2±0.2
Row 8	12.9±0.2	14.1±0.1	13.0±0.1	12.2±0.2	11.6±0.2	10.8±0.4	7.8±0.3
Tarsus length	87.4±0.3	87.9±0.2	83.2±0.3	75.9±0.4	74.8±0.7	68.0±0.4	56.5±0.9
Sex comb teeth	0.2±0.1	0.5±0.1	2.9±0.3	8.3±0.4	8.1±0.5	11.8±0.3
Ta. tr. rows	0.0±0.0	0.1±0.0	0.8±0.1	3.2±0.2	2.6±0.3	6.1±0.2
Ti. tr. rows	0.0±0.1	0.0±0.0	1.1±0.2	3.3±0.2	4.0±0.2	5.0±0.1

* Row 2 lists the number of transverse rows.

The entries in each column include the mean±S.E. for 13 characters. The mean (and standard error) for the rows in Sam-Ore is based upon 20 legs, for the other characters upon 50 legs, or 27 legs and 102 legs respectively for Pc, etc.; the mean for each character in Scx is based upon a sample of 86 legs. For abbreviations and other details see Table 1.

changed into a first leg. However, transformation from a third leg to a first leg was not as complete for any one of the 13 characters in most of the genotypes as it was in the second leg.

The difference between the means, for each of the 13 characters, between the first leg of Sam-Ore males and Pc, Scx, esc, esc-Scx and Scx-Pc males was significant (at 2 S.E.) for all but one character (row 5.5 in Scx-Pc) with the extra sex comb genotypes always more third leg-like than first leg-like. The difference between the means of esc-Scx-Pc third legs and Sam-Ore first legs was significant for eight values. The greatest difference between the means was in the length of the tarsi and the number of bristles per row in rows 1–8. Even in these characters, however, there were changes making the third leg like a first leg. Of the characters normally not present in the third leg, i.e., sex comb teeth, tarsal and tibial transverse rows and more than one bractless bristle in row 5.5, only the tibial rows were significantly less in esc-Scx-Pc third legs than in Sam-Ore first legs. Thus it seems that for these characters the extra sex comb factors are almost as effective

in changing a third leg to a first leg as there are in changing a second leg into a first leg.

In spite of the difference in action in the two legs, the extra sex comb mutants appear to transform the whole leg, whether it be a second or a third leg, into a first leg not just to induce the formation of a sex comb. If all of the genotypes are

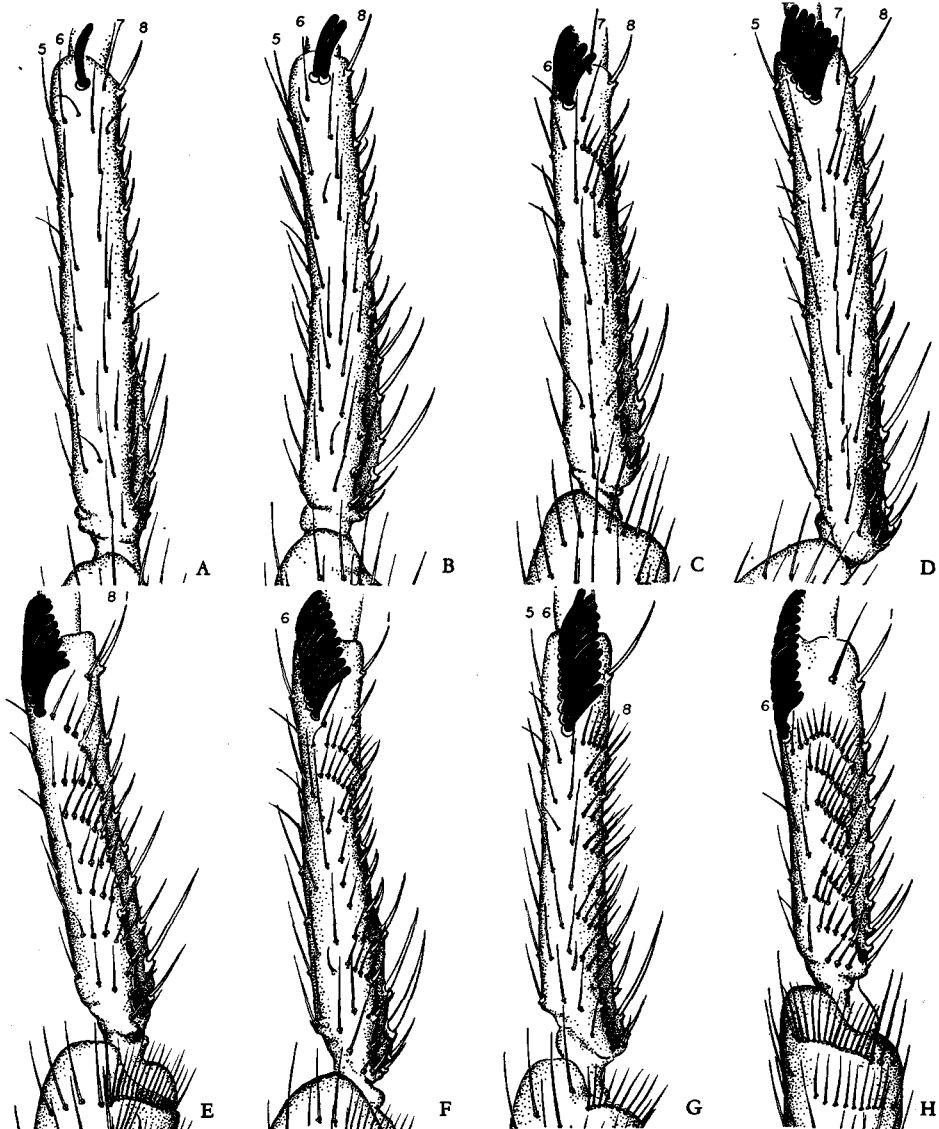


FIGURE 4.—Stages in transformation of a male third leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-H—Examples of the metatarsus and distal end of the tibia from third legs of males with one or more extra sex comb factors (A-B from *Scx*, C, E, G, and H from *esc-Scx* and D and F from *Scx-Pc*). For details see Figures 1A and D and 2.

ranked (taking into consideration the variance as before) for the 13 characters of the third leg the mean ranks place the genotypes in a graduated series:

$$\text{Sam-Ore} > \text{Pc} > \text{Scx} > \text{esc} > \text{Scx-Pc} = \text{esc-Scx} > \text{esc-Scx-Pc}$$

and the linearity is correlated with the increase in number of extra sex comb factors (Figure 3).

The difference in order of the mean ranks among the genotypes showed that the mode of action of the extra sex comb mutants must be different in the second and third legs. The greatest difference in the second leg occurred between the one- and two-factor compounds; in the third leg the difference was as great between *esc* and *Scx*, and the two- and three-factor compounds as it was between the one- and two-factor compounds. Secondly, the order of *Scx* and *esc* were reversed in the third leg as compared to the second leg (second leg—mean rank of *Scx* = 3.35, and *esc* = 2.88; third leg—mean rank of *Scx* = 2.58 and *esc* = 4.19). Furthermore, the difference between *Scx* and *esc* in transforming the two legs into first legs was expressed to the greatest degree in those characters which normally are not present in the posterior legs, e.g., the sex combs and transverse rows (Tables 1 and 4).

The difference between *esc* and *Scx* is shown most clearly in the penetrance and expressivity of the sex comb character. In *Scx*, 80.7 percent of the second legs had sex combs with an average of 5.9 teeth per comb in the legs with combs, while only 22.1 percent of the third legs had sex combs with a mean of 2.1 teeth per comb. This means that fewer third legs had sex combs and of those legs with combs, the number of teeth per comb was less in the third leg than the second leg. Therefore, both penetrance and expressivity are lower in the third leg than the second leg. In *esc* 92.7 percent of the second legs had combs with 5.9 teeth per comb on the legs with combs and 70.7 percent of the third legs had combs with 4.0 teeth per comb. In *Pc* 38.5 percent of the second legs had combs with 1.6 teeth per comb and 14.7 percent of the third legs had combs with 1.1 teeth per comb. Thus in the third legs of *esc* and *Pc*, penetrance is more affected than expressivity. *Scx* behaves more like *esc* in expression of the sex combs in the second leg, but more like *Pc* if the third leg is considered.

(1) *Systematic variation within a genotype*: Complete chaetotaxal studies of the tarsus and distal end of the tibia using relatively large samples were made only for two genotypes, *Scx* and *Scx-Pc* (Table 5). The third leg of *Scx* flies, if separated either on the basis of those with sex combs vs. those without sex combs, or by other criteria (as in Table 5) show little systematic variation. This could be interpreted as lack of correlation in expression of the various characters. However, if only the characters that are not normally present in the posterior legs are considered, there is some evidence for a systematic change within the *Scx* genotype.

In the *Scx-Pc* males the evidence of a systematic change in the third leg is more convincing although even in this genotype only nine of the 13 characters showed the expected increase or decrease parallel with an increase in number of sex comb teeth, in spite of the fact that the number of teeth per comb ranged from 0–15.

TABLE 5

Evidence of a systematic change in the expression of 13 characters in the third leg and in one- and two-factor genotypes

Genotype	No sex combs in 2 & 3	3rd leg not affected	Scx/+ Transverse rows in tarsus	Sex comb teeth	0-6 sex comb teeth	Scx/Pc 7-9 sex comb teeth	10-15 sex comb teeth
Phenotype Sample	8	39	20	19	9	24	13
Row 1	11.6	11.5	11.6	11.3	11.7	10.8	11.2
Row 2*	11.9	11.7	11.7	11.6	11.7	11.0	11.5
Row 3	9.5	9.7	9.7	9.8	9.0	9.3	9.1
Row 4	7.1	7.3	7.2	7.2	7.0	7.0	7.0
Row 5	6.6	7.0	6.9	7.0	6.4	5.8	5.5
Row 5.5	1.1	1.2	1.3	1.7	3.4	3.6	3.6
Row 6	6.8	7.0	6.9	6.7	6.1	5.0	5.0
Row 7	10.3	10.2	10.0	9.7	7.3	7.3	6.5
Row 8	13.5	14.1	13.8	13.8	13.0	11.7	10.5
Tarsus length	83.1	83.5	83.8	82.1	78.9	74.5	73.7
Sex comb teeth	0.0	0.0	0.0	2.1	4.2	8.2	11.5
Ta. tr. rows	0.0	0.0	0.1	0.1	2.1	3.1	4.6
Ti. tr. rows	0.0	0.0	0.0	0.1	2.4	3.8	3.7

* Row 2 lists the number of transverse rows.

The 86 legs of Scx male were divided into four groups based upon phenotypic changes in the third leg. The 46 legs of Scx-Pc males were separated into three groups, 0-6, 7-9 and 10-15 sex comb teeth in the third leg. For details see Tables 1 and 2.

Such results are indicative of a large variance in the expression of each of the characters.

(2) *Covariance*: Only 15 of the 139 legs from Pc males had evidence of a chaetotaxal change, 14 with one or two sex comb teeth and one with a tibial row but no sex comb teeth. In Scx, 67 of the 86 legs were without combs; in spite of this the number of teeth was correlated with the length of the tarsus ($r = 0.45\ddagger$). Five males had transverse rows: one leg with four sex comb teeth had a tibial and a tarsal row, one leg, also with four teeth had only a tibial row; one leg with two teeth had a tibial row, one leg with one tooth had a tarsal row and one leg without any teeth had a tarsal row. The mutual relationship in the expression of the characters in the third leg becomes evident, by correlation tests, in the other genotypes. A high degree of correlation was found between two variates (six comparisons) for esc, esc-Scx and Scx-Pc, and a greater degree of correlation in esc-Scx-Pc in the third leg than in the second leg (Table 6). A significant correlation in Scx-Pc for all six pairs in the third leg and only three pairs in the second leg (Table 3) is further proof that there is an upper limit in the expression of each character. This threshold is reached for most of the characters in the second leg but not the third leg in Scx-Pc. In esc-Scx-Pc the threshold is reached for most characters, particularly the tibial rows, in both legs. This is expressed as a lack of correlation in either bivariate or trivariate comparisons.

As in the second leg, transformation of a third leg into a first leg in compounds of two factors, or three factors, is far greater than would be expected from their actions singly (Table 4). This is seen most clearly in the tarsal transverse rows:

in single factor genotypes there is less than one row, in two factor compounds the average is three rows and in the three factor compound the average is six rows. Thus each of these mutants has subthreshold effects which are only expressed in compounds with one another.

B. Changes in the metatarsus and tibia of the female meso- and methathoracic legs

The changes from the female second or third leg into a first leg include: (1) a decrease in the length of the metatarsus and a decrease in the number of bristles per row; (2) induction of transverse rows of bristles on the anterio-ventral and ventral surfaces of the metatarsus and distal end of the tibia. As these changes are not as obvious as the changes in the male, such as formation of a sex comb, it had formerly been thought that the extra sex comb mutants were sex-limited in their expression. However, the transformation of the posterior legs into female first legs occurs in the same manner as in the male and the final results are just as profound.

TABLE 6

Correlation in expression of the extra sex comb characters in the third leg of males with one or more extra sex comb mutants

	<i>esc/esc</i> n=82	<i>esc/esc;</i> <i>Scx/+</i> n=53	<i>Scx/Pc</i> n=50	<i>esc/esc;</i> <i>Scx/Pc</i> n=32
Sex comb teeth to length	0.25*	0.48†	0.39†	0.23
Sex comb teeth to tarsal rows	0.73†	0.64†	0.55†	0.51†
Sex comb teeth to tibial rows	0.50†	0.39†	0.48†	0.24
Length to tarsal rows	0.25*	0.37†	0.34*	0.62†
Length to tibial rows	0.13	0.08	0.29*	0.00
Tibial to tarsal rows	0.52†	0.27*	0.46†	0.01
Sex comb teeth to tarsal rows to tibial rows	0.65†	0.39*	0.54*	0.26

* The correlation (r) is significant at the five percent level.

† The correlation (r) is significant at the one percent level.

Mesothoracic leg: Comparisons of the length of the metatarsus and chaetotaxy of the metatarsus and distal end of the tibia of the female second leg for the seven genotypes are presented in Table 7 and examples of types in Figure 5. The principal change was in the length of the metatarsus and the transverse rows on the tibia and tarsus, which as in the male, changed proportionately with the increasing number of extra sex comb factors. If all of the characters are ranked (in the same manner as before) the mean ranks form a graduated series for the genotypes:

$$\text{Sam-Ore} > \text{Pc} > \text{esc} > \text{Scx} > \text{Scx-Pc} = \text{esc-Scx} > \text{esc-Scx-Pc}$$

The seriation of genotypes is the same as for the male second leg and consistent with the increasing number of extra sex comb factors (Figure 3).

The difference between the female and the male, e.g., the higher mean rank in the female for Sam-Ore, Pc, esc and Scx, and the lower mean rank for the female in the compounds may be because of sexual dimorphism in expression of the

TABLE 7
Comparisons of the chaetotaxy of the metatarsus and distal end of the tibia and length of the metatarsus of the first leg of Sam-Ore females and second legs from females of seven different genotypes

Genotype Sample	1st leg	Sam-Ore 20	2nd leg	Pc/+ 20	Scx/+ 20	esc/esc 20	Scx/Pc 20	esc/esc; Scx/+ 20	esc/esc; Scx/Pc 6
Row 1	8.0±0.0	11.6±0.1	12.6±0.2	11.5±0.3	11.1±0.2	10.0±0.1	10.0±0.2	10.0±0.2	9.2±0.4
Row 2	7.2±0.2	10.5±0.2	10.7±0.2	10.1±0.3	10.3±0.1	7.8±0.2	8.9±0.2	8.9±0.2	8.0±0.3
Row 3	5.7±0.2	6.9±0.1	7.4±0.2	6.9±0.2	7.7±0.1	6.6±0.1	6.7±0.2	6.7±0.2	6.0±0.0
Row 4	4.3±0.1	6.1±0.1	6.2±0.1	5.5±0.2	5.7±0.1	5.2±0.1	5.4±0.1	5.4±0.1	5.2±0.2
Row 5	4.7±0.2	6.1±0.1	6.1±0.1	6.0±0.1	5.9±0.1	5.2±0.1	5.4±0.1	5.4±0.1	5.0±0.0
Row 5.5	1.0±0.0	1.0±0.0	1.1±0.1	1.0±0.0	1.0±0.0	1.2±0.0	1.0±0.1	1.0±0.1	1.0±0.0
Row 6	5.4±0.1	7.0±0.2	6.7±0.1	6.7±0.2	7.2±0.1	6.1±0.1	6.1±0.1	6.1±0.1	5.2±0.2
Row 7	7.1±0.2	9.5±0.1	10.3±0.2	9.0±0.2	10.2±0.1	7.1±0.1	7.5±0.2	7.5±0.2	6.8±0.2
Row 8	8.1±0.1	11.8±0.2	12.2±0.2	11.7±0.3	11.5±0.2	9.3±0.2	8.7±0.2	8.7±0.2	8.2±0.2
Tarsus length	54.1±0.4	79.0±0.5	76.2±0.8	69.8±1.1	69.8±0.9	62.9±0.4	54.8±0.4	54.8±0.4	53.5±0.6
Ta. tr. rows	7.9±0.1	...	0.5±0.2	2.9±0.8	0.5±0.3	8.4±0.3	7.8±0.4	7.8±0.4	8.3±0.2
Ti. tr. rows	6.0±0.1	...	1.4±0.3	2.6±0.5	0.8±0.3	5.7±0.1	5.6±0.2	5.6±0.2	5.8±0.1

The entries in each column include the mean ± 1 S.E. for 12 characters: rows 1-8, tarsal transverse rows, and length of the tarsus. For abbreviations and other details see Table 1 and Figure 1B and C.

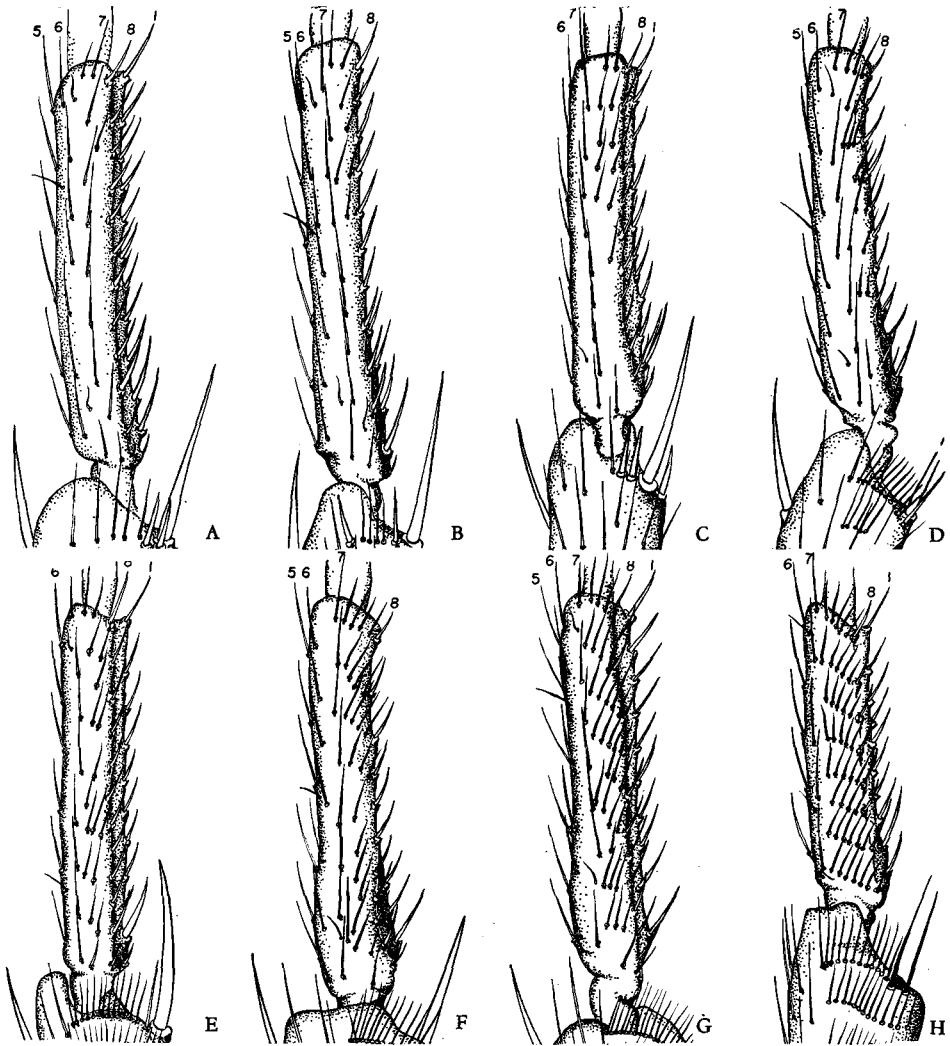


FIGURE 5.—Stages in transformation of a female second leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-H—Examples of the metatarsus and distal end of the tibia from second legs of females with one or more extra sex comb factors (B from Pc, A, E, and G from Scx; C, D, and F from esc; and H from esc-Scx-Pc). The newly induced or transformed bristles of the transverse rows are in solid black; the bristles characteristic of the second leg are in outline. For details see Figures 1B and C.

characters, or it may be due only to sampling error. A difference between the female and male in the expression of certain of the characters is suggested from comparison of individual characters in the two sexes.

The length of the metatarsus of the second leg decreases from approximately 77 to 45 units in the male while in the female the decrease is only from 79 to 54 units (Tables 1 and 7). In the first legs of Sam-Ore, the difference between the

two sexes in the length of the metatarsus is about 12 percent. In the second leg the difference between Sam-Ore females and males is only three percent while the difference between the second leg of *esc-Scx-Pc* females and males is 15 percent. The tarsus length of the female is reduced in size, as compared to Sam-Ore, by 32 percent and the male by 42 percent. This difference in decrease in length of the metatarsus in the two sexes is undoubtedly one of the reasons for the number of bristles per row, in rows 1–8, showing a greater decrease in the males than the females.

The bractless bristles (row 5.5) do not increase proportionately with the increase in number of extra-sex comb factors in the female. This would be expected if the extra sex comb mutants were sexually dimorphic in expression, for row 5.5 in the female first leg never has more than one or two bristles.

Except in *esc*, the number of tibial and tarsal transverse rows is usually greater in the female than in the male posterior legs of extra sex comb flies especially in the one- and two-factor genotypes. Such a difference would be expected for the tarsal rows because of the sexual dimorphism in number of rows in the first leg—an average of six rows in the male and eight in the female. In the tibia, however, both the female and male first legs had an average of six tarsal rows, thus a consistently higher number of rows would not be anticipated in the female posterior legs of the extra sex comb genotypes.

This dimorphism in expression of the various characters probably accounts for the higher mean rank of the females in the one-factor genotypes and the male in the three-factor genotype, with the females being higher in some but not all of the two-factor genotypes.

Metathoracic leg: Comparisons of the length of the metatarsus and the chaetotaxy of the metatarsus and distal end of the tibia of the female third leg are presented in Table 8 and Figure 6. As was found previously the changes in the expression of the extra sex comb phenotypes were proportional to the number of factors which when ranked form a graduated series for the genotypes:

$Pc > Sam-Ore > Scx > esc > esc-Scx > Scx-Pc > esc-Scx-Pc$

The seriation in the third leg of the females differs from the seriation in the second leg of both sexes and the third leg of the males (Figure 3). However, as the general pattern is the same as for the male third leg, the differences can probably be accounted for, to a large extent, by sampling error.

The most pertinent fact from ranking the genotypes is that in the female, as in the male, the rank of *Scx* and *esc* is reversed in the third leg as compared to the second leg. This means that in the females as in the males *esc* and *Scx* have different modes of expression in the second and third legs.

Finally, as was also found for the males, the threshold for expression of the extra sex comb phenotype was virtually reached in the second leg in two-factor compounds, but not until the three-factor compounds in the third leg.

C. Changes in the coxa and femur

In the wild type fly, the three pairs of legs differ considerably in the shape and structure of the coxa (Figures 7 A-C). The coxa of the first leg is more tubular,

TABLE 8

Comparison of the chaetotaxal pattern of the metatarsus and distal end of the tibia and length of the metatarsus of the third leg of females from seven different genotypes

Genotype Sample	Sam-Ore 20	Pc/+ 20	Scx/+ 20	esc/esc 20	Scx/Pc 20	esc/esc; Scx/+ 20	esc/esc; Scx/Pc 6
Row 1	11.6±0.2	12.3±0.1	11.6±0.1	10.4±0.2	11.2±0.2	10.5±0.2	9.8±0.3
Row 2*	11.8±0.1	12.4±0.2	11.7±0.1	10.5±0.1	11.1±0.1	10.4±0.1	10.0±0.3
Row 3	10.5±0.2	11.1±0.1	10.2±0.1	9.8±0.2	9.6±0.1	10.1±0.1	9.0±0.5
Row 4	7.4±0.1	7.9±0.1	7.9±0.2	7.1±0.2	7.3±0.1	7.1±0.2	7.2±0.3
Row 5	7.1±0.1	7.7±0.1	7.3±0.1	6.1±0.1	6.8±0.1	6.3±0.1	6.0±0.3
Row 5.5	1.0±0.0	1.4±0.1	1.0±0.0	1.0±0.0	1.1±0.1	1.0±0.0	1.0±0.0
Row 6	7.5±0.1	7.7±0.2	7.3±0.2	7.3±0.2	6.9±0.1	7.0±0.2	6.7±0.2
Row 7	10.5±0.2	10.7±0.2	10.0±0.3	10.3±0.2	8.4±0.2	9.5±0.2	8.3±0.5
Row 8	12.9±0.2	14.4±0.2	13.9±0.3	12.9±0.2	11.7±0.2	12.6±0.4	11.0±0.5
Tarsus length	90.6±0.4	89.3±0.5	85.7±0.4	75.5±1.0	77.9±0.5	74.3±1.2	65.0±1.7
Ta. tr. rows	0.3±0.1	0.4±0.2	0.3±0.1	7.1±0.4	3.2±0.5	7.3±0.8
Ti. tr. rows	0.8±0.3	0.4±0.2	0.3±0.2	4.8±0.1	3.4±0.4	4.8±0.3

* Row 2 lists the number of transverse rows.

The entries in each column include the mean±1 S.E. for 12 characters. For abbreviations and other details see Table 1.

longer and more separated from the body than the coxae of the posterior legs. The principal difference in the femurs is in the complete proximal-distal row of larger, longer bristles on the postero-dorsal surface of the femur of the first leg but absent in the posterior legs.

The coxae and femurs of the posterior legs were transformed into first leg-like segments only in compounds of several extra sex comb mutants. Transformation occurs in the same manner and is parallel with transformation of the tibiae and metatarsi (Figures 7 D-G). However the changes are not as great as in the more distal segments. The legs of both the female and male are affected to the same extent by the factors or their compounds.

Scx and Pc: The flies of these two genotypes have little if any changes in the structure or chaetotaxy of the coxa or femur of either posterior leg indicative of transformation from a second or third leg into a first leg.

esc: The coxae of neither the second or third leg show any changes indicative of transformation of these segments into first leg segments. The femur usually has the characteristic chaetotaxy of the second or third leg. Not infrequently, however, a row of bristles on the postero-dorsal surface of the mesothoracic femur is more distinct and the bristles are somewhat longer. There is no evidence of two or three longer preapical bristles on the dorsal surface of the femur.

Scx-Pc: The coxae of both the second and third legs had very slight morphological changes, if any, in the direction of the first leg. Many flies—even those with profound changes in the metatarsus—show no evidence of a row of bristles on the postero-dorsal surface of the femur. In others, two or three bristles are in the proper position and long enough to be considered evidence of a chaetotaxal change in the direction of a first leg. In a very few flies the row of longer bristles is complete and not distinguished from the row of bristles in the first leg.

esc-Scx: The coxa of the second leg shows considerable transformation toward a first leg coxa so that in the majority of the flies it is intermediate in structure and chaetotaxy between the first and second leg. The row of bristles on the posterior-dorsal surface of the femur may be entirely missing or present to a varying

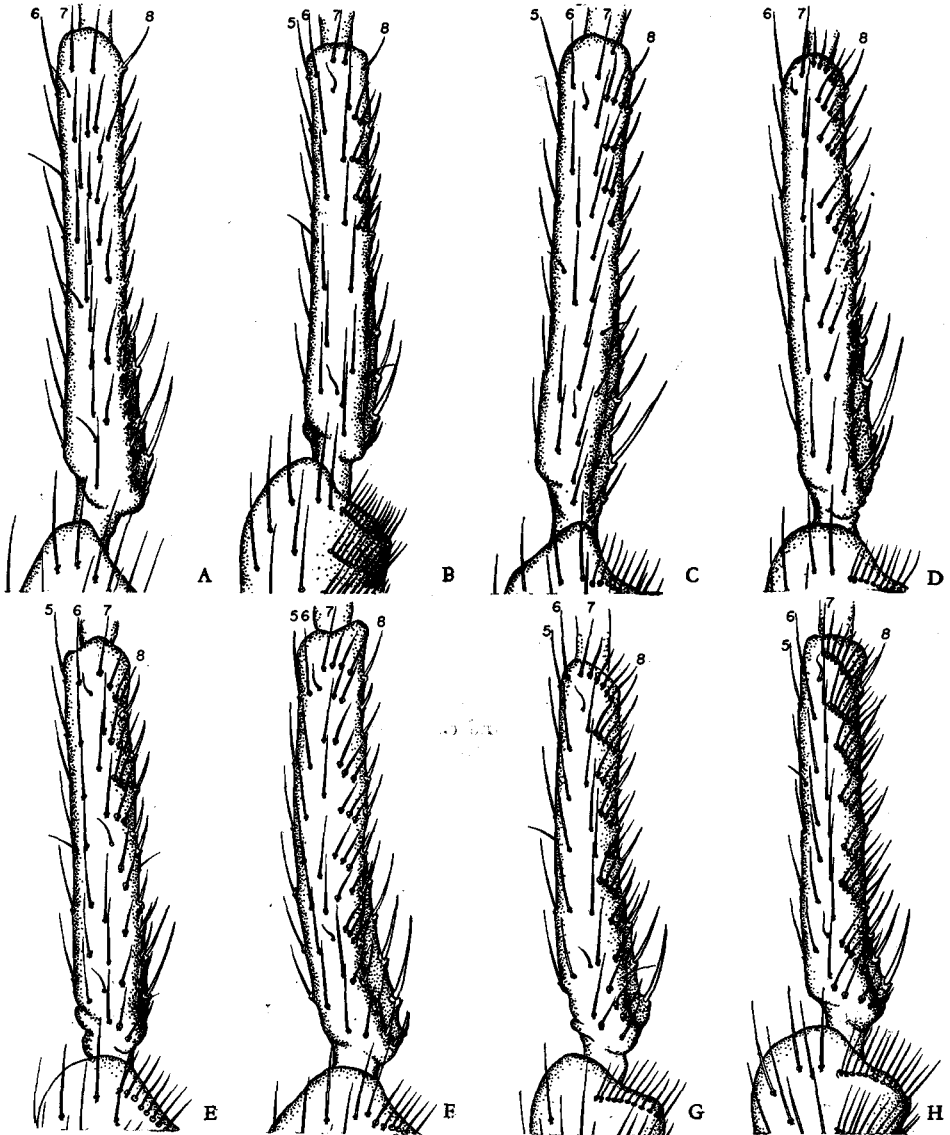


FIGURE 6.—Stages in transformation of a female third leg into a first leg through changes in size of the tarsus and chaetotaxy of the tarsus and tibia. A-H—Examples of the metatarsus and distal end of the tibia from third legs of females with one or more extra sex comb factors (A and B from *Scx*; C from *esc*; D and F from *Scx-Pc* and E, G, and H from *esc-Scx-Pc*). For details see Figures 1B and D and Figures 5.

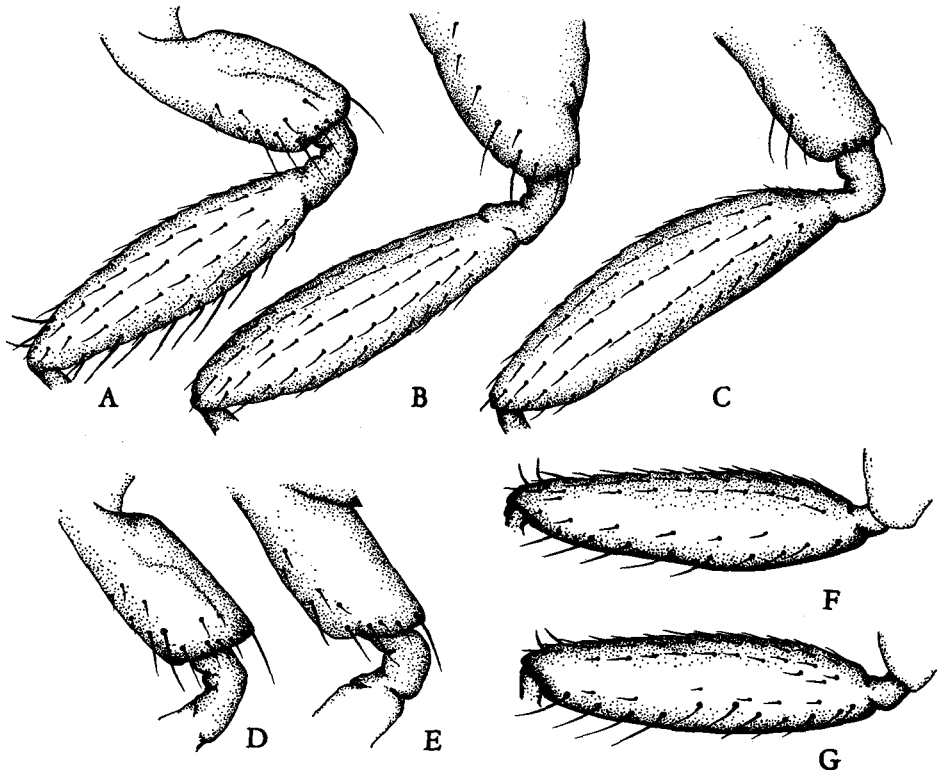


FIGURE 7.—Drawings of the most proximal segments of the three legs of Sam-Ore and extra sex comb males depicting transformation of the coxa and femur of the second leg into coxa and femur characteristic of the first leg. A.—Anterio-lateral view of the coxa and femur of a first leg of a Sam-Ore male showing the structure and chaetotaxy of the coxa and the complete row of bristles on the postero-dorsal surface of the femur. B.—Same for the second leg. The complete row of bristles is absent from the femur. C.—Same for the third leg. D.—The coxa of a first leg from an *esc-Scx-Pc* male (anterior view). E.—A second leg from the same fly showing transformation of the coxa into a first-leg coxa. F.—The femur of a first leg from a *Scx-Pc* male (dorsal view). G.—The femur of a second leg from the same fly showing the row of bristles on the postero-dorsal surface of the femur (dorsal view).

degree. However, the group (usually three to four) of longer bristles on the dorsal surface is, in the majority of the legs examined, well differentiated. The coxa of the third leg has less change than the coxa of the second leg, and there is never any indication of rows of bristles on the femur.

esc-Scx-Pc: The changes in the coxa of the second leg are more profound than in *esc-Scx* flies. The coxa is frequently like a first leg coxa (Figure 7 E), but even in the flies with the greatest transformation in the direction of the prothoracic segment, it is only about 60 percent as long as the first leg coxa and never as well separated from the body. The majority of the flies have a complete row of bristles on the postero-dorsal surface of the femur of the second leg and frequently the group of longer preapical bristles on the dorsal surface (Figure 7G). The third

legs of *esc-Scx-Pc* flies show little indication of morphological changes in the coxa and femur indicative of transformation of these segments into first leg segments.

It may be that the crippling of the legs, which occurs in many of the *esc-Scx-Pc* flies, is partially responsible for some of the morphological changes interpreted as homoeotic changes. However, as the observations were made primarily on legs which were not crippled, it seems reasonable to conclude that the changes in the coxa and femur of the posterior legs of *esc-Scx-Pc* flies are homoeotic changes just as the sex combs and transverse rows are homoeotic changes. Furthermore, it seems reasonable to assume also that the changes in the posterior legs of *esc-Scx-Pc* flies are the result of the transformation of these legs into first legs, just as they are in the two-factor compounds where leg-crippling was not encountered.

DISCUSSION

The sex combs are taxonomically important morphological structures in many but not all of the species of the *Sophophora* subgenus of *Drosophila*. In some species they occur only on the metatarsus, in others they are on both the first and second tarsal joints of the male first legs. A great variety of types of combs and range in numbers of teeth per comb occur in all of the species-groups (c.f., Figure 10, STERN 1954b). SPIETH (1952) proposed that the sex combs function as claspers during courtship and mating, and pointed out the concordance in type of comb and type of mating behavior. There is little doubt that sex combs are of selective value and it can be presumed that they have been derived from an ancestral form or ancestral forms. The different types could arise either by a slow elaboration of new genetic systems or by more sudden changes initiated by one or a very few genetic changes.

It is well known that the number of teeth per comb in *D. melanogaster*, as well as in other species, varies from strain to strain and that sometimes, but not always, there is a response to selection (c.f., BRASTED 1941; MATHER and DOBZHANSKY 1939; PREVOSTI 1955; SLIFER 1942). On the other hand, many mutants are known which in one step reduce the comb to one or two teeth, change the whole morphology of the comb or induce the formation of new combs (c.f., BRIDGES and BREHME 1944; HANNAH and STRÖMNAES 1955; STERN 1954b; STRÖMNAES unpublished; STURTEVANT 1929). If a single mutant can have such drastic effects upon differentiation of the sex comb, "it seems entirely possible that the evolutionary processes which diversified the sex comb features in different species began with the same kind of sudden changes . . . namely the response of mutated genes to pre-existing developmental prepatterns and the creation of new prepatterns by mutations." (STERN 1954b).

The concept of prepatterns was first proposed by STERN and HANNAH (1950) to interpret differentiation of the sex combs in gynanders of *D. melanogaster*, and has been further developed by these authors in studies of both bristles and sex combs (HANNAH-ALAVA and STERN 1957; STERN 1954a, b, 1956a, b, 1957; STERN and SWANSON 1957). From such studies it was concluded that there are at least

two ways in which genes may determine the bristle patterns. The genes may affect the paths of development in such a way as to bring about, early in development, different over-all organization of a region, i.e., different prepatterns in different genotypes. Or the differential effect of different genes may not set in until after the establishment of a given, unchanged prepatter. In other words, "in the first alternative different bristle patterns would originate in consequence of different prepatterns; in the second, the bristle pattern would depend upon the genetically controlled competence of the hypodermal cells to respond or not to respond to the singularities of the constant prepatter." (STERN 1957).

Many of the mutants of *Drosophila* can be classified as either "prepatter" or "terminal-pattern" mutants. Among the many mutants which remove bristles, most are probably of the latter type; that is, the prepatter for development of a bristle in a specific position is present but for some reason the bristle does not develop. The mutant *split*—in which the bristles are sometimes doubled and sometimes missing—is probably an example of a terminal-pattern mutant and also an example of the complex morphogenetic relationships in differentiation of the phenotype. Histological studies by LEES and WADDINGTON (1942) have shown, firstly, that three instead of two divisions occur, producing four instead of two cells at each bristle site and secondly, that orientation of this group of four cells in the hypodermis determines whether no bristle, one bristle or two bristles are finally differentiated. In one *split* strain, bristles are rarely formed because most of the groups of four cells lie below the surface of the epithelium and do not develop into bristles. Obviously the prepatter for development of a bristle at each bristle site is presented in *split* flies, but the final pattern is dependent upon a second morphogenetic process—the position and orientation of the presumptive bristle cells.

The homoeotic mutants which change the development of a segmental appendage into another type are probably the best example of prepatter mutants. Among the homoeotic mutants several are known which change the legs into different types or even transform other completely different organs into legs: *aristapedia* (BALKASCHINA 1929) in which the arista becomes a tarsus, *Antennapedia* (LEWIS 1956) in which the antennae becomes a leg, *bithorax* (BRIDGES and MORGAN 1923) in which the third leg in some respects becomes a second leg (along with other changes), *bithoraxoid* (LEWIS 1951) in which an abdominal third leg is formed (as well as other changes) and the extra sex comb mutants which change the posterior legs into first legs.

In understanding the results from the extra sex comb genotypes and their application in analysis of prepatter mutants, several important questions are raised: Why are sex combs differentiated only on the first leg and not on the second and third legs of normal flies? How do the extra sex comb genotypes cause the differentiation of sex combs in legs not normally possessing them?

If it is assumed, for example, that the prepatter for sex combs is already present in all of the legs but that "intensity" (c.f., STERN 1954b) is the deciding factor in the expression of the final phenotype, the lack of sex combs on the pos-

terior legs could be interpreted as an intensity below the threshold of expression in these legs. According to this hypothesis the extra sex comb genotypes would respond to lower intensities than the non sex comb genotypes. Another interpretation is that a diffusible "sex comb substance" produced somewhere in the body is responsible for the formation of the sex combs (c.f., BRASTED 1941; SLIFER 1942). According to this hypothesis, an increase in the sex comb substance in the extra sex comb flies would induce the formation of sex comb teeth in the posterior legs. Either a gradient in the substance itself or a gradient in threshold of response in the legs would account for lack of combs in the posterior legs of normal flies and their presence in extra sex comb flies.

Both hypotheses are based, in part, upon the assumption that only the sex combs are the product of the action of the extra sex comb mutants. Such schemes would not account for the homoeotic effects of the extra sex comb mutants, for they transform a second or third leg into a first leg and all segments are affected. Neither would such schemes explain the interactions of *Antennapedia*, *aristapedia* and the *extra sex comb* mutants in the production of sex combs upon the antennal legs (see STERN 1954b for details). Thus it seems likely that the extra sex comb factors act by changing the prepattern of the embryonic legs, and to this changed prepattern the cells in the male genotype respond by formation of sex comb teeth.

SUMMARY

1. A morphological and chaetotaxal study was made of the legs of flies with one or more of the extra sex comb mutants, extra sex comb (*esc*) a second chromosome recessive, Polycomb (*Pc*) and Extra sex comb (*Scx*) two homozygous lethal third chromosome dominants. Comparisons were made between seven genotypes: wild type, *esc/esc*, *Pc/+*, *Scx/+*, *esc/esc;Scx/+*, *Scx/Pc* and *esc/esc;Scx/Pc*.

2. The primary action of each mutant is to change the posterior legs into first legs by changes in the size, shape and chaetotaxy of all of the segments. Although there was a considerable range in expression of each of the characters, a change in any one character was accompanied by a proportional change in another character, that is the expression of the characters are covariant.

3. The most conspicuous character in the extra sex comb genotypes is the sex combs on the metatarsi of the second and third legs of the males. These mutants also induce in both sexes transverse rows of bristles on the distal end of the tibia and metatarsus and a complete row of bristles on the femur, characters normally present only in the first legs. In addition there are changes in the size, shape, and chaetotaxy of the coxae and in the number of bristles per row and the length of the metatarsi in the posterior legs.

4. The extent of change in the posterior legs is proportional to the number of extra sex comb factors. A complete transformation of a second leg into a first leg is achieved only in the compound of all three mutants. The augmented action of the mutants in compounds as compared to their action singly is indicative of sub-threshold effects which in the single factor genotypes contribute to variance in

expression of the phenotype, but in compounds interact to produce full expression of the extra sex comb characters.

5. Transformation of a second or third leg into a first leg occurs in both sexes, but in the males the posterior legs become male first legs while in the females the posterior legs are transformed into female first legs.

6. The three mutants can be differentiated from one another by differences in penetrance, expressivity and action in the compounds.

7. From a consideration of the phenogenetics of the extra sex comb mutants and by comparison to other homoeotic mutants, it is proposed that they are "pre-pattern" mutants.

LITERATURE CITED

- BALKASCHINA, E. I., 1929 Ein Fall der Erbhomöosis (die Genovariation "Aristopedia") bei *Drosophila melanogaster*. Arch. Entwicklunsmech. Organ. **115**: 448-463.
- BRASTED, A., 1941 An analysis of the expression of the mutant 'engrailed' in *Drosophila melanogaster*. Genetics **26**: 347-373.
- BRIDGES, C. B., and K. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552**.
- BRIDGES, C. B., and T. H. MORGAN, 1923 The third chromosome group of mutant characters of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **327**.
- DOBZHANSKY, TH., 1930 The manifold effects of the genes Stubble and stubbloid in *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **54**: 427-457.
- GRIMSHAW, P. H., 1905 On the terminology of the leg-bristles of Diptera. Ent. Mo. Mag. **41**: 173-176.
- HANNAH, A. and Ø. STRÖMNAES, 1955 Extra sex comb mutants in *D. melanogaster*. Drosophila Inform. Serv. **29**: 121-123.
- HANNAH-ALAVA, A., 1958 Morphology and chaetotaxy of the legs of *Drosophila melanogaster*. J. Morphol. (In Press)
- HANNAH-ALAVA, A., and C. STERN, 1957 The sex combs in males and intersexes of *Drosophila melanogaster*. J. Exptl. Zool. **134**: 533-556.
- LEES, A. D., and C. H. WADDINGTON, 1942 The development of the bristles in normal and some mutant types of *Drosophila melanogaster*. Proc. Roy. Soc. London B. **131**: 87-110.
- LEWIS, E. B., 1951 Pseudoallelism and gene evolution. Cold Spring Harbor Symposia Quant. Biol. **16**: 159-174.
- 1956 Report on the mutants *Antp^B* and *Antp^{Y^u}*. Drosophila Inform. Serv. **30**: 76-77.
- LEWIS, E. B., and R. GRELL, 1953 Report on "multiple" chromosomes. Drosophila Inform. Serv. **27**: 57-58.
- LEWIS, P., 1947 *Pc*: Polycomb. Drosophila Inform. Serv. **21**: 69.
- MATHER, K., and TH. DOBZHANSKY, 1939 Morphological differences between the 'races' of *Drosophila pseudoobscura*. Am. Naturalist **73**: 5-25.
- PREVOSTI, A., 1955 Variacion geografica de caracteres cuantitativos en poblaciones britanicas de *Drosophila subobscura*. Genet. Iberica **7**: 3-44.
- SLIFER, E. H., 1942 A mutant stock in *Drosophila* with extra sex combs. J. Exptl. Zool. **30**: 31-40.
- SPIETH, H. T., 1952 Mating behavior within the genus *Drosophila* (Diptera). Bull. Am. Museum Nat. Hist. **99**: 395-474.

- STERN, S., 1954a Two or three bristles. *Am. Scientist* **42**: 213-247.
- 1954b Genes and developmental patterns. *Caryologia* (Suppl.) **6**: 355-369.
- 1956a Genetic mechanisms in the localized initiation of differentiation. Cold Spring Harbor Symposia, Quant. Biol. **21**: 375-382.
- 1956b The genetic control of developmental competence and morphogenetic tissue interactions in genetic mosaics. Wilhelm Roux' Arch. Entwickl. Org. **149**: 1-25.
- 1957 The role of genes in differentiation. Proc. Intern. Genet. Symp. Suppl. Vol. of Cytologia, 1957: 70-72.
- STERN, C., and A. HANNAH, 1950 The sex combs in gynanders of *Drosophila melanogaster*. Portugaliae Acta Biol. Ser. A. (R. B. Goldschmidt) 798-812.
- STERN, C., and D. L. SWANSON, 1957 The control of the ocellar bristle by the scute locus in *Drosophila melanogaster*. J. Fac. Sci. Hokkaido Univ. Series VI. **13**: 303-307.
- STRÖMNAES, Ø., and A. HANNAH, 1955 Mounting of *Drosophila* legs. *Drosophila Inform. Serv.* **29**: 179.
- STURTEVANT, A. H., 1929 The genetics of *Drosophila simulans*. Carnegie Inst. Wash. Publ. **399**: 1-62.