DEVELOPMENTAL GENETICS OF THE MUTANT COMBGAP IN *DROSOPHILA MELANOGASTER.* I: EFFECT ON THE MORPHOLOGY AND CHAETOTAXY OF THE PROTHORACIC LEG*

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THE genetic basis of cellular differentiation in higher organisms can be studied at different levels: (a) at the molecular level, limiting the study to changes in the process of cellular metabolism and physiology (e.g., studies of GALL and CALLAN, see CALLAN 1963; DAVIDSON *et al.* 1965; BEERMANN 1964, 1965; PAVAN 1965; SCHULTZ 1965; CLEVER 1966), or to differential protein realization (PAIGEN and GANSCHOW 1965); (b) at the developmental level, tracing the gene effects from their first appearance (studies of HADORN and his collaborators, see HADORN 1965, 1966); or (c) at the level of pattern differentiation (STERN 1954a, 1965, 1968) at the final step of gene-controlled cell product. This last category of investigation forms the basis of the present study on genetic control of cellular differentiation in higher organisms.

STERN (1956) has proposed that pattern differentiation in higher organisms is a consequence of several gene-controlled steps which, preceding the final pattern formation, evoke a "regional differentness" termed prepattern, and the realized pattern is the result of localized cellular response to that prepattern. According to this concept, the mutant genes should exert their influence on differentiation either by changing the prepattern itself or by changing the ability of cells to respond to the prepattern stimuli. Earlier studies have shown that most of the mutants affecting the bristle patterns are responsible for initiating a change in the terminal step or steps of pattern formation (TOKUNAGA and STERN 1965; STERN 1968). However, the behavior of the mutant gene ey^D in genetically produced mosaics (STERN and TOKUNAGA 1967) and in transplants (TOKUNAGA 1968) has reinstated the possibility for the existence of a prepattern gene.

The effects of prepattern *vs.* competence genes can be resolved by the time of their action in development. The distinction can be made either by analysis of the interaction between different pattern mutants (LEES and WADDINGTON 1942; ANDERS 1955; MUKHERJEE 1965; MUKHERJEE and MITRA 1967) or by mutants in genetic mosaics (STERN 1936, 1956, 1968; STERN and HANNAH 1950; TOKUNAGA 1961, 1962, 1968; MUKHERJEE and STERN 1965). MUKHERJEE

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(1965) has shown that the effects of interaction of genes can unmask certain important underlying events which otherwise remain cryptic in the normal process of pattern formation; for example, the formation of a new array of secondary transverse rows of bristles on the prothoracic basitarsus of *sx–en*. This work presents the results of genetic analysis of another mutant, combgap, of *Drosophila melanogaster*. Its phenotypic effects on the bristle pattern of the prothoracic legs, singly and in combination with other pattern mutants are strikingly different. The action of the mutant combgap (*cg*), which is distinguishable from that of other mutants in its effect on the sexcomb and bristle pattern, forms the basis of the prediction that it initiates a change in the basitarsal chaetotaxy in a manner conforming with its effects on the prepattern stimulus.

MATERIALS AND METHODS

The wild-type strain Oregon-R (Ore), the mutant combgap (*cg*), and five other bristle pattern mutants of *Drosophila melanogaster* described below formed the materials for the present study. *cg* (*2*:71.1) is an autosomal recessive mutant which induces an increase in the teeth number in the sexcomb in male prothoracic legs and eliminates the fourth longitudinal vein of the wings distal to the posterior crossvein. The homozygous *cg* female is sterile, and therefore the stocks were maintained in heterozygous condition as *cg c/U* and *cg c/Cy*. The mutant curved (*c*) was used as an additional marker, and upturned (*U*) and Curly (*Cy*) are the balancers (LINDSLEY and GRELL 1968). Observations for mutants as well as the wild type will be restricted to their effects on the basitarsal bristle patterns and morphology of the prothoracic leg. A brief description of the mutants used for studying interactions is given below.

1. Engrailed (*en*, *2*:62.0) causes the formation of a cleft at the tip of the scutellum, and forms on the basitarsus of the foreleg a secondary sexcomb in mirror-image fashion to the primary (BRASTED 1941). The mean number of teeth in the primary sexcomb in *en* (of *pr en* stock) males is about 11, as compared to about 10 in Ore; that in the secondary is about 5.

2. Polycomb–Extra Sexcomb complex (*Pc–Scx*, *3* : ?): Both Polycomb (*Pc*) and Extra Sexcomb (*Scx*) initiate jointly or singly the formation of sexcombs on the second and third legs, in addition to the one on the first. The stock *th st Pc–Scx* p^p *ss/D* has been used in the present study; the homozygous *Pc–Scx/Pc–Scx* is lethal (HANNAH-ALAVA 1958).

3. Transformer (*tra*, *3*:44.0–45.3): In homozygous condition *tra* changes the *XX* female into a phenotypic male and produces male external genitalia and sexcombs on the forelegs (STURTEVANT 1945). *tra* has no effect on the *XY* (or *X0*) male.

4. Sexcombless (*sx*, *1*: locus to the right of *sn*) causes absence or reduction in the number of sexcomb teeth in male forelegs. *sx* males are sterile, poorly viable, and rarely come out of the pupal case (MUKHERJEE 1965). In addition to typical teeth, intermediate bristles appear in the sexcomb area.

5. Eyeless-dominant (ey^D, *4*: reverse tandem duplication) causes the formation of abnormal eyes and of a large number of teeth in the primary sexcomb. The teeth are oriented either in parallel rows or in extreme condition in clusters (STERN and MUKHERJEE 1964). In the female basitarsus of ey^D several extra bristles are found near the junction of the first two tarsal elements. Since flies homozygous for ey^D are lethal, the stock is maintained in heterozygous condition, *T(1;4)* w^m/ey^D.

These mutants were combined individually with *cg* by the usual procedure. The different combination stocks synthesized were *cg/cg;Pc–Scx/+*, *sx/Y;cg/cg*, *en cg/en cg*, *cg/cg*; *tra/tra*, and *cg/cg*; $ey^D/+$, respectively, and they will be referred to as *cg–Pc–Scx*, *sx–cg*, *en–cg*, *cg–tra*, and *cg–*ey^D, respectively.

All flies were raised in standard Drosophila medium and reared at 24° ± 1°C. A drop of living yeast was added to the culture bottles prior to use. The stock cultures of the wild-type (Ore) and mutant strains were made in parallel condition. Flies eclosed during the 14th to 15th

day were taken out and fixed in 70% ethyl alcohol in small fixing tubes. Prothoracic legs were dissected out in 90% ethyl alcohol and mounted in André medium or Euparal between two cover glasses. Since *sx* males are unable to eclose, *sx* and *sx-cg* imagines were dissected out from the pupal case. The preparations were examined and recorded by camera lucida drawings and photographs under a binocular compound microscope (Zeiss) using an 8× ocular and a 40× objective.

RESULTS

Morphology and chaetotaxy of the basitarsus of combgap prothoracic legs: A normal male basitarsus consists of 6 to 7 transverse rows, 8 longitudinal rows of bristles (1′ and 1 to 7), a sexcomb of about ten teeth and a stout central bristle. The female basitarsus lacks the last two items but has an extra transverse row instead (Figures 1A, B). The bristle patterns of male and female basitarsi of the *cg* mutant are somewhat different from those of the normal flies and will be described in detail below (Figures 1C, D).

General morphology of the basitarsus: The basitarsal element of *cg* is frequently inflated and overlaps proximally with the tibia and distally with the second tarsal segment. The marginal line between the tibia and basitarsus or that between the second tarsus and the basitarsus is frequently absent or incomplete. The length of the basitarsus is decreased significantly in both sexes in comparison with the respective sexes of the wild type (see below).

Bristle patterns of basitarsus. The sexcomb: While the mean numbers of sexcomb teeth in the four non-combgap strains (Ore, *c*, *cg c/U*, and *cg c*/Ore) are more or less similar, it is significantly higher in *cg* males than in each of the four genotypes ($P < 0.01$, Table 1). There is no overlapping in the distributions of teeth numbers in the combgap and non-combgap strains (range for non-*cg* =

TABLE 1

Mean numbers and distributions of primary sexcomb teeth in Ore, cg, *various single mutants, and* cg-*combinations*

Genotype	Distribution of teeth 8	9	10	11	12	13	14	15	16	17	18	19	20–32	Mean±S.E.	N
Part A															
Ore	1	10	12	6	1	..	..	..	..	..	..	..	..	0.8±0.16	30
c/c	2	5	14	7	2	..	..	..	..	..	..	..	..	10.0±0.15	30
cg c/U	..	7	12	10	1	..	..	..	..	..	..	..	..	10.1±0.15	30
cg c/Ore	..	3	10	12	5	..	..	..	..	..	..	..	..	10.6±0.15	30
cg c/cg c	..	..	..	..	1	9	5	2	10	3	0	3	15	17.7±0.62	48
Part B															
*en**	..	1	4	15	7	2	1	..	..	..	..	..	..	11.2±0.18	30
*en–cg**	..	..	1	3	2	4	6	7	3	1	1	0	2	14.4±0.44	30
Pc–Scx	1	6	30	11	4	..	..	..	..	..	..	..	..	10.2±0.11	52
cg–Pc–Scx	..	..	1	1	2	2	12	9	2	1	..	..	..	14.1±0.25	30
tra	..	1	8	7	3	1	..	..	..	..	..	..	..	10.7±0.20	30
cg–tra	..	..	1	2	8	1	5	1	1	2	..	..	1	13.5±0.60	22

* The mean numbers of secondary sexcomb teeth in *en* and *en–cg* are 4.9±0.26 and 0.8±0.37, respectively.

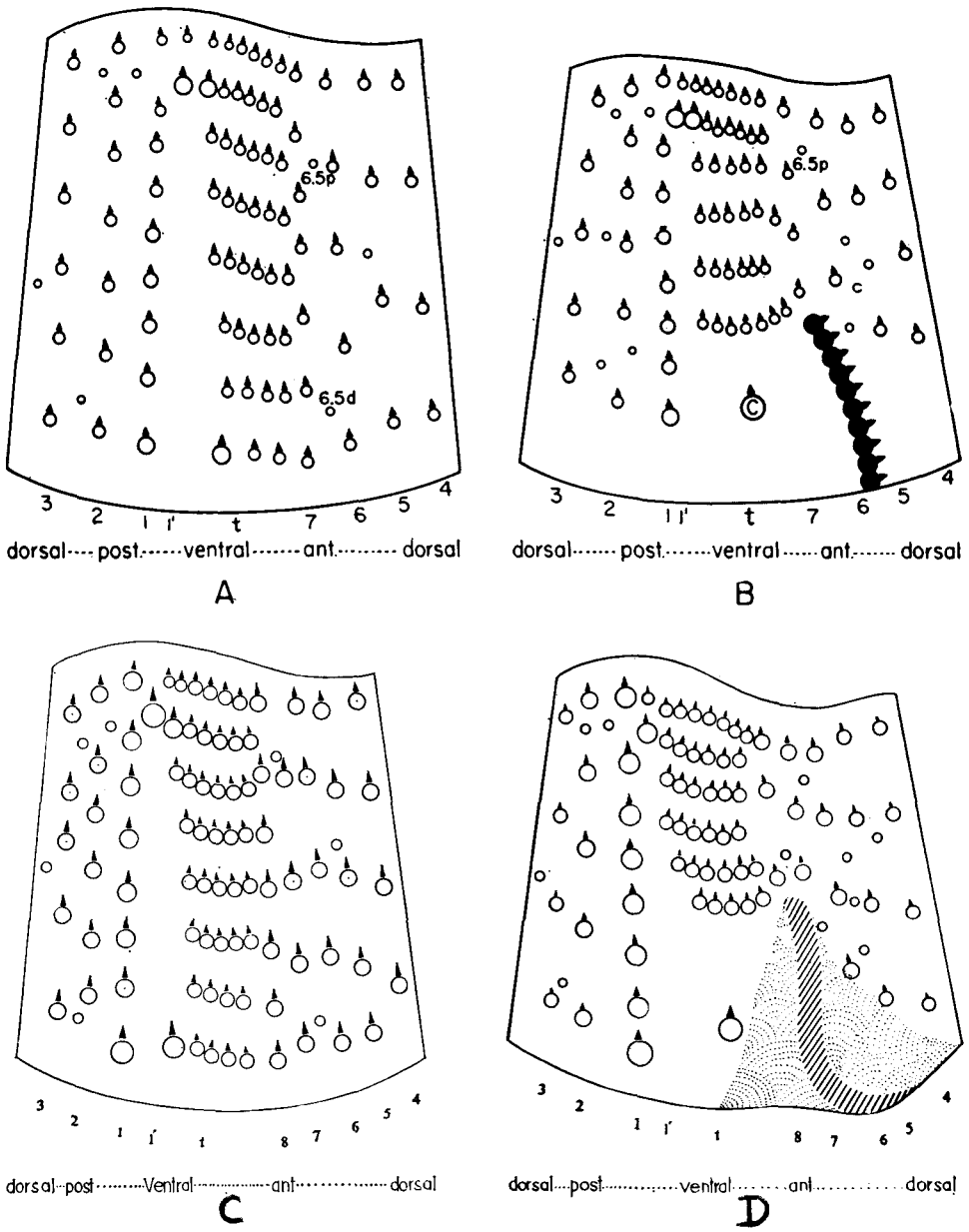

FIGURE 1.—Diagrammatic presentation of the bristle patterns on the prothoracic basitarsus of (A) normal female, (B) normal male (after MUKHERJEE 1965), (C) *cg* female, and (D) *cg* male. Magnifications approximately 300× (Figures 1 A & B) and 425× (Figures 1 C & D).

The basitarsi have been presented as cut lengthwise between the longitudinal rows 3 and 4 and laid out flat. In (D) cross-hatched region shows the usual arrangement of sexcomb teeth, and the stippled region presents the potential area for the formation of *cg* sexcomb. t = transverse rows; Numbers 1′ and 1–8 denote longitudinal rows; open circles with triangle = normal bristles with bracts; open circles = bractless bristles; 6.5p and 6.5d = proximal and distal 6.5 bractless bristles; solid circles with triangle in (B) = sexcomb teeth; C = central bristle.

10–12, for *cg* = 12–32). The sexcomb in *cg* males shows great variation, in number as well as orientation, of teeth on the basitarsus; they can be grouped into three classes: (a) They may be aligned in a single row of 12 to 20 teeth, curved antero-posteriorly in contrast to a more or less straight row in Ore males (Figure 2A). Such curved orientation may possibly be a device for utilizing the maximum area available for the placement of the teeth during development. (b) They may form two distinct rows in the primary sexcomb-forming area, and the total number of teeth in those basitarsi varies from 16 to 22 (Figure 2B). (c) Finally, they may show an irregularly clustered arrangement with one or more teeth appearing outside the cluster (total number = 22–32, Figure 2C). The sexcomb teeth may extend to regions away from the usual sexcomb area and one or more teeth may appear between the 5th and 6th or 4th and 5th longitudinal rows or even in the second tarsus. The type c is less frequent than the others. In type c, in addition to the increased number of teeth, a few intermediate type bristles and many extra bristles are frequently observed around the sexcomb area and especially distal to the last transverse row; the normal orientation of the teeth and also of other bristles is greatly distorted.

Transverse rows of bristles: Table 2 gives the mean number of transverse rows, of bristles per row, and of bristles in the 2nd and last rows in the basitarsi of Ore and *cg* males and females. Since *c/c*, *cg c/U*, or *cg c/Cy* genotypes do not show significant deviations from Ore in the number of bristles in transverse and longitudinal rows, only comparisons between Ore and *cg* have been presented (Table 2). The reason for presentation of the second and last transverse rows is not only for their ease of identification but also for conveying an impression of the degree of constancy of bristle number in terms of individual rows. The mean number of

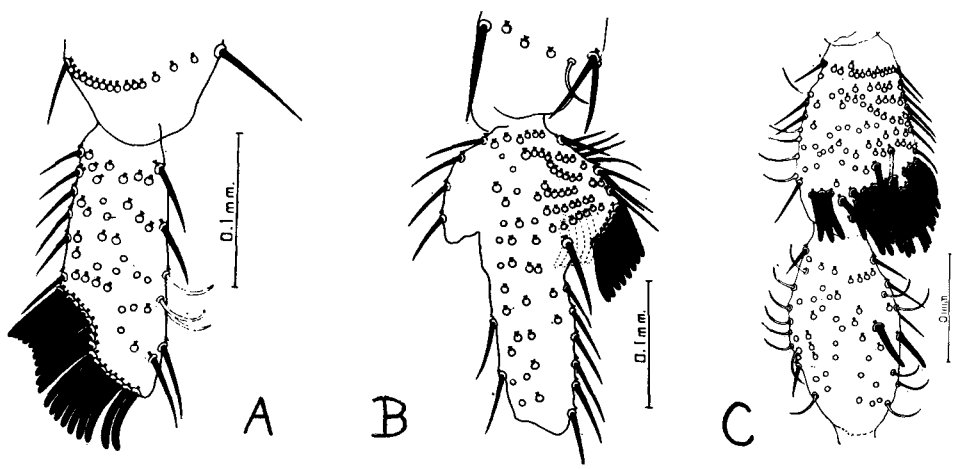

FIGURE 2.—Basitarsi of *cg* male forelegs showing orientation of sexcomb teeth, longitudinal rows of bristles and bractless bristles. (A) sexcomb teeth aligned in a long curved row, (B) sexcomb teeth in two rows, one on ventral and the other on dorsal side; teeth on the dorsal side are shown in dotted outline; suture between the basitarsus and the 2nd tarsal element is absent, (C) sexcomb teeth oriented in more than two rows and form a cluster at the distal end. Two sexcomb teeth are seen on the 2nd tarsal element.

TABLE 2

Basitarsal bristle patterns of the prothoracic legs in Ore *and* cg *males and females*

Bristle patterns*	Ore (30) ♂♂	Ore (30) ♀♀	*cg* (48) ♂♂	*cg* (48) ♀♀
Mean of tr. rows	6.3±0.16	8.6±0.13	6.3±0.12	7.8±0.08
Mean number bristles in row 2 (tr.)	4.5	4.5	6.0	5.8
Mean number bristles in the last row (tr.)	7.2	4.6	5.9	5.0
Number bristles/tr. row	4.6	4.6	5.8	5.1
Mean number long. rows (excluding 1′ row)	7.0†	7.0†	8.5	7.8
Number bractless bristles between 1–4 long. rows	4.7	3.9	4.6	4.0
Number bractless bristles between 4–7 long. rows	5.0	3.0	6.0	2.3
Total number bractless bristles on basitarsus	9.7	6.9	11.6	7.0

Number in parentheses gives total number of legs examined.
* tr. ≡ transverse; long. ≡ longitudinal.
† Number is invariably seven.

bristles per transverse row in the *cg* male (and to some extent in the female) shows an average increase of about one bristle per row as compared to that in normal strains, but there is no difference in the mean number of transverse rows between Ore and the mutant strain. The mean numbers of bristles in the second transverse row of both males and females of *cg* are also higher than in Ore; in contrast, the number of bristles in the last transverse row is decreased in *cg* males but increased in *cg* females. Thus it is clear that in *cg*, unlike in *sx*, there is a net increase in the total number of bristles.

Longitudinal rows of bristles: Earlier investigations with normal strains as well as with sexcomb mutants, e.g., *sx*, *en*, *Pc–Scx*, etc. have shown that the number of longitudinal rows is invariably eight (1′ and 1–7 rows) and the number of bristles is also not greatly variable. On the contrary, the number (mean = 8.5) of longitudinal rows (excluding 1′ row) varies in *cg* males from 8 to 11 and in females from 8 to 9 (mean = 7.8, Figure 1C, D; Table 2).

The individual longitudinal rows in Ore and *cg* cannot be directly homologized with each other from the present data. However, it has been observed that the first four longitudinal rows of *cg* flies and location of bractless bristles between them correspond in pattern and number with those present on the dorsal side of the basitarsus of Ore flies (Table 3). The extra longitudinal rows which are present on the basitarsus of *cg*, therefore, appear to have been interposed either between the longitudinal rows 5 and 7 or between the 7th longitudinal row and the anterior part of the transverse rows. The mean number of bristles in most of the longitudinal rows and in both sexes of *cg* are significantly reduced compared to those in Ore. The 8th longitudinal row is regularly reduced compared to those in Ore. The 8th longitudinal row is regularly present on *cg* basitarsi and has an

TABLE 3

Distributions of longitudinal rows of bristles in Ore *and* cg

Longitudinal rows	Mean number of bristles per row: Ore (30) ♂♂	Ore (30) ♀♀	*cg* (48) ♂♂	*cg* (48) ♀♀
Row 1	8.3	9.7	7.2	7.8
Row 2	5.2	7.0	5.2	6.2
Row 3	5.0	6.0	4.1	4.8
Row 4	4.1	4.9	3.6	4.3
Row 5	3.6	5.1	3.9	4.5
Row 6	3.0	6.0	3.5	4.7
Row 7	4.1	6.7	3.7	4.6
Row 8	..	..	3.7	3.4

average of 3.7 and 3.4 bristles in the male and female, respectively (Table 3). Other additional rows are present in a less regular manner.

Bractless bristles: The total number of bractless bristles on Ore male basitarsi is usually 9 or 10 (mean = 9.7) and that on the Ore female is about 7 (Table 2). This difference is attributable mainly to the additional bractless bristles in the region between longitudinal rows 5 and 6 (region 5.5), and between 6 and 7 (6.5). In *cg* males, the number of these bristles is increased further by about two (mean = 11.6), although no significant difference is apparent in Ore and *cg* females. The data in Table 2 (lower part, compare rows 6–9) suggest that one of the two additional bractless bristles in *cg* males is located somewhere between longitudinal rows 4 and 7, and the other must have been formed between the row 7 and the array of transverse rows. A detailed observation shows that in fact the bractless bristles in the region 6.5 of the basitarsus are usually two, as in *sx* males and normal females (MUKHERJEE 1965), or three, as in *dsx* males (HILDRETH 1965). The increase in the 6.5 bractless bristles in the *cg* male should not be correlated with the feminizing effect as it is with *sx*, but it is possible that as a consequence of multiplication of the longitudinal rows, the bractless bristles have also multiplied in number.

Correlation tests were made for (a) width of the basitarsus *vs.* the number of longitudinal rows, (b) the number of sexcomb teeth *vs.* the sum of the numbers of bristles on the last longitudinal row plus the bristles around the sexcomb, and (c) width of the basitarsus *vs.* the total number of sexcomb teeth. In all three tests the correlation coefficients were positive and high ($r = 0.81$ to 0.95) and significant at the 1% level. The results support the thesis that sexcomb size, general bristle pattern, and morphology of the basitarsus are developmentally interrelated and that *cg* acts early enough during the course of determination sequences in development to cause a change in all subsequent phases of bristle pattern development.

Effect of interaction of cg *with* en, sx, tra, *and* Pc–Scx *on basitarsi of the male prothoracic legs. Morphological changes:* The data presented in Table 4 show that as in the mutant *cg* by itself, in all combinations of *cg* the length of the basitarsus is reduced and the width increased as compared to the corresponding non-

TABLE 4

Length and width, in ocular micrometer units, of basitarsi of the prothoracic legs in Ore *and the different mutant males* (one ocular unit = 4.2 μ)

Genotypes	Number	Mean length ± S.E.	Mean width ± S.E.
Ore	30	48.5 ± 0.28	10.8 ± 0.53
cg	48	40.5 ± 0.52	16.3 ± 0.41
en	30	45.5 ± 0.25	11.8 ± 0.29
en–cg	30	39.7 ± 0.33	14.3 ± 0.26
Pc–Scx	52	44.4 ± 0.37	11.7 ± 0.33
cg–Pc–Scx	30	40.4 ± 0.55	13.5 ± 0.29
tra	30	46.1 ± 0.20	10.7 ± 0.30
cg–tra	22	40.1 ± 0.86	14.3 ± 0.46

cg types. In *sx–cg*, however, the legs are abnormally developed and show striking morphological changes, so much so that no measurements of the length or width were possible, but it was obvious that the legs were greatly reduced in length and enlarged in width. Frequently, the tarsal elements in *sx–cg* become indistinguishable from each other (Figure 4). This effect, attributed partially to both *sx* and *cg*, is synergistically augmented in *sx–cg*. Certain circular or semicircular whorl-like structures (Figure 4), similar to those reported by MUKHERJEE (1965) for the *sx–en* combination, are also observed in *sx–cg*. The significance of this whorl formation is not clear, although its occurrence is frequent, and its location very specific.

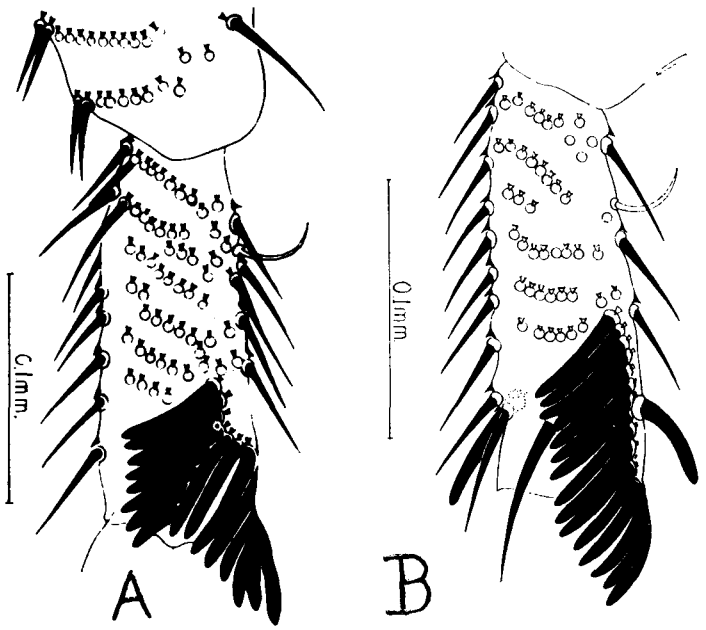

FIGURE 3.—Basitarsi of the prothoracic legs of *en–cg* males showing (A) two sets of sexcomb and (B) enlarged primary sexcomb and only one secondary tooth.

The sexcomb: It is evident from Table 1 (Part B) that the number of sexcomb teeth in all combinations is increased over that in non–*cg*. The mean numbers in all cases are significantly different from that in Ore or from those in the corresponding non–*cg*, as tested by ordinary t-test ($P < 0.01$). Since the distribution of sexcomb teeth number in *cg* does not show a conventional (normal) pattern (STERN, personal communication), the usual t-test may not be valid; but by an application of the Central limit theorem (CRAMÉR 1946), one can use the τ table (normal) to test the significance of the t-statistic in the present case. This τ test also shows that the mean numbers of teeth in *cg* and non-*cg* types are significantly different. The χ^2 test for homogeneity performed to identify the pattern of distribution of teeth among *cg* and non-*cg* types shows that all χ^2 values for non-*cg* samples are highly significantly different from that for *cg*. These facts support the contention that the distribution of teeth in *cg* has significantly shifted to the right (higher number of teeth) as compared to those of all non-*cg* types. As in *cg*, the range of variation in the number of teeth in all combinations is also widely spread out on both sides of the mean (Table 1). The mean numbers in *cg* combinations are, however, greater than that in Ore but lower than in *cg*.

Apart from these general interaction effects, in all combinations (except *sx–cg*) the teeth are arranged very frequently in a single semicircular row and only very rarely in the two rows usual for *cg*. This difference may be the consequence of slightly reduced number of teeth in the sexcomb of the *cg* combinations as compared to *cg*.

FIGURE 4.—Photograph of the basitarsus of an *sx–cg* male showing lumpy structure of tarsal elements and orientation of sexcomb teeth and other bristle patterns.

The mean number of teeth in the secondary sexcomb of *en* male prothoracic legs is about 5 but that in the combination *en–cg* is sharply reduced (mean = 0.8, $P < 0.01$) and not increased. The variability in the number of primary sexcomb teeth is also very high in *en–cg* (range = 10–20). It appears, therefore, that the increase in the number of primary sexcomb teeth in *cg* may underlie a mechanism of utilization of the prospective potency of the sexcomb-forming cells of the secondary sexcomb region, i.e., the increase in number of primary sexcomb teeth might have been made at the cost of the cells normally determined for the formation of secondary sexcomb teeth. This idea finds support from the fact that in the few cases of *en–cg* in which two sexcombs were found (Figures 3A.B), both were present at or near the primary sexcomb area, so much so that it became difficult to classify the second set as either primary or secondary. Interestingly, unlike the effect in *en–ey*^{D} (MUKHERJEE 1965), the number of teeth in the secondary region of the basitarsus of *en–cg* does not show a corresponding increase with the increase in that of the primary, and yet the overall bristle numbers on the basitarsus, especially those on the ventral surface, have increased considerably as in *cg*.

In *sx–cg* the mean number of teeth in the sexcomb has been found to be 5.4, and the range is very wide (Table 5); in no other combination of *sx* was the mean so high and the range so wide (MUKHERJEE 1965). It is evident from Table 5 and Figure 4 that the effects of *sx* and *cg* are superimposed in the *sx–cg* combination. The sexcomb teeth in *sx–cg* are arranged in a highly distorted manner rather than in discrete rows. Only in some cases are distinct rows of teeth observed and in those cases the number of teeth exceeds ten. The mean number of intermediate bristles is also significantly increased ($P < 0.01$) in *sx–cg* by almost a factor of two (Table 5). In regard to the other two combinations, *cg–Pc–Scx* and *cg–tra*, no marked difference from *cg* is observed in the arrangement and distribution of

TABLE 5

Mean number and distribution of sexcomb teeth and intermediate bristles in sx *and* sx–cg *males*

Genotypes	Distribution of teeth															Mean ± S.E.	Number
	Sexcomb teeth																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
sx	14	11	5	..	..	..	..	..	..	..	..	..	..	..	..	1.05 ± 0.09	30
sx/sc^{s}*·Y*	6	14	5	3	2	..	..	..	..	..	..	..	..	..	..	1.4 ± 0.10	30
sx/0 (X0)	14	25	11	1	..	..	..	..	..	..	..	..	..	..	..	0.9 ± 0.09	51
sx–cg	1	1	4	2	2	7	3	3	3	1	2	0	0	0	1	5.4 ± 0.54	30
	Intermediate bristles*																
sx	6	3	6	4	1	..	..	..	..	..	..	..	..	..	..	1.6 ± 0.20	20
sx/sc^{s}*·Y*	4	6	7	3	..	..	..	..	..	..	..	..	..	..	..	1.5 ± 0.22	20
sx/0 (X0)	8	12	24	5	2	..	..	..	..	..	..	..	..	..	..	1.6 ± 0.14	51
sx–cg	2	3	8	8	6	2	1	..	..	..	..	..	..	..	..	2.9 ± 0.23	30

* In *cg*, these bristles are very irregularly present and that only in type c (see text); therefore, their mean and distribution have not been presented here.

TABLE 6

Transverse row, longitudinal row, and bractless bristles of the prothoracic legs in* Ore *and mutant males*

Genotypes	Transverse rows		Mean number long. rows	Number bristles in long. row			Bractless bristles		
	Mean	Bristles/Basit.		Row 1	Row 7	Row 8	1–4	4–7	Total†
Ore	6.3±0.15	35.3	7.0±0.00	8.3	4.1	..	4.7	5.0	9.7
cg	6.3±0.12	38.3	8.5±0.15	7.2	3.5	3.7	4.6	6.0	11.6
en	6.3±0.09	33.9	7.0±0.00	8.5	3.8	..	3.7	4.9	8.6
en–cg	6.1±0.01	35.5	8.6±0.08	8.2	3.5	3.4	4.4	4.8	9.9
Pc–Scx	6.5±0.21	33.3	7.0±0.00	8.9	4.1	..	4.3	5.7	10.0
cg–Pc–Scx	6.1±0.11	34.0	8.3±0.08	7.5	3.6	3.4	4.9	5.3	10.2
sx†	6.8±0.02	23.1	.....	..	..	..	..	..	..
sx–cg‡	5.9±0.09	27.9	.....	..	..	..	..	..	..
tra	6.3±0.09	31.4	7.0±0.00	9.0	3.2	..	4.3	4.9	9.2
cg–tra	5.9±0.09	35.7	8.0±0.19	7.9	4.1	3.4	3.8	4.8	9.0

* *N* same as in Table 4.
† Total number of bractless bristles actually observed.
‡ For these two strains $N = 30$ and in them counts of longitudinal row and bractless bristles were not possible due to distortion of basitarsi.

sexcomb teeth, although both the mean number and the distribution are shifted toward slightly but significantly lower values.

Transverse rows of bristles: The mean number of transverse rows in all *cg* combinations, except *sx–cg*, does not differ much from those in the corresponding single mutants (Table 6). However, the total number of bristles per basitarsus in the combinations is lower than that in *cg* but higher than those in non-*cg* types. In *sx–cg* the mean number of transverse rows is decreased in comparison with that in *sx*, but the total number of bristles on the basitarsus does not show as much increase as in other combinations; the mean number of bristles per row is, however, increased to almost double that in *sx*. The numbers of bristles on the second and last rows, in different combinations, do not show any significant difference from the respective non-*cg* types or from Ore.

Longitudinal rows of bristles: As in *cg*, the mean number of longitudinal rows, in *cg* combinations, is always greater than 7 (Table 6), and the mean numbers of bristles in rows 7 and 8 are more similar to each other than to Ore. However, the mean number of bristles in row 1 tends to be smaller in the presence of *cg* (Table 6).

Bractless bristles: The mean numbers of bractless bristles in regions 1–4 and 4–7 in different combinations are more or less similar to each other, to Ore, as well as to the corresponding single mutants, although their number in the 4–7 region in *cg* is greater by one bractless bristle (Table 6). The total number of bractless bristles on the basitarsus in different combinations, except *en–cg* and *cg–tra*, also does not show an increase from that in their corresponding single mutants. In *en–cg* and *cg–tra* the sum of the number of bractless bristles in region 1–4 and in region 4–7 is lower than the total number of bractless bristles actually

present on the basitarsus. This indicates that at least in certain combinations, as in *cg*, bractless bristles may also appear between the 7th longitudinal row and the transverse rows. Thus it seems likely that in *cg* as well as in certain combinations additional bractless bristles are indeed formed but that some reorganization of the normal pattern of these bristles may also have taken place in the combinations studied.

cg–ey^D *interactions:* In the *cg–ey^D* combination the number of teeth in the sexcomb is greatly increased when compared to that in either *cg* or *ey^D*; the range of the number may be from 25 up to 100 or more. The sexcomb teeth are highly clustered together, obliterating the individuality of teeth and their pattern. The number of central bristles in *cg–ey^D* is also more than one, which is apparently an effect of *ey^D*. On the other hand, the *cg* effect is also realized from the great increase in the number of longitudinal rows and inflated condition of the basitarsi; in *cg–ey^D* flies the distal region of the basitarsi is more widened than in *ey^D*. It may be assumed from the morphological changes undergone in the *cg–ey^D* combination that effects of these two genes are additive and/or synergistic.

DISCUSSION

Differentiation of a biological pattern invites the existence of a precise genetic regulation at the intra- and intercellular level. How this regulatory system should work under one unified principle is still conjectural. One possible way among others is to consider that not all genes of a genome are active at one time and that different sets of genes may have been set apart in time and space to perform a stepwise sequential series of activities within a tissue. Studies on different aspects of developmental genetics (as noted in introduction) in Drosophila and other organisms have led to the important idea that differentiation of a biological pattern should require differential activation of and an interaction between two sets of genes—the so-called prepattern genes which are primarily responsible for singularization of certain cellular islets and the so-called terminal pattern genes which provide proper competence to the cells for the realized pattern. This idea, which has been a subject for test in various laboratories, is derived from the works of STERN (1954b, 1956). Previous studies have attested to the existence of both these genes in normal and mutant forms (STERN and MUKHERJEE 1964; STERN and TOKUNAGA 1967; STERN 1968; MUKHERJEE 1965). LEES and WADDINGTON (1942; see also WADDINGTON 1962) have shown that different mutant genes may be arranged in order of their level of action during development of the tissue. The development of bristle apparatus, for example, follows a sequence of several well-defined stepwise developmental reactions, starting from isolation of bristle-forming cells from ordinary epidermal cells to the formation of different grades of pigmentation leading to the formation of a rigid pointed bristle or a tooth (STERN 1954b). A mutant may act at any step in development after the isolation of the bristle-forming cell, or it can act before a bristle cell is determined.

MUKHERJEE (1965) predicted that formation of an enlarged sexcomb in the mutant *ey^D* might not be a simple consequence of additional teeth formation, but

might be a result of a complex organizational change in the basitarsal morphology; this prediction was substantiated later by STERN and TOKUNAGA (1967) and by TOKUNAGA (1968). As in the case of ey^D, the altered bristle pattern in the basitarsi of *cg* is not a result of reorganization of bristles, but has been caused by actual induction of new sites of bristle organs. This is borne out by several observations: First, the number of longitudinal rows is increased from 7 (excluding 1′) to at least 8 and up to 11. Secondly, actual count of the total number of bristles on the basitarsus (and also on other segments of the prothoracic leg) shows a decidedly higher number. Finally, high positive correlations exist not only between the number of sexcomb teeth and number of extra bristles within the sexcomb area, but also between the teeth number and width of the basitarsus, and also between the width of the basitarsus and number of longitudinal rows. Interaction studies with the five mutants further attest to the conjecture that new sites of bristle organs have been induced by *cg*. An increase in the number of transverse rows and induction of extra bristles (e.g., intermediate bristles) in the sexcomb area has been reported in *sx* (MUKHERJEE 1965), but in that case the increase was accompanied by a decrease in the number of bristles elsewhere (viz., number of bristles per transverse row), indicating a net constancy in the bristle number and a change in the organization of the bristle pattern. MUKHERJEE ascribed that change to an altered response of the competent cells to the prepattern singularity.

The observed changes in the pattern as well as the number of bristles in *cg*, however, do not indicate a constancy in the pattern or number of bristles and teeth. It is noteworthy that this change in the prospective potency of bristle-forming cells is not haphazard, but follows the precise cell lineage observed in the wild-type basitarsi (TOKUNAGA 1962). These facts, therefore, suggest that the effects may be attributed to a change in the preceding cellular organization; and the mutant *cg* may possibly be considered to be a prepattern mutant. This idea finds further support from unpublished observations on genetic mosaics, which reveal that except in very large patches (e.g., whole basitarsus) of homozygous *cg/cg* tissue (marked by yellow body and bristle color), the mutant *cg*, in *cg*/+ background, fails to manifest itself. Only in very large patches (thought to have resulted from early somatic crossing over) was a *cg*-type effect observed. Clearly, such an effect shows the nonautonomous behavior of *cg* and is indicative of an early action of the mutant.

Some of the *cg* effects mentioned above are unique and make them distinct from the effects of ey^D. Firstly, the number of longitudinal rows in *cg* has increased remarkably; secondly, the number of transverse rows remains unaffected, and finally, unlike ey^D, intermediate bristles form in *cg* and its combinations. It appears, therefore, that the two mutants act at different levels of development, although their subsequent effects and end results may be the same. It is likely that the mutant *cg* has a wider range of action than ey^D, so that while it may primarily initiate an increase in the stimulus, it may secondarily affect the response as well. Examples of such dual action of the same mutant have been provided by Hairy-wing (GOTTLIEB 1964) and dumpy (KING 1964) in *D. melano-*

gaster. SONDHI (1963) has discussed other examples of this in other species of Drosophila.

The increase in the number of longitudinal rows in *cg* and its combinations is an interesting phenomenon not hitherto found in any mutant forms of *D. melanogaster*. The formation of sexcomb teeth is related by cell lineage to the transverse rows and the last one or two longitudinal rows of bristles (TOKUNAGA 1962). An increase in the prepattern gradient of sexcomb pattern, as it presumably takes place in *cg*, by an interaction with normal response provided by the competent wild-type gene, should enhance the ability to utilize the bristle cells in tooth-forming processes and thereby increase the "reserve" (or pool) of potential tooth-forming cells. The reaction involved in the interaction between the stimulus and the response may reach a saturation point after which many of the reserve cells may remain unused and differentiate as ordinary macrochaetae of longitudinal or transverse rows or even as intermediate bristles. A similar situation can be encountered if the defective response provided by a mutant of some competence gene fails to carry out its reaction with the stimulus to completion, resulting in nonutilization of many precursor reserve cells and leaving an array of extra bristles only. An example of this situation is known from the formation of extra transverse rows in *sx–en* and *sx–en–ey*^{D} (MUKHERJEE 1965).

It has been seen that in no combination of *sx* did the number of teeth exceed five (MUKHERJEE 1965). Even in *sx–ey*^{D} the mean number of teeth was 2.9 and in *sx*/+;*tra*/*tra* it was 3.7. In *sx–cg* the number was in many cases as high as in Ore and the mean was raised to more than five times that in *sx*. Despite this increase in teeth number, the number of intermediate bristles is increased additively. As evident from some less distorted basitarsi, inhibition of tooth formation by *sx* in *sx–cg* also results in the formation of still more longitudinal rows as compared to their number in *cg* alone. This fits with the proposed mechanism for the formation of extra secondary transverse rows in *sx–en* (MUKHERJEE 1965) and of extra longitudinal rows in *cg* reported here. Thus taking the facts and conjectures together, it seems reasonable to surmise that the defective response of *sx* in *sx–cg* is due to the enhanced stimulus from a *cg*-induced altered prepattern singularity, which, although it tends to restore the normal pattern of sexcomb, fails to provide a sufficient effective threshold of reaction to all precursor bristle cells; consequently, extra bristles are formed as longitudinal rows, as extra bristles in the sexcomb area, or as intermediate bristles. Such an effect is expected and can be predicted if *cg* acted at an early stage in the cell lineage from bristle cell determination to tooth formation.

The direct correspondence of the increase (as in *en–ey*^{D}) or decrease (as in *sx–en–ey*^{D}) in the number of secondary sexcomb teeth with that in the primary (MUKHERJEE 1965) substantiates the fact that the genesis of the two sexcombs is highly coordinated (BRASTED 1941; TOKUNAGA 1961). In *en–cg* such a direct relationship between the two sexcombs is lost; the teeth in the secondary sexcomb are either absent or extremely reduced in number. This could perhaps be explained by assuming an independent behavior of *en* and *cg* in the two regions in

a manner similar to autonomous pleiotropy (STERN and TOKUNAGA 1968). Alternatively, a more likely explanation would be to consider the observed effect in terms of stepwise sequential determination (WADDINGTON 1962; HADORN 1966). As pointed out by URSPRUNG (1966), the first step in determination is the period when prepattern singularity of the cells is fixed in morphogenesis. It may then be a reasonable surmise that the cells taking part in both primary and secondary sexcombs have a common determination at the first step; a second step in determination then specifies the distribution of cells into the two sexcombs. This implies that the inhibition of the secondary sexcomb in *en–cg* may be a consequence of concentration in the primary zone of all or a major part of the singularized cells determined for teeth formation. In the context of a possible mechanism of pattern formation, URSPRUNG (1966) has proposed that there may be a possibility of singularization of the cells at one site within the developing disc and only an active or passive cell migration can take them to the appropriate place of the final pattern. Although direct evidence for cell migration in insects is still lacking, evidence both from a cell lineage study of TOKUNAGA (1962) and from the precision in pattern formation in mixed cellular types after dissociation of imaginal discs (GARCIA-BELLIDO 1966; TOBLER, quoted in HADORN 1966) are sufficient to indicate that in Drosophila bristle pattern development, active cell migration may be a regular event. If such a phenomenon actually exists in the morphodynamics of sexcomb development, the idea of concentration of the singularized cells determined for teeth formation at the first step in the primary zone in *en–cg* would find a valid support.

The observed effects of *cg* in *cg–tra* are also compatible with the early action of *cg*. It was seen in previous work (MUKHERJEE 1965) that in the presence of *tra*, the single dose of *sx*(*sx*/+;*tra*/*tra*) forms 3 to 4 times as many teeth as in *sx*/*Y*. It was argued that the single dose of normal *X*-chromosomal genes acts on the *tra*-induced unmasked prepattern and leads to a higher response than *sx* to the prepattern stimulus, and that *tra* may be considered to act relatively earlier than *sx*. The relatively early action of *tra* also finds support from the work of ANDERS (1955) on the lozenge-clawless-transformer combination (see HADORN 1965). As mentioned above, studies on genetic mosaics with *cg*, on the other hand, provide evidence primarily for an early action of *cg*, but also they suggest that the action of the mutant *cg* may extend over a wide range in development. This broad range of action of *cg* may then overlap in time with the relatively early action of *tra*.

SUMMARY

The effect of the mutant combgap (*cg*) on the basitarsi of the forelegs of *Drosophila melanogaster* has been examined singly as well as in combination with five different sexcomb mutants (*sx*, *en*, *tra*, *Pc–Scx*, and ey^D), in order to understand the mode and level of action of *cg* during differentation. The mutant *cg* greatly increases the sexcomb size (mean teeth number = 17.7) as compared to that in normal males (mean = 9.8). This increase in teeth number is accom-

panied by: (a) widening of basitarsi, (b) increase in the number of longitudinal rows of bristles, and (c) increase in the number of bractless bristles. Furthermore, *cg* causes an overall increase in the total number of bristles on the basitarsi in both sexes. Positive correlations were observed between the number of sexcomb teeth and number of extra bristles, between the width of basitarsi and the total number of sexcomb teeth, and between width of basitarsi and number of longitudinal rows. The mean numbers of sexcomb teeth and longitudinal rows of bristles are increased in all combinations with *cg*. In *sx–cg* the mean number of sexcomb teeth shows a five-fold increase over that in *sx* and the basitarsus is more distorted than in *sx*. The secondary sexcomb in *en–cg* is abruptly reduced despite an increase in the primary. Effects of *cg* and ey^D are synergistically augmented in *cg–*ey^D, although they can be distinguished from each other at various points. It has been suggested that *cg* initiates a change in the potential sexcomb-forming area, and that it acts relatively early in morphogenetic determination of the sexcomb. It has been proposed that *cg* may also be one of the few prepattern-changing mutants so far known, and that its action may have been determined as early as the first step in the singularization process of pattern formation, although the action may extend over a wide range.

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