STUDIES ON THE EXPRESSION OF THE TRANSFORMER GENE OF *DROSOPHILA MELANOGASTER*¹

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STURTEVANT's transformer gene (*tra*) of *Drosophila melanogaster* is a third chromosomal recessive which has no effect on males but converts homozygous females into flies which resemble normal males. These transformed females show malelike abdominal tergites, anal plates, external genitalia, and the typical male abdominal coloration. Transformed females possess male sex combs, and dissection reveals normal male genital ducts, accessory glands and an ejaculatory bulb. The testes, however, while showing the characteristic yellow pigmentation of the sheath and the normal attachment to the ejaculatory duct, are greatly reduced in size and fail to develop the usual coiled condition. Although these "males" mate with females, their testes fail to develop sperm, and they are thus completely sterile. STURTEVANT (1945) pointed out two ways in which XX *tra/tra* individuals resemble normal females; they are generally larger than males and are found more often in young than in older cultures (as are normal females which have a slightly shorter developmental period than males). STURTEVANT located the *tra* gene in chromosome III between scarlet (44.0) and clipped (45.3).

By suitable crosses STURTEVANT transferred the gene to an attached-X stock and found the XXY *tra/tra* individuals to be phenotypically identical to flies of genotype XX *tra/tra*. Thus, the Y chromosome is without effect upon the action of *tra*. MULLER (1955) found XXYY *tra/tra* individuals (containing two Y^S·X·Y^L chromosomes) to be sterile, and XY *tra/tra* individuals (containing one Y^S·X·Y^L chromosome) to be fertile. Thus, the sterility of transformed females cannot be due to insufficient doses of Y chromosomal fertility factors or to the presence of a third chromosomal recessive gene which causes male sterility when homozygous. NOVITSKI (1951) synthesized a stock carrying "transformer" and an unstable ring X chromosome (X^{c2}, *In(1)w^{pc}*), which is frequently lost during early cleavage. The loss of the ring X produces XXY *tra/tra* mosaics which contain large patches of XY *tra/tra* tissue. Of the fertile mosaics (8 out of 24) none produced female offspring carrying the ring X chromosome. This finding indicates that the testes of the fertile "male" mosaics were composed, at least in part, of cells having the XY AA constitution characteristic of normal males, and only these cells underwent normal spermatogenesis.

STURTEVANT also established a triploid stock carrying transformer, and XXX *tra/tra/tra* individuals were found to resemble the diploid transformed females,

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although they had larger wing cells and ommatidia characteristic of triploid females. Studies of the effects of various doses of *tra* and Hermaphrodite, a dominant gene which converts females into sterile hermaphrodites (GOWEN and FUNG 1957; FUNG and GOWEN 1957), suggest that *tra* and *Hr* may be alleles.

Several studies have been made concerning the effects of the transformer gene on characteristics which show sexual dimorphism. STURTEVANT (1945) showed that in transformed individuals homozygous for *scute*³ (which is lethal in females) or *facet* (which is less extreme in females than in males) or *eosin* (which is darker in females) these genes are expressed as though acting in females rather than males. FOX (1956) reported the presence of a peptide in *D. melanogaster* males which is absent in females. When transformed females were tested chromatographically, the peptide was found to be present (MUNYON 1958; FOX, MEAD, and MUNYON 1959). LIEB (1946) found that transformed females show complete dosage compensation for the genes *apricot*, *forked*, *Bar*, and *scute*, in which respect the flies behave as normal females, possessing a double dose of compensators on the X chromosome.

Evidence which suggests that transformed females are intersexual has been obtained by MUNYON (1958). Chromatographic analyses demonstrated a greater amount of glutamine in *D. melanogaster* females than in males, while the latter possess more β -alanine and tryptophan-proline (which were measured together as a single spot). The amounts of these substances in *tra* females was found to be intermediate between normal males and females.

The purpose of this communication is to supply further information concerning the effect of transformer upon the morphology of the testis and upon size and sex comb morphology, traits which exhibit a sexual dimorphism. An observation made by DOBZHANSKY (1929), that each cell comprising the wings of *Drosophila* possesses a single bristle, makes possible an estimate of the total number of cells in the wing. Since the relative sizes of wings and body vary in direct proportions and respond to both nutritional and genetic changes (ALPATOV 1930; ROBERTSON 1959), wing measurements serve as a useful index of over-all size.

The sex combs of *D. melanogaster* males present a striking example of sex-limited characters, since the corresponding regions of the first tarsal segments of the female forelegs show the typical hypodermis or setae found on the other legs. Although little is known concerning the details of sex comb development, it appears from the work of HANNAH-ALAVA and STERN (1957) that both sexes possess a potential sex comb region, but only male cells are able to respond to this "prepattern". That the environment influences sex comb development is indicated by the fact that the number of teeth in the sex combs of wild type males increases with increasing temperature (COMBS 1937).

MATERIAL AND METHODS

The stock used in the series of experiments is designated as: FMA3/*w^av*; *tra/In(3LR) Ubx*¹⁵⁰. The FMA3 chromosome (LEWIS 1958) is a tandem direct attached-X of great structural complexity which is marked with yellow². Thus,

attached-X females carry yellow², and males are hemizygous for apricot and vermilion; both sexes are heterozygous for transformer and a third chromosomal dominant, Ultrabithorax, which is lethal when homozygous. (For a description of γ^2 , w^a and v , see BRIDGES and BREHME 1944; for *Ubx*, see bx^D in BRIDGES and BREHME 1944.) Since individuals homozygous for *Ubx* are inviable and super-females (triplo-X) seldom survive to adulthood, the offspring in this stock appear in the following ratio: 2 yellow-bodied females:3 apricot-eyed males:1 yellow-bodied "male". The latter class corresponds to the females homozygous for *tra* which are converted into males. Two thirds of the w^a males and all of the γ^2 females also show the *Ubx* phenotype. Therefore *tra/Ubx* males must be selected for the male parents if the offspring are to appear in the ratios referred to above. Stocks were maintained at 18° or 25°C, and in each experiment flies from each temperature group were compared. The only exception concerns the flies used for the body weight determinations which were reared at 28°C.

The body weights of γ^2 females, w^a males, and *tra* females reared at 28°C were determined from the mean weights of groups of 4–10 flies placed in glass piglets and weighed to the nearest 0.0001 gm with a Chainomatic balance. In order to cut down variability among females due to ovaries of varying size, all flies were weighed within three hours after eclosion.

The flies were collected daily, and the *tra* females and w^a males were placed in small glass vials containing culture medium and were aged for various periods of time. The reproductive systems of individuals from one to 30 days of age were dissected out in *Drosophila* Ringer's solution, fixed in Carnoy's (6:3:1) for 15 minutes, hydrolyzed in 1N HCl for 15 minutes, and stained with Schiff's reagent. Whole mounts prepared in this manner were examined with normal bright field and phase contrast optics. Figures 1, 3, 4, and 6–8 were made using a Leitz Ortholux microscope equipped with a Zeiss drawing apparatus.

Comparison of the number of teeth in the sex combs of w^a males and *tra* females grown at 18°C and 25°C was made on fresh forelegs mounted directly in Permout on microscopic slides. The number of teeth was counted at a magnification of 400×. Estimation of the mean size of the wing cells was made with right wings mounted in Permout on microscope slides. Using a compound microscope equipped with a 40× objective and 10× eyepiece containing a counting grid, the number of bristles was counted within an area of 10,000 μ^2 on the upper surface of the wing. The area selected (see Figure 1) has a bristle density which is representative of the entire wing, except for the proximal portion (DOBZHANSKY 1929). Since one bristle corresponds to one cell, the mean cell area in μ^2 was determined by dividing the number of bristles by 10,000.

The relative differences in the wing areas of γ^2 females, w^a males, and *tra* females were determined as follows: Using an inverted Seibert Promi projection microscope, wings mounted in Permout on microscope slides were projected at a magnification of approximately 45× and the wing outlines traced on paper. The shape of the wing was found to be elliptical, as shown in Figure 1, and the wing area calculated from the formula, $AREA = \pi(\frac{1}{2}A)(\frac{1}{2}B)$. Measurements A and B were made to the nearest 0.5 mm on the drawing, and the wing area

FIGURE 1.—Right wing of *Drosophila melanogaster*, showing its elliptical shape (dotted line). Wing area calculated from $\pi (\frac{1}{2}A)(\frac{1}{2}B)$, which gives the area of an ellipse. Bristle density (cell number) was determined within the crosshatched area, which contains 10,000 μ^2 .

so calculated converted into μ^2 . The comparison of the lengths and widths of the wings of the three types of flies was based on the measurements of A (length) and B (width). The average number of cells in one wing (both sides included) was calculated by dividing twice the mean area of a group of wings by the mean cell size. After these estimates were made, it appeared that no significant difference existed between the number of wing cells of the *tra* females grown at 25°C and those grown at 18°C. Since there seemed to be a greater difference between *w^a* males grown at 25°C than those grown at 18°C, a second group of *w^a* male wings was studied. In this determination measurements of wing area and bristle density were made on individual wings, and the mean values for cell number were tested for a significant difference.

RESULTS AND CONCLUSIONS

Examination of cultures and collection of specimens at daily intervals is too infrequent to permit accurate estimation of the developmental times of the individuals of various genotype. However, the minimum developmental times may be of interest. At 25°C γ^2 females and *w^a* males begin to eclose on the ninth day after egg deposition although in relatively small numbers, and *tra* females first appear on the tenth day. At 18°C the developmental periods of all three types of individuals are increased by 15 days on the average. At each temperature the transformed females had viabilities somewhat lower than the normal males and females.

Body weights: The body weights of γ^2 females, *tra* females, and *w^a* males were found to be 1.058, 0.866, and 0.787 mg, respectively. These values represent the means of 40–60 flies of each type, and the standard error in each case is less than 1.0 percent. Since all three values were found to be highly significantly different from each other, the *tra* female is intermediate in weight between the γ^2 female and *w^a* male, being 9.2 percent heavier than the male and 18.1 percent lighter than the female.

Sex combs: The average number of teeth in the sex combs of *w^a* males was found to be 12.44 at 25°C and 12.08 at 18°C, while the sex combs of *tra* females

have 10.20 teeth at 25°C and 9.87 teeth at 18°C. The number of sex combs examined was 38, 39, 42, and 50, respectively. In each case, the standard error of the mean is between 1.2 and 1.7 percent. The difference between the *w^a* males and *tra* females at each temperature is very highly significant, with a significant difference between *w^a* males at 25°C and 18°C ($P = 0.035$), and between *tra* females at 25°C and 18°C ($P = 0.011$). These data show that temperature affects sex comb development of *w^a* males and *tra* females to the same degree, both types of individuals having approximately three percent fewer teeth per sex comb at 18°C than at 25°C. The *tra* females have 18 percent fewer teeth than the *w^a* males at each temperature. All of the sex combs examined showed uninterrupted rows of teeth.¹

Wings: Figure 2 shows the relations between the area, width, length, cell area and total cell number for the wings of *w^a* males, *tra* females, and γ^2 females reared at 18°C and 25°C.

Considering first the wing area, it was found that at 25°C the wings of the γ^2 female are 19 percent larger than the wings of the *w^a* male and five percent larger than the wings of the *tra* female, both differences being highly significant. At 18°C, however, the wings of the γ^2 female are more variable, are nine percent larger than those of the *tra* female, and are only six percent larger than those of the *w^a* male. The wings of *w^a* males, *tra* females, and γ^2 females increased in area 35 percent, 21 percent, and 25 percent, respectively at 18°C. Thus, as is evident in Figure 2A, all three types of individuals show an inverse relationship between wing area and temperature, and as temperature decreases, the sexual dimorphism between *w^a* males and γ^2 females decreases. From these data it is evident that temperature has a greater effect upon the wing size of *w^a* males than upon the wing size of *tra* or γ^2 females.

Analysis of the lengths and widths of the various wings brought out the following differences. At 25°C the wings of the γ^2 female are the same length as those of the *tra* female but are approximately two percent wider, while they are 11 percent longer and seven percent wider than the wings of the *w^a* male. At 18°C, however, the wings of the γ^2 female are three percent longer and five percent wider than the wings of the *tra* female, but they are now only three percent longer and are the same width as the wings of the *w^a* male. These changes are due to the wings of *w^a* males, *tra* females, and γ^2 females being 22, 15, and 16 percent longer, and 17, 8, and 10 percent wider respectively at 18°C than at 25°C. Examination of Figures 2B and 2C will demonstrate these differences and also the fact that the

¹ HANNAH-ALAVA pointed out in 1958 (J. Morph. **103**: 281-310) that a sexual dimorphism occurs with respect to the number of transverse rows of bristles found upon the metatarsus of the prothoracic leg. The number of rows is eight in females and six in males. These observations led us to study this feature of the chaetotaxy of males, females, and transformed females reared at 21°C. The mean numbers of transverse rows of metatarsal bristles observed were 6.05 ± 0.07 ($N = 39$) for males, 6.34 ± 0.09 ($N = 29$) for transformed females, and 8.12 ± 0.11 ($N = 25$) for normal females. The number of transverse rows of metatarsal bristles is significantly greater in transformed females than in males. Thus with respect to sex comb morphology and the number of transverse rows of metatarsal bristles transformed females resemble males, but this resemblance is *not* a perfect one.

FIGURE 2.—(A) area, (B) width, (C) length, (D) cell area, and (E) cell number of the right wings of *w^a* males, *tra* females, and *γ²* females. For each genotype, the left bar represents the 25°C mean and the right bar the 18°C mean. The number within each bar represents the number of individuals measured, while the brackets represent the standard error of the mean. The figure above each 25°C bar represents the percentage of the 18°C value. A dotted line connecting two or more bars indicates that the values are not significantly different.

increase in wing dimensions at 18°C is greater for *w^a* males than for *tra* or *γ²* females. The finding that the wing dimensions of normal males and females vary inversely with temperature agrees with various other studies, such as those made by ALPATOV (1930) and IMAI (1934); and the observed decrease of the sexual dimorphism shown by the wings has previously been observed by ALPATOV (1930).

Calculation of the surface area of the wing cells showed *tra* and *γ²* females to have cells of the same area at each temperature. The areas of the wing cells of *tra* and *γ²* females are 12 percent and six percent larger than the cells of the *w^a* males at each temperature, respectively (see Figure 2D). Both in terms of wing cell size and the response of the developing wings to temperature, the transformed individuals clearly resemble normal females more closely than males.

Examination of Figure 2E demonstrates that the number of wing cells of the *tra* female is the same at each temperature. The slight difference (four percent) between the wing cell numbers of *γ²* females reared at the two temperatures agrees with results obtained by ROBERTSON (1959), who concluded that the response of female wing area to temperature is expressed almost entirely in

changes of cell size. Our data show a significant difference in the total number of cells of the w^a male wing (seven percent more cells at 18°C than at 25°C). Since the wings of the γ^2 female are larger than the wings of the *tra* female at each temperature, and since the number of wing cells is the same at each temperature for the same genotype, the wing cell number of the γ^2 female must be greater than that of the *tra* female at each temperature.

In light of the preceding data concerning the wings of the three types of individuals, the following conclusions may be drawn. At 25°C the wings of the γ^2 female are longer and wider than those of the w^a male because the female wings have larger cells and probably have more cells as well. The γ^2 female wings are wider but have the same length as the wings of the *tra* female because the latter has fewer cells. At 18°C the wings of the γ^2 female are the same width but are longer than those of the w^a male, because the female has larger cells and probably more cells. At 18°C the female wings are longer and wider than those of the *tra* female because the wings of the latter have fewer cells. Thus, at each temperature the wings of the *tra* female have fewer cells than those of the γ^2 female, while the w^a male wings have fewer cells and smaller cells than the γ^2 female wings. Considering the effect of temperature, the wings of the γ^2 female and w^a male are longer and wider at 18°C than at 25°C, primarily due to increased cell size although cell number is also increased slightly. The transformed female wings, however, are larger at 18°C completely due to increased cell size.

Reproductive systems: The reproductive system of the w^a male is shown in Figure 3. The terminology used to designate the various parts is that of MILLER (1950). The testes, seminal vesicles, and vasa deferentia of freshly dissected w^a males show only a very faint yellow coloration, in contrast to wild type testes which are decidedly yellow. Examination of fresh testes and Feulgen-stained whole mounts with bright field and phase contrast optics revealed the characteristic histology. The testis wall consists of an inner layer of squamous epithelial cells and an outer layer of large, flattened sheath cells, containing small pigment granules which give the testis its color in the adult individual. These pigment granules are visible only in freshly dissected testes, however, and they are evidently water soluble. STERN and HADORN (1939) have shown that the sheath cells originate in the gonad and that during development some of them migrate posteriorly and cover (thus coloring) the vasa deferentia once they attach to the gonads. The vasa deferentia originate from the posterior (genital) imaginal disc and grow forward to the gonads. The spermatogonia were observed under phase contrast in the distal tip of the testis, while spermatocytes, spermatids, and bundles of spermatozoa fill the remainder of the testis (MILLER 1950). The reproductive systems of individuals reared at 18°C were larger than those of flies which developed at 25°C.

The reproductive system of the transformed female (Figure 4) is identical to that of the normal male except for the testes. The poorly developed testes are always attached to seminal vesicles and vasa deferentia of normal appearance. These three structures are pale yellow in freshly hatched individuals (as they are in wild type males) and quickly darken to a deep butter yellow color. In

FIGURE 3.—The reproductive system of a *w^a* male. t, testis; sv, seminal vesicle; vd, vas deferens; ag, accessory gland; Dej, ejaculatory duct; Bej, ejaculatory bulb.

FIGURE 4.—The reproductive system of a *tra* female, showing the greatly reduced and uncoiled testes. The labelling is the same as in Figure 3.

mature transformed individuals the testes are a much brighter yellow than are those of wild type males or *w^a* males. Whether this coloration is due to the presence of excess yellow pigment or to the normal amount of pigment compressed in the smaller testes has not been determined, however. One bit of evidence that suggests a normal pigment content is the finding that the outer sheath of

the testis of a *tra* female is generally thicker than in normal males, and in some areas may show two or three layers of pigment-containing sheath cells. This piling up of the cells of the outermost layer of the testis sheath suggests that the sheath cells develop autonomously, irrespective of the amount of germinal material.

The inner layer of the wall of the testes of transformed females is normal in appearance, showing numerous small nuclei which seem to bulge slightly from the thin layer of protoplasm. Within the distal tip of the testis appears a mass of small cells with dense Feulgen-positive nuclei. Whether these cells are spermatogonia or oögonia could not be determined. The remainder of the testis contains vacuoles of various sizes, scattered nuclei, and blobs of degenerative Feulgen-positive material. No cells resembling spermatocytes or later stages of spermatogenesis were found.

A very interesting finding which prompted most of this study was the presence of the structures shown in Figures 5–8 in a few “testes” of transformed females. These Feulgen-positive spheres are almost certainly endopolyploid nurse cell nuclei which develop during the process of oögenesis. Using the classification of KING, RUBINSON, and SMITH (1956), the nuclei ranged in size from those typical of stages 4 to 8. The largest nuclei observed were about $12\ \mu$ in diameter and were spongy in appearance (Figure 5). In some instances the oöcyte nucleus (which is characterized by a condensed, deeply staining karyosome) was also evident (Figures 7 and 8). The number of nurse-like cells present in any given group varied from 12–15 (the number in a normal developing egg chamber is 15). In several specimens the testes were filled with randomly scattered groups of

FIGURE 5.—A photomicrograph of nurse-like cells in the distal portion of the “testis” of a *tra* female. Eight of the 12 cells present in this specimen are visible. The upper arrow points to the inner sheath (note small nuclei) and the lower arrow points to the outer sheath of the “testis”.

FIGURE 6.—The distal portion of a “testis” from a *tra* female showing fusion of several nurse cell-like nuclei.

FIGURE 7.—The entire “testis” of a *tra* female showing “nurse cell nuclei” and an “oocyte nucleus” (arrow.)

FIGURE 8.—The “testis” of a *tra* female showing several groups of “nurse cell nuclei” and an “oocyte nucleus” (arrow).

these nurse-like cells (Figure 8). All of the cells within each group seemed to be in roughly the same stage, as determined by their nuclear morphology. However, cells from differing groups differed markedly in their morphology. In one case (Figure 6), several nurse-like nuclei had fused into large irregular masses of chromatin. All testes containing nurse-like cells were found in flies between nine and 27 days of age.

Further study of this phenomenon has been hampered by the low frequency with which it seems to occur. Out of 372 pairs of “testes” examined from individuals reared at 25°C, only 12 have shown these nurse-like cells (a frequency

of about three percent). In each case, both of the testes contained these cells. The reproductive systems of 87 transformed females reared at 18°C have been examined, but nurse-like cells were not found in any of them. Whether or not the lower temperature suppresses formation of the nurse-like cells cannot be decided, however, because of the small size of the sample. With regard to the effect of development at 18°C upon the male-like internal genitalia of *tra* females, they seem to behave as do normal males in that the reproductive systems are larger than those formed in flies reared at 25°C; but no striking morphological changes were evident.

In order to determine the size of the larval gonads of transformed females, 63 third instar larvae were collected from a stock maintained at 25°C and separated into two groups: those possessing large gonads and those with small gonads. Normally, *Drosophila* larvae may be easily classified according to sex since the male gonads are much larger than those of the female. Of the larvae having small gonads, 20 developed into γ^2 females and nine developed into *tra* females; while all of those with large gonads (34) developed into *w^a* males. Thus, the larval gonads of transformed females are small, and consequently they are not behaving as male gonads in terms of growth.

DISCUSSION

With respect to the majority of characteristics so far studied, transformed females resemble males. Among these male characteristics (in addition to the over-all behavior) are the abdominal coloration, the tergite pattern, the external genitalia, the internal accessory sexual organs, the sex combs, and the possession of the male peptide. However, transformed females do have about 20 percent fewer teeth in their sex combs, and young transformed flies are intermediate in mass between males and females of the same age. Furthermore, the amounts of β -alanine, tryptophan and proline found in *tra* females are intermediate between male and female values. Transformed females resemble females in terms of both wing size and the differential growth response of the wings at 18 and 25°C. Transformed females also react in the female fashion with respect to dosage compensation and to the genes *sc^s*, *fa* and *w^a*. Finally, since in rare instances germ cells in the testes of transformed females do undergo developmental processes reminiscent of oögenesis but never show any spermatogenesis, it is clear that oögonia are not transformed to spermatogonia. Transformed females are therefore intersexes of an extreme male type.

A hypothesis which explains the behavior of the transformer gene can be obtained by a modification of BRIDGES' balance theory of sex determination. According to BRIDGES (1934) the sexual phenotype results from the interaction of female determining genes which reside mainly on the X chromosome and male determining genes mainly located on the autosomes. Presumably these genes exert their action through the formation of nondiffusible substances, since clear-cut demarcation between male and female tissue in *Drosophila* gynandromorphs rules out the existence of sex hormones. To explain the effect of *tra* on the sexual phenotype one may assume that the + allele directly or indirectly leads

to an enhanced activity of the female determining substances produced by the X chromosomal genes, or suppresses the masculinizing action of the substances produced by the autosomal genes. It is impossible to decide at present which of these suggestions is more likely.

Thus, one could suppose that each X chromosome in the presence of one or more + alleles of *tra* produces twice the concentration of "feminizing factors" that it does in the absence of + alleles. Conversely, the autosomes in the absence of the + allele of *tra* might produce twice the concentration of "masculinizing factors" they do in the presence of one or more + alleles. If the ratio between feminizing and masculinizing factors is what counts, either model will fit the facts. The observation that the phenotypic expression of XX *tra/tra* individuals is so constant suggests that the ratio in question is much closer to that found in normal males than in triploid intersexes.

There are in the literature many examples of genes which enhance or suppress the effectiveness of nonallelic genes. In *Neurospora crassa*, for example, gene *td₂₄* forms a modified tryptophan synthetase which is readily inactivated by certain metals. A nonallelic gene (*su-24*), by modifying the metal metabolism of the fungus, prevents the enzyme from being poisoned (YANOFSKY 1960). Here then is an example of a gene which enhances the effectiveness of the product of a second gene. An example of a gene which inhibits, either primarily or secondarily, the action of nonallelic genes is found in *Escherichia coli*. The + allele of gene *i* inhibits the action of adjacent genes which are responsible for the production of the enzymes β -galactosidase and galactoside permease (PARDEE, JACOB and MONOD 1959).

According to the above hypothesis, an XX AA individual is a female because in the presence of one (or more) + alleles of *tra* the feminizing factors produced by X chromosomal genes occur at concentrations which overcome the action of masculinizing factors produced by autosomal genes. The *tra* gene is presumed to be inactive physiologically. In XX *tra/tra* flies the masculinizing factors arising from autosomal genes occur at concentrations that overcome the action of the feminizing factors produced by sex-linked genes. Both masculinizing and feminizing factors might act as inducers which determine whether the male or female pattern of differentiation shall be followed by the imaginal discs which produce the tergites, external genitalia and genital ducts of the organism. One might expect that inducers would not affect the germ cells which are set aside very early in development.

The fact that females are larger than males, show dosage compensation, etc. may result from their containing in double dose certain genes which have no effect on sexual phenotype and which are found in males in single dose because these genes reside on the X chromosome. The presence or absence of an active allele at the transformer locus would not affect the expression of these genes.

It is of interest that the locus of the transformer gene is close to that of certain genes (Polycomb, Extra sex comb, proboscipedia, tetraltera and tetrapter) which seem to affect the pattern of differentiation of specific imaginal discs. It remains for future research to determine whether or not this association is fortuitous.

SUMMARY

Drosophila melanogaster females homozygous for the transformer gene are compared with normal males and females in terms of wing form at 18°C and 25°C and sex comb morphology. It is shown that transformed females have approximately 20 percent fewer teeth in their sex combs than normal males, are intermediate in mass between males and females, and resemble females in terms of wing size and response of differential growth of the wings at 18° and 25°C. Transformed individuals also resemble females in that some of their germ cells occasionally undergo developmental processes resembling oögenesis but never spermatogenesis. A hypothesis is put forward to explain the action of *tra*; the + allele either suppresses the masculinizing action of substances produced mainly by autosomal genes, or enhances the activity of feminizing substances produced mainly by sex-linked genes.

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