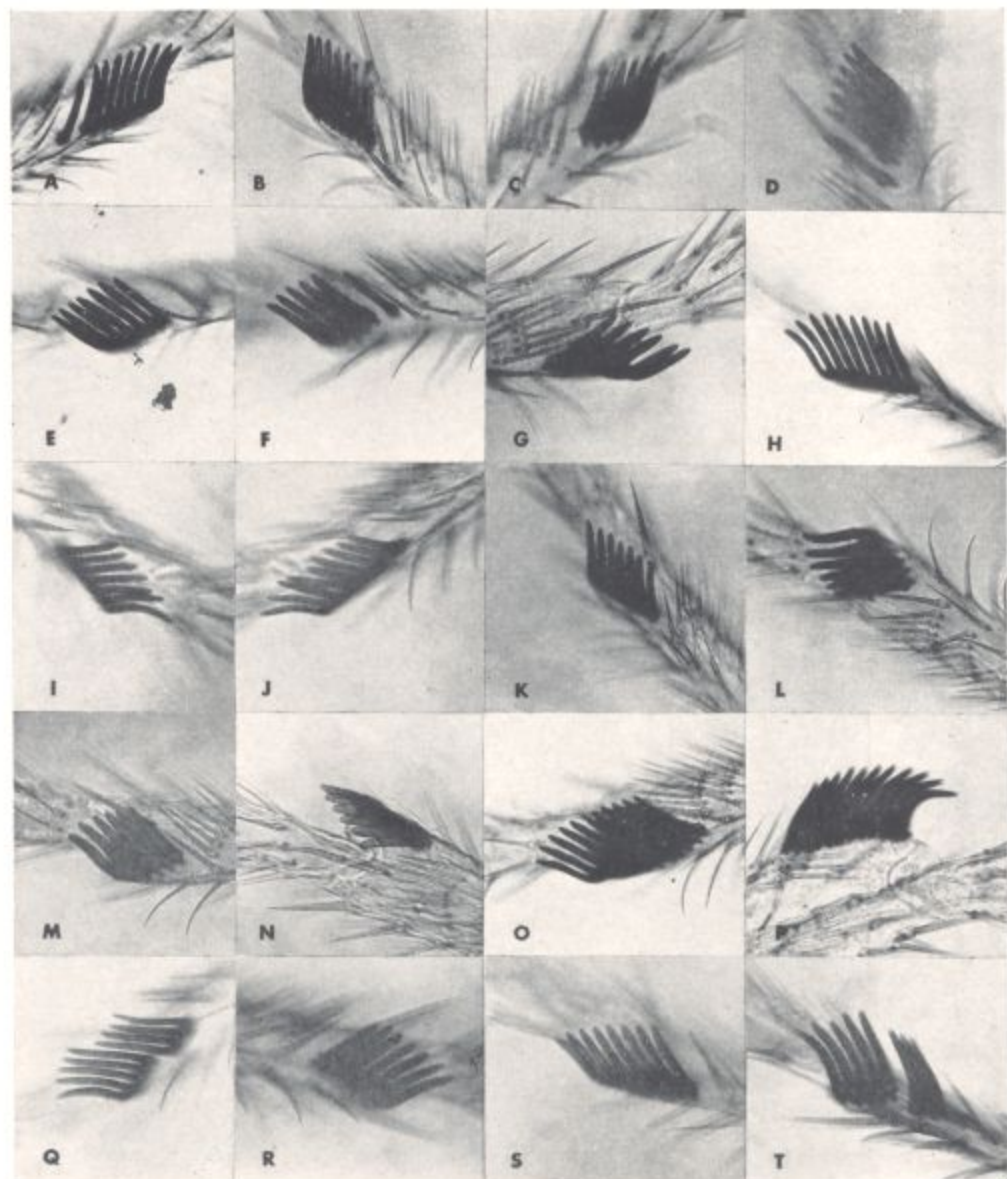


Sex Combs in Unbalanced
Male Forms in
Drosophila Melanogaster

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SEX COMBS

Figure 1

Photographs of sex combs of third chromosome aneuploids, unbalanced male forms, and ordinary diploid males. *A* and *H*—left and right combs of 2-6 hypo-diploid male; *B*—*H7L* hyper-diploid male; *C* and *D*—left and right combs of *H7L* hyper-intersex; *E*—*H7-13* hypo-diploid male; *F*—*H7-13* hypo-intersex; *G*—*85CR* hyper-intersex; *I* and *J*—left and right combs of *2R* hyper-diploid male; *K*—*12L* hyper-diploid male; *L* and *M*—*12L* hyper-intersex; *N*—*85CL* hyper-intersex; *O* and *P*—left and right combs of normal diploid male; *Q*—super-male; *R* and *S*—left and right combs of control *2X3A* intersex; *T*—right comb of control *2X3A* intersex.

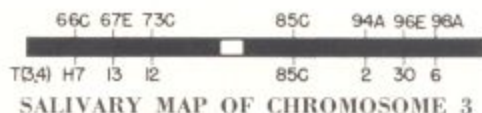
SEX COMBS IN UNBALANCED MALE FORMS OF *DROSOPHILA MELANOGASTER*

SARAH BEDICHEK PIPKIN*

SINCE studies of the terminalia of neither second nor third chromosome triploid aneuploids revealed a definite qualitative sex shift^{15,16,17}, the sex combs of certain male or intersex aneuploids have been investigated. This work was undertaken to compare the sex combs of hyper-intersexes carrying in excess of $2X3A$ various fragments of chromosome 3 with their sibling $2X3A$ intersexes to learn if a modification toward maleness occurred in the former. Sex combs of males hypo-diploid for a short section of chromosome 3 were examined to see if a change in the female direction had taken place. Sex combs of hyper-diploid males and super-males were studied since these represent unbalanced male forms where no sex shift is expected.

Materials and Methods

The 3,4 translocations used and methods of obtaining the triploid aneuploids have been described elsewhere^{16,17}. A diagram of the third chromosome with the salivary map points of breakage of the translocations used is given in Figure 2. Either the left-hand fragment (L) or the right-hand fragment (R) of the respective translocations + $2X3A$ was present in a given hyper-intersex. Figure 3A shows the genetic composition of a hyper-intersex and of its sibling control $2X3A$ intersex. Sex combs of the following long-region hyper-intersexes were examined: $12L + 2X3A$, $85CL + 2X3A$, $85CR + 2X3A$, $89ER + 2X3A$. Short-region hyper-intersexes studied included $H7L + 2X3A$, $2R + 2X3A$, $30R + 2X3A$, and $H6R + 2X3A$. Sex combs



SALIVARY MAP OF CHROMOSOME 3

Figure 2

Diagram of the salivary map of chromosome 3, showing below, the points of breakage of 3,4 translocations, and above, the corresponding locations on the salivary map.

of $30R + 2X3A$ and $2R + 2X3A$ hyper-intersexes were of interest because their mean sex type (scored according to the external genitalia) was slightly but significantly more male than the mean sex type of their respective $2X3A$ intersex siblings¹⁰. The genetic composition of hyper-diploid and hypo-diploid males, respectively, is shown in Figure 3B and C. Sex combs of hyper-diploid males carrying one of the following fragments in excess of $1X2A$ were studied: $2R$, $H7L$, and $12L$. Sex combs of hypo-diploid males with the section between $T(3,4) A2$ and $T(3,4) H6$ haploid and also those with the section between $T(3,4) H7$ and $T(3,4) A13$ haploid were examined. The latter hypo-ploid males were chosen for study because approximately one-fourth possessed rotated genitalia. The region $H7-13$ is very nearly the same region studied by Patterson *et al.*¹², who reported hypo-diploid males with frequently rotated and imperfect genitalia.

Super-males and control normal diploid males here studied were taken from the $y^2; ru ca$ triploid stock used in third chromosome aneuploid studies.

Whole forelegs of flies were stored in 70 percent alcohol and mounted either in euparal or clarite. Right legs were cut shorter than left so that these could

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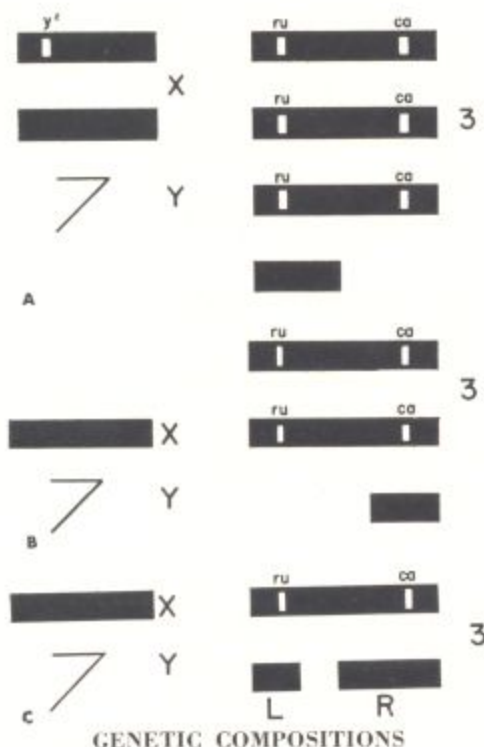


Figure 3

A—Genetic composition with respect to X and third chromosomes of a hyper-intersex; *B*—Genetic composition with respect to X, Y, and third chromosomes of a hyper-diploid male; *C*—Genetic composition with respect to X, Y, and third chromosomes of a hypo-diploid male.

be distinguished on the slide when the sex comb prongs were counted. Where possible, sex combs of 25 individuals of each genotype were examined.

Results

Photographs of sex combs of certain aneuploids, the normal diploid male (*IX2A*), the super-male (*IX3A*), and the ordinary *2X3A* intersexes are presented in Figure 1. Sex combs of the unbalanced male forms (aneuploids, super-male, and *2X3A* intersexes) differ from the sex combs of the normal diploid male by a reduction in prong number; presence of occasional or, in some instances, rare gaps in the comb (divided combs); and, in the case of the super-male, an increase in the asymmetry of prong number in right and left combs of the same individual. The

sex comb teeth of diploid males are pressed close together, and the comb possesses a characteristic curve not usually seen in combs of intersexes and aneuploids. In the latter, the teeth are spread apart and appear more stubby and thick than the teeth of the normal male comb, though Hannah-Alava and Stern¹¹, from actual measurements of intersex and diploid male combs, found no difference in thickness of corresponding prongs.

A comparison of mean tooth number in the sex comb of the control diploid male and in those of the various chromosomally unbalanced male forms is given in Table I. Here it is of interest that the mean tooth number in hyper-intersexes carrying a short right-hand end fragment of chromosome 3 (*i.e.*, *2R*, *30R*, or *H6R*) does not differ significantly from the mean tooth number of sex combs of the corresponding *2X3A* sibling control intersexes. All chromosomally unbalanced male forms have fewer teeth in the sex comb than the control diploid male. The degree of reduction of tooth number is correlated with the degree of chromosomal imbalance. Super-males (*IX3A*) have the fewest sex comb prongs and the greatest qualitative disturbance of the comb. Hyper-intersexes with longer fragments usually showed greater reduction in tooth number than hyper-intersexes with shorter fragments. Gaps in the sex comb were extremely rare in ordinary *2X3A* intersexes but were found in hyper-intersexes *89EL*, *H7L* (Figure

TABLE I. Mean tooth number of the sex combs of hyper-intersexes, their corresponding control sibling *2X3A* intersexes, normal diploid males, hyper-diploid males, and super-males

HYPER-INTERSEX		CONTROL INTERSEX (2 × 3A)
12L	6.50 ± 0.17	7.58 ± 0.16
85CL	6.09 ± 0.16	6.99 ± 0.14
2R	7.50 ± 0.24	7.40 ± 0.15
30R	8.00 ± 0.23	7.58 ± 0.22
6R	8.12 ± 0.23	8.00 ± 0.21
H7L	(5-6) (2 flies)	
89EL	(7.6) (1 fly)	
89ER	(7.10) (1 fly)	
HYPO-DIPLOID ♂		CONTROL DIPLOID ♀ (1X2A)
H7-13	8.57 ± 0.14	10.84 ± 0.14
2-6	(9.9) (1 ♀)	
HYPER-DIPLOID ♀		SUPER-♀ (1X3A)
2R	9.27 ± 0.11	8.56 ± 0.22
12L	(7.7) (1 ♀)	
H7L	(6-9) (3 ♀)	

1D); hypo-intersexes *H7-13* (Figure 1F); hypo-diploid males *2-6* and *H7-13*; and most frequently, in super-males, where seven of 40 combs studied were of this type. The gap in a divided comb may occur at different places in the comb. The teeth in proximal and distal combs numbered 3,4; 4,4; 6,2; 5,3; 4,3; 8,2; 7,8, respectively, in seven super-males showing divided sex combs. In the last case, an additional prong was present on the extensor surface of the tarsus near prong 5. The presence of a prong "out of line" with the rest of the comb was also observed in the hyper-intersex *89EL*.

The asymmetry of sex comb prong number characteristic of both normal diploid males and of ordinary *2X3A* intersexes is likewise present in the aneuploids' sex combs and is actually more pronounced in the super-male sex combs. Of 26 normal diploid males, 16 showed differences in prong number between right and left legs of the same individual, the difference ranging from one to three prongs. In ordinary *2X3A* intersexes, 51 of 82 examined displayed such asymmetry. In super-males, 15 of 20 combs studied were asymmetrical.

Discussion

A reduction in sex comb tooth number indicating a shift from the 8-pronged intersexual comb in the female direction has been found by Pipkin¹⁵ in the case of certain X-chromosome triploid aneuploids which have rudimentary combs of one or two prongs. On the other hand, an increase in comb teeth from four to six in triploid females carrying the hermaphrodite gene, *Hr*, in one third chromosome to 10 to 12 teeth in sex combs of triploid females homozygous for its allele, *tra*, studied by Gowen and Fung⁹, demonstrates an obvious modification in the male direction, using the sex comb tooth number as a criterion of the degree of sex shift.

No rudimentary sex combs were found on hyper-triploid females carrying *3X3A* plus short or long fragments either of chromosome 2¹⁶ or of chromosome 3^{16,17}, although Bridges¹ reported that individuals with *3XA* (determined cytologically) were similar to *2X3A* intersexes. According to external genitalia studies, no sexual shifts were observed in any of the surviving hypo-intersexes involving sections of chromosome 2¹⁶ or of chromosome 3^{16,17}. It was therefore concluded that male-determining factors present both in chromosome 2 and in chromosome 3 were responsible for the shift toward maleness in ordinary *2X3A* intersexes¹⁷. Hyper-intersexes bearing either of two short

right-hand end regions of chromosome 3 showed mean sex types (classed according to the external genital ducts) slightly more male than those of sibling control intersexes¹⁶, but the mean sex comb tooth number of these hyper-intersexes did not differ significantly from the mean sex comb tooth number of sibling control intersexes. Furthermore, the hyper-intersexes with longer fragments showed the greater reduction in number of sex comb teeth. Hypo-diploid males did possess fewer sex comb teeth than control diploid males, but this reduction is not taken as a shift toward femaleness but rather as the result of a growth disturbance caused by aneuploidy. A lesser reduction in mean sex comb tooth number was likewise found in hyper-diploid males, and a more pronounced reduction in super-males, where no sex shifts are expected. Thus the studies of sex combs and of external genital ducts of third chromosome hyper-intersexes are in agreement in failing to indicate a definite shift toward maleness.

On the other hand, it may be that no increase in sex comb tooth number should be expected in autosomal hyper-intersexes. To anticipate such an increase supposes that the reduction in ordinary *2X3A* intersex sex combs, as compared with the tooth number in diploid males, is due to intermediate sexual development, an inhibition of the development of the comb owing to female development within the same individual. It may be argued that the sex combs of triploid intersexes represent a complete male development, the small reduction in prong number being due to a growth disturbance dependent upon the unbalanced *2X3A* genotype but not upon a feminizing (hormonal) influence. The following are some reasons for thinking that triploid intersexes possess a complete male development in the early differentiated sex comb (with the exception of the very rare, extreme female-type intersexes which lack combs): (1) Dobzhansky² found that selection of a triploid line yielding male-type intersexes was more effective and proceeded more rapidly than selection of a triploid line yielding female-type intersexes. (2) Although extremely rare type VI *2X3A* intersexes lacking sex combs and occurring in two to three percent of the highest selected female-type intersex producing line were described by Dobzhansky and Bridges¹, Pipkin¹⁵ found that the mean prong number of sex combs of triploid intersexes of the extreme male type I did not differ significantly from the mean sex comb prong number of intersexes of the female type IV (with imperfect female genitalia, sex combs). Hannah-Alava and Stern¹¹ reported a similar finding for sex combs of male-like, intermediate, and female-like triploid intersexes. (3) Similarly, Hannah¹⁰ found that Strapped, a digenic male-limited character, was expressed in both male- and female-type triploid intersexes. (4) Pipkin¹⁶ found the variation in total time of development of male-type and female-type triploid intersexes to be the same. Furthermore, Gowen and Fung⁹ stated that the triploid intersex genotype,

2X3A, is little affected by *Hr*, the hermaphrodite gene, and *tra*, its recessive allele, both of which strongly modify development in the male direction in diploid and triploid females, but fail to affect the diploid male.

Another case in which it is possible that a growth disturbance accounts for the reduction of mean sex comb tooth number is the finding by Stern and Hannah¹⁹ that sex combs of mosaic legs or segments of legs of gynandromorphs averaged 1.3 prongs fewer than those of their diploid male siblings, these authors concluding, "whether this influence derives from a quantitatively specific nature of the female tissue, transmitted to the male tissues in a hormonal way, or whether the quantitative differences in general metabolism and duration of development which characterise the two normal sexes are instrumental, must remain an open issue."

Recognizing the predominantly male-like character of *Drosophila* triploid intersexes, Goldschmid⁷ described them as "male" intersexes in contrast with 2X2A diploid intersexes homozygous for an intersex mutant which he termed "female" intersexes. He considered 2X3A triploid intersexes to be sex mosaics in time, male development preceding female development, a view supported by the work of Dobzhansky and Bridges². Although contested and defended, this theory emphasizes the essentially male nature of most triploid intersexes in *Drosophila*.

Four mechanisms have been found which modify the sexual development of ordinary 2X3A intersexes: (1) Dobzhansky², working with a triploid line producing intersexes ranging from sex types I to V, found that culturing intersexes at 20° C. favored a shift toward maleness; at 28° C. a shift toward femaleness. (2) Dobzhansky² located a particular third chromosome which in one dose shifted the mean sex type of intersexes in the female direction. (3) Fung and Gowen⁵ reported female-type triploid intersexes to have higher numbers of fourth chromosomes than intermediate and male-type intersexes. (4) In one cross, Dobzhansky and Schultz⁴ found that the mean sex type of intersexes arising from 1X2A eggs does not differ from the mean sex type of intersexes arising from 2X2A eggs. The former intersexes lack a Y chromosome; the latter possess one. These authors concluded that the Y chromosome does not influence the sexual development of intersexes. Pipkin¹⁴ crossed males of 20 different wild strains to an inbred $y^2 v f$ (yellow-2, vermilion, forked) triploid line. In six of these crosses, the wild-type intersexes, coming from 1X2A eggs, were significantly more male-like than the intersexes displaying the recessive marker mutants $y^2 v f$, arising from 2X2A eggs. In the remaining 14 crosses, the frequencies of sex types of wild-type and $y^2 v f$ intersexes were homogeneous (did not differ significantly). The author concluded that these variations in sex types of the two kinds of intersexes were owing to dominant modifier(s) in some of the wild-type X chromosomes, although the possibility

of modifiers in the Y chromosome was not completely ruled out. Further crosses of wild-type males to triploids homozygous for the mutant y^2 (yellow-2), carried out in connection with other experiments, have confirmed the observation that, in a number of crosses, the yellow-2 intersexes (from 2X2A eggs) are significantly more female-like than the wild-type intersexes (from 1X2A eggs) (Pipkin, unpublished). Other explanations than the one given by Pipkin¹⁴ may account for these data. Possibly intersexes arising from 1X2A eggs are sometimes more male-like than their sibling intersexes arising from 2X2A eggs because of the action in the 1X2A eggs before fertilization of autosomal male-determining genes which have twice the dosage of X chromosome genes in these eggs. This explanation was applied by Goldschmid⁴ to some results found in his Bd-Mn intersexes. He further pointed out that a case of diploid intersexuality has been studied by Sturtevant²⁰ in which male-determining genes acted in the egg before fertilization. Since the frequency of 1X2A eggs is much higher than the frequency of 2X2A eggs, this explanation would partially account for the preponderance of male-type intersexes in most triploid lines. However, intersex-triploid mosaics^{8,18} prove that the decision of sex determination comes after fertilization as in the case of diploid males and females. Finally, the yellow intersexes arising from 2X2A eggs may be more female-like in some cases because there may be a tendency of fourth chromosomes to segregate into the 2X2A eggs with a higher frequency than into the 1X2A eggs.

Summary

Sex combs of hyper-intersexes of the genotype 2X3A with a fragment of chromosome 3 possessed no more prongs than their control sibling 2X3A intersexes. An examination of sex combs of other unbalanced male forms, hyper-diploid males and hypo-diploid males, the aneuploidy involving sections of chromosome 3, and supermales (1X3A), revealed a slight reduction in sex comb prong number as compared with ordinary diploid males. It is suggested that the 2X3A intersex sex comb in all but the extreme female-type intersex represents a complete male development. The reduction in prong number is thought to be due to a growth disturbance caused by the unbalanced genotype. Some factors influencing the degree of sexuality of 2X3A intersexes are discussed.

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