

THE EFFECT OF A SUPERNUMERARY X CHROMOSOME ON MEMBERS OF THE BAR SERIES OF DROSOPHILA¹

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Studies on the bar series of *Drosophila* have, until the present time, been carried out entirely on flies of normal diploid chromosome constitution. The phenomena of polyploidy and heteroploidy make possible an investigation of the expression of the bar gene and its allelomorphs in flies having an aberrant chromosome complex.

As a result of occasional failure of the X chromosomes to disjoin during maturation of the egg, individuals having three X chromosomes and the normal complement of autosomes may be produced. These individuals, known as superfemales, are of extremely low viability, and die at various stages of development, rarely attaining maturity (Li 1927).

L. V. MORGAN (1922) isolated a yellow stock in which the X chromosomes of the female appeared to be permanently attached. In addition to the attached X chromosomes, a Y from the father was present. It was found that occasionally the X's disjoin, giving normal diploid daughters in which one X comes from the mother, the other from the father.

When double-yellow attached-X females (\widehat{yy}) are mated to normal males, a small percentage of superfemales may be expected among the progeny. Furthermore, as the result of infrequent disjunction of the X's, normal diploid females are produced. Since these two classes of flies differ only in the presence or absence of an extra X chromosome, a comparison of the two affords an excellent medium for a study of the effect of a supernumerary X chromosome on the expression of any character.

EXPERIMENTAL PROCEDURE

Three members of the bar series were studied: (1) bar (*B*); (2) double-bar (*BB*); (3) infra-bar (*B*[†]).

Bar series. The flies on which facet counts were made were obtained in an experiment of HERSH, KARRER, and LOOMIS (1930) on the possible effect of supersonic vibrations on mutation rate. In the experiment *ct f B* males were inbred with \widehat{yy} females for eight generations. The males were

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then subjected to treatment of 285,000 vibrations per second and mated to \widehat{yy} females in pair matings.

Among the progeny of these flies were 290 superfemales, and 30 heterozygous females resulting from disjunction of the X chromosomes. Facet counts were made on these two classes, and also on a number of superfemales obtained from control matings in the experiment. The frequency distribution in the different progeny classes is given in table 1.

Double-bar series. $f BB$ males were inbred with \widehat{yy} females for six generations. As in the bar series, pair matings were used throughout the experiment (see table 1).

Infra-bar series. $f B^i$ males were inbred with \widehat{yy} females for four generations. Pair matings were used in the experiment (see table 1).

Temperature control. In all the experiments the flies were raised at 25°C. In the bar series, several times in the course of the experiment, the temperature rose to 28°C for short intervals, but never fell below 25°C. In the BB and B^i series the average fluctuation was $\pm 0.5^\circ\text{C}$. On a few occasions and for very short periods, the temperature varied as much as 2°C.

Culture medium. In all the experiments a 1 percent banana-agar medium in 8 dram vials was used. The food was inoculated with a small amount of yeast foam. The parent flies were permitted to lay eggs over a 6 to 7 day period, and then transferred to fresh vials. Two or three broods were obtained from each set of parents. The progeny were examined and counted every third day over a 10 to 15 day period after emergence of the first fly from the pupa case. The superfemales are easily recognized by a number of definite and constant characteristics (BRIDGES 1922). The disjunctive females, on the other hand, are quite normal in appearance, but may be identified by the gray body color and heterozygous eye.

*Facet counts.*² In the BB series, facet counts were made directly, using a binocular dissecting microscope with 10 \times eyepiece and 24mm objective. In the B and B^i series dissections of the cornea were made, following a modification of the procedure of R. K. HERSH (1924) and STURTEVANT (1925). The flies were placed in a 5 percent solution of KOH for 24–48 hours for clearing. The cornea was then removed by fine needles and mounted in water upon a slide. Three or four radial incisions were made in order to flatten the somewhat convex surface. The cornea was then projected on a Bausch and Lomb projecting apparatus and the facets counted. Error was minimized by blocking out each facet as counted. The facet counts were checked by making recounts of 12 eyes in each series, a few days after the initial counts. In all cases the error was found to be slightly less than 1 percent.

² The facet counts in the bar series were made by Miss EDITH I. SCRIBNER, who has kindly consented to publication of the data in this paper.

Temperature variations have already been considered. They occurred quite at random, and did not appear to be of such magnitude as to obscure any marked effect on facet number.

No effort was made to control the number of eggs laid per vial. Considering the length of the egg-laying period a crowded condition no doubt prevailed. This condition, however, was constant in all the experiments. LUCE (1931) has shown that crowding markedly reduced facet number in B^1 . Whether crowding might act differentially in diplo- and triplo-X flies is unknown.

EXPERIMENTAL DATA

In table 1 the progeny results are summarized. The last column of the table gives the data of DOBZHANSKY (1928) on the effect of temperature on production of superfemales. These data are for 24.5°C, a temperature comparable with the one used in the present experiment. In DOBZHANSKY'S experiments sooty males were mated to $\bar{y}\bar{y}$ females.

TABLE 1
Table showing frequency distribution of individuals in progeny.

	B SERIES	BB SERIES	B ¹ SERIES	DATA OF DOBZHANSKY AT 24.5°C
$\bar{y}\bar{y}$ females	12,189	19,054	4,625	3,262
<i>f</i> males	13,598	17,184	5,261	3,645
Superfemales	290	21	61	101
Yellow males	28	21	1	1
Disjunctional females	30	22	2*	2
Total	26,135	36,302	9,950	7,011
Percent superfemales	1.11	0.058	0.61	1.4
Percent disjunction	0.22	0.12	0.03	0.04

* These two females were inbred to fB^1 brothers in order to obtain a sufficient number of diploid heterozygous females for facet counts.

It is apparent that large differences in relative numbers of superfemales, and also in the frequency of disjunction of X chromosomes, occur in the three series. These differences may be explained by the fact that the system of inbreeding used was designed primarily to render the flies of any one series isogenic with each other. It did not eliminate genetic differences between members of different series to any appreciable extent. It is likewise necessary to consider possible direct effects of the different bar allelomorphs on viability and disjunction. These allelomorphs are known to affect viability in different degree.

When the production rate of superfemales in the different series is compared with DOBZHANSKY'S data, there is, in general, considerable disparity.

His data, however, are in fairly close agreement with the results on the bar series. As regards disjunction rate, DOBZHANSKY'S data accord well with the data in the infra-bar series. These comparisons, however, have little meaning due to the genetic heterogeneity of the flies in the two experiments.

In table 2 are summarized the essential statistics on facet number in the three series, together with their standard errors.

TABLE 2
Summary of data on facet number.

	B^i SUPER ♀	B^i HET. ♀	BB SUPER ♀	BB HET. ♀	B SUPER ♀	B HET. ♀
Mean facet number	558.5 ±12.1	475.0 ±11.7	70.8 ±2.7	44.4 ±1.1	550.8 ±4.3	395.3 ±18.8
Standard deviation	85.5 ±8.6	82.5 ±8.3	11.2 ±1.9	8.1 ±0.8	78.3 ±3.1	90.0 ±13.2
Coefficient of variability	15.3	17.4	15.8	18.4	14.2	22.8
Number of flies	50	50	17	53	327	23

In each of the three series, a supernumerary X chromosome produces a significant increase in facet number, taking the diplo-X heterozygous female as base of reference. The percentage increase, however, differs for the three bar allelomorphs. It is 16.7 percent for B^i , 39.4 percent for B , and 59.2 percent for BB . However, as the table shows, the order of absolute increase in facet number as affected by a supernumerary X chromosome is $B > B^i > BB$.

DISCUSSION

The simplest and most apparent interpretation of the increase in facet number produced by an additional X chromosome, would be that in the triplo-X fly there are present two wild-type allelomorphs of bar, as compared with one such allelomorph in the diploid heterozygous female. If we look upon the wild-type allelomorph of bar as a differential for facet production, the increase in facet number is to be expected. Evidence, however, has been adduced (BRIDGES 1917, STURTEVANT 1925) that the normal allelomorph of bar plays no part in facet production. STURTEVANT has suggested the possibility that no normal allelomorph of bar exists. In either of these cases the data require an alternative explanation. The wild-type eye is the resultant of the interaction of a number of genes distributed throughout the chromosomes. Some of these genes may well have their loci in the X chromosome (HERSH 1929). From this standpoint of

genic balance, the eye which is produced is the somatic expression of a balance among the effects of these genes, some having positive and others negative effects on facet production. If in the X chromosome the internal balance is such that the net tendency is in the plus direction facets are added. Conversely if minus modifiers preponderate, facet number is reduced. From the data here cited it appears that the balance of modifiers in the X chromosomes of the stocks investigated favor facet production.

How does this interpretation relate itself to other studies on facet number? All studies on members of the bar series have shown that males have somewhat larger eyes than the homozygous females. In considering the effect of the addition of an X chromosome it is necessary that we use the heterozygous and not the homozygous female as base of reference. The reason for this is at once apparent. The bar gene and its allelomorphs are powerful inhibitors of facet production. The presence of two bar genes in the female as against one in the male easily obscures whatever effect the additional X chromosome in the female might have on facet production. Females heterozygous for any allelomorph of the bar series always have much larger eyes than males having the same allelomorph. Here then the only differential is an additional X chromosome in the female, and the results are explained by a preponderance of plus modifiers in this chromosome.

The data available on the wild-type eye, however, are somewhat conflicting. R. K. HERSH (1924) in a study over a range of temperatures found that the wild-type eye is very slightly larger in the male than in the female at all temperatures studied. STURTEVANT (1925), on the other hand, observed that at 25°C the female eye is significantly larger than the male eye. The data of KRAFKA (1920) are extremely meager but interesting insofar as they may be used to corroborate the results of either of the above investigators. If KRAFKA'S experiments are taken singly, his one experiment, in which a sufficient number of flies was used, is in accord with R. K. HERSH. However, when all data at one temperature are grouped they confirm STURTEVANT.

The discrepancies in the data on the wild-type eye are not readily attributable to error on the part of any of the investigators. In all experiments the flies were inbred for a number of generations. The technique in facet counting was such as to give a high order of precision to the counts. The differences no doubt arise from genetic differences in the stocks used. The disparity in the data is suggestive of the possibility of a reversal in sexual dimorphism. ZELENY (1921) has shown that there is a marked reduction in sexual dimorphism in bar resulting from selection. KRAFKA'S data (1920) on *BB* indicate further that sexual dimorphism is a function of external factors. In the experiments there is a progressive decrease in

sexual dimorphism of the eye with increase in temperature, and a possible reversal between 29°C and 31°C.

The interpretation of the results presented in this paper, namely—that an additional X chromosome increases facet number because in the X chromosome the plus modifiers of facet number predominate—is consonant with all existing data on the bar series. The conflicting data on the wild-type eye, when compared with the condition in the bar series, indicate that we may be dealing with two totally or partially different systems of eye modifiers in the X chromosomes. The one system is stable as evidenced by the consistency of all data on the bar series which show that the eye of the heterozygous bar female is considerably larger than the male. This system may, as seems probable, consist of a single plus modifier acting directly on the bar system, and appears to be a constant feature of all stocks investigated. The second system is labile, due, presumably, to the fact that it consists of a number of minor modifiers mutating at random either in a plus or minus direction, and appears to act on the general facet-producing mechanism. This second system accounts for the disparity in the results of the investigators on facet number in the wild-type eye.

SUMMARY

1. The effect on facet number of a supernumerary wild-type X chromosome is reported for three members of the bar series, namely, bar, double-bar, and infra-bar. The facet number in superfemales, having two wild-type X chromosomes and one X chromosome carrying bar or one of its allelomorphs, is compared with the facet number in normal diploid flies heterozygous for the same allelomorph.

2. In each of the three series, a supernumerary wild-type X chromosome increases facet number markedly.

3. The results are interpreted in terms of genic balance. In the X chromosome plus modifiers of facet number predominate, thereby increasing facet production.

4. This conclusion is tested by comparing males showing one of the bar allelomorphs with females heterozygous for the same allelomorph, and in all cases the interpretation is tenable.

5. With respect to sexual dimorphism in the wild-type, the data are conflicting, indicating that we are dealing with different systems of facet number modifiers in the wild-type stocks and in bar and its allelomorphs.

LITERATURE CITED

- BRIDGES, C. B., 1917 Deficiency. *Genetics* 2: 445-465.
1922 The origin of variation in sexual and sex-limited characters. *Amer. Nat.* 56: 51-63.
DOBZHANSKY, T., 1928 The effect of temperature on the viability of superfemales in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. Washington* 14: 671-675.

- HERSH, A. H., 1929 The effect of different sections of the X chromosome upon bar eye in *Drosophila melanogaster*. Amer. Nat. **63**: 378-382.
- HERSH, A. H., KARRER, E. AND LOOMIS, A. L., 1930 An attempt to induce mutation in *Drosophila melanogaster* by means of supersonic vibrations. Amer. Nat. **64**: 552-559.
- HERSH, R. K., 1924 The effect of temperature upon the full-eyed race of *Drosophila*. J. Exp. Zool. **39**: 43-52.
- KRAFKA, JOSEPH JR., 1919 The effect of temperature upon facet number in the bar-eyed mutant of *Drosophila*: Part I. J. Gen. Physiol. **2**: 409-432.
- LI, JU-CHI, 1927 The effect of chromosome aberrations on development in *Drosophila melanogaster*. Genetics **12**: 1-58.
- LUCE, W. M., 1931 The temperature-effective period in infra-bar and its heterozygotes. J. Exp. Zool. **59**: 467-498.
- MORGAN, L. V., 1922 Non-criss-cross inheritance in *Drosophila melanogaster*. Biol. Bull. **42**: 267-274.
- STURTEVANT, A. H., 1925 The effects of unequal crossing over at the bar locus of *Drosophila*. Genetics **10**: 117-147.
- ZELENY, C., 1921 Decrease in sexual dimorphism of bar-eye *Drosophila* during the course of selection for low and high facet number. Amer. Nat. **55**: 404-411.