EXCHANGES AT THE BOBBED LOCUS OF *DROSOPHILA MELANOGASTER*[1]

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IN this paper evidence will be presented that crossing over at the bobbed (*bb*) locus of *Drosophila melanogaster* generates a variety of mutant alleles. Some of these alleles are distinguishable from one another and their parental alleles in the degree of their effect as judged by bristle size, abdominal etching and viability. These observations are in harmony with the genetic organization of the bobbed locus proposed by RITOSSA, ATWOOD and SPIEGELMAN (1966). Their paper presents evidence which identifies the bobbed locus with the nucleolus organizer (*NO*), that segment of DNA which codes for ribosomal RNA. Their model proposes that the r-DNA is intercalated in the chromosome as a linear array of tandem repeats. The various *bb* mutants of different strengths are considered as phenotypic manifestations of different sized deletions of the region. Bobbed mutants may frequently arise and revert because tandem repeats present opportunities for unequal pairing and subsequent crossing over between homologous chromosomes or intrachromosomal exchange will generate products of unequal lengths. On the other hand, the data presented here for *bb* are incompatible with the "master-slave" organization for genetic units in general that has been proposed by CALLAN (1967) and elaborated by WHITEHOUSE (1967). The appearance of the paper by RITOSSA *et al.* stimulated the experiments presented here, since two X ray-induced mutants obtained for other purposes proved to be eminently suitable for the detection of exchanges at the *bb* locus. A sketch of the method and summary of the results have appeared in abstract form (SCHALET 1967).

The following list includes symbols for the important mutants and special chromosomes used in this study. Additional information concerning these and other genetic variations that will be mentioned can be found in LINDSLEY and GRELL 1968; SCHALET and FINNERTY 1968.

ac: achaete (bristles)
B: bar (eye shape)
bb: bobbed (bristles)
l(1): recessive lethal in *X* chromosome
mal: maroonlike (eye color)
sc: scute (bristles)
su(f): suppressor of forked (bristles)
y: yellow (body color)

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$B^{S}Y$	A *Y* chromosome that causes extreme B phenotype; carries *su(f)*+ and *l(1)20*+.
y+*Ymal*+	A *Y* chromosome that carries *l(1)J1*+, *y*+, *ac*+, and covers mal^{12}.
y+*Y*	A *Y* chromosome that carries *l(1)J1*+,*y*+,*ac*+.
Y^{bb-}	A *Y* chromosome nonfunctional for *bb*+ (see RITOSSA 1968).
$In(1)sc^{8}$	An *X* chromosome inversion with left break between *ac* and *sc;* right break between *bb* and centromere.
In (1)dl-49	An *X* chromosome inversion. $In(1)dl\text{-}49/In(1)sc^{8}$ heterozygote suppresses crossing over throughout *X* chromosome.
$In(1)B^{M1}$	An *X* chromosome inversion with weak alleles of *B* and *bb*. $In(1)dl\text{-}49+B^{M1}/In(1)sc^{8}$ heterozygote suppresses crossing over throughout *X* chromosome.
In(1)FM6	An *X* chromosome inversion complex and crossover suppressor; carries *B* and an allele of *y*.

ORIGIN AND CHARACTERIZATION OF PARENTAL *bb*-BEARING CHROMOSOMES

The two *bb* chromosomes used in this study were originally detected in *Inversion(1)sc*^{8}, *sc*^{8} *B* chromosomes as single exceptional individuals. One appeared as a yellow body color (*y*) fly and the other as a maroonlike eye color (*mal*) fly among female progeny of X ray-treated $In(1)sc^{8}$,

GENETIC AND CYTOLOGICAL RELATIONSHIPS IN DISTAL PORTION OF sc^{8} CHROMOSOMES WITH PARTIAL BOBBED DEFICIENCIES

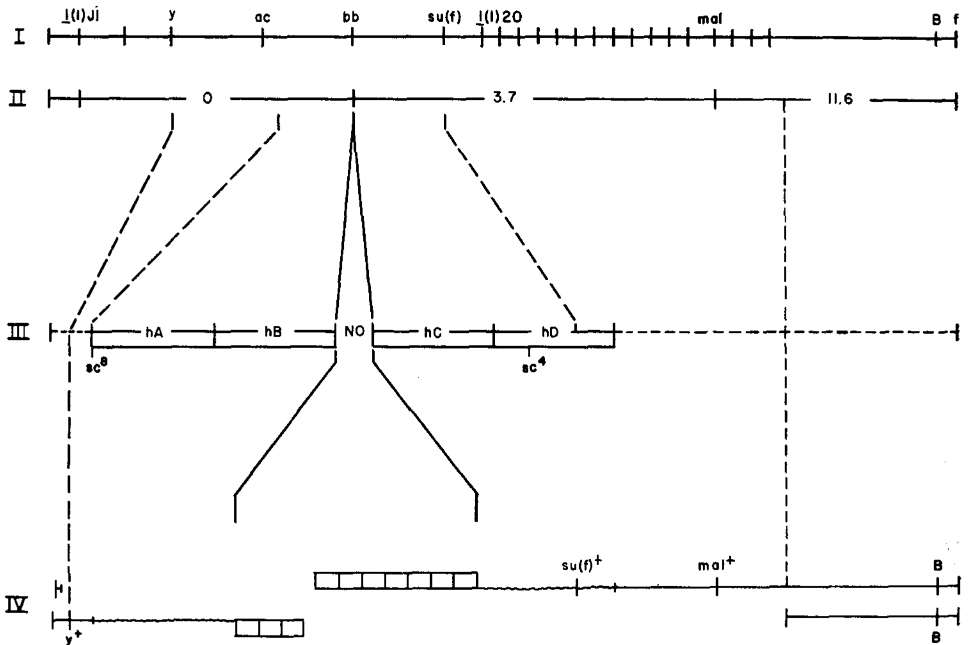

FIGURE 1.—I, Genetic markers; II, Linkage relations derived from $Df(1)y^{X2}$ sc^{8} mal^{1} *f v cv*/sc^{8} mal^{F3} females crossed to *Y*/*y v f* mal^{1} *su(f)* males. mal^{F3}/mal^{1} females have wild-type eyes; III, Cytogenetic map of heterochromatic region of mitotic X (redrawn from COOPER 1959). Placement of *su(f)* uncertain; IV, Schematic representation of parental *bb* chromosomes. Above—$Df(1)y^{X2}$, below—$Df(1)mal^{12}$.

sc⁸ B/y+Ymal+ males crossed to *In(1)sc^{S1}sc⁸, y sc^{S1} sc⁸ mal^{F2}* females. From the evidence presented below it will be seen that the mutant-bearing chromosomes may properly be designated as $Df(1)y^{X2}$, y^{X2} sc^8 B and $Df(1)mal^{12}$, sc^8 B mal^{12}, respectively. Hereafter they will be referred to simply as the y^{X2} and mal^{12} chromosomes. Furthermore, the various loci to be mentioned below are designated as being to the left or to the right of the *bb* locus in the orientation presented by the sc^8 inversion chromosome (Figure 1). In tests for loci to the left of *bb*, the y^{X2} chromosome was shown to lack the normal alleles of lethal(1)J1 [*l(1)J1*], achaete bristles (*ac*) and to be missing the Hairy-wing (*Hw*) effect associated with the left breakage point of the sc^8 inversion. In addition, y^{X2} displayed a *bb* phenotype when compounded with viable and lethal *bb* alleles. Loci to the right of *bb*, such as suppressor of forked [*su(f)*] and lethal(1)20 [*l(1)20*] appeared to be functioning normally and this was confirmed by the viability, fertility, and normal appearance of males carrying y^{X2} and *y+Y* which covers *l(1)J1, y, ac* and *bb* but none of the loci to the right of *bb*. In the sc^8 *B/y+Ymal+* parental male a spontaneous gonial exchange, between the distal heterochromatic region of the sc^8 inversion and the arm of the *Y* that carried the *KS* male fertility complex together with the *mal+* region, could also have produced a yellow appearing F_1 female. Consequently, the y^{X2} chromosome was tested for the presence of the full *KS* complex but this test proved to be negative. No attempt was made to determine the capping element of y^{X2}.

For mal^{12}, the normal alleles of melanized body (*mel*), short wing (*sw*), and outheld wings (*ot*) to the right of *mal*, and at least 12 lethal loci between *mal* and *su(f)* were missing. The normal allele of *su(f)* was also missing. When compounded with viable and lethal *bb* alleles, mal^{12} displayed a viable or near lethal *bb* phenotype. However, the normal alleles of loci to the left of *bb* and the *Hw* effect were present. From these results it seemed reasonable to conclude that y^{X2} was a deficiency extending into the *bb* region from the left and that mal^{12} was a deficiency extending into the *bb* region from the right (Figure 1).

SELECTION OF RECOMBINANT OFFSPRING

The observation that the y^{X2}/mal^{12} female heterozygote was viable and fertile with a *bb* phenotype provided a tool with which to select for exchanges at the *bb* locus. Reference to Figure 1 will make it clear that when y^{X2} sc^8 B/sc^8 B mal^{12} females are crossed to *In(1)dl-49, y* sn^{X2} bb^l/B^SY males, no viable male offspring are produced in the absence of an exchange at *bb*. Neither the *l(1)J1* of the y^{X2} chromosome nor the loci to the right of *l(1)20* of the mal^{12} chromosome are covered by B^SY. However, an exchange at *bb* which produces a recombinant chromosome carrying the normal portion of the mal^{12} chromosome to the left of *bb* and the normal portion of the y^{X2} chromosome to the right of *bb* will yield viable male offspring. Such males will be phenotypically *bb+* because of the *bb+* carried by B^SY. All nonrecombinant, regular female offspring as well as those produced by an exchange at *bb*, of the type just described, could yield viable female offspring which would be *bb* in appearance. Any *bb+* females produced by maternal or paternal nondisjunction would also carry a B^SY which gives a narrow shaped eye. Thus, they would be distinguishable from the regular females heterozygous for an *X*-borne *B* which gives them a wider appearing eye.

Virgin females, up to three days of age, and males were distributed among 124 numbered half-pint bottles so that each culture contained about 30 females and 30 to 40 males. Parents from this first set were transferred twice to fresh cultures at intervals that varied from 4 to 6 days. However, by the end of 8 to 12 days most of the parental females had become mired in the medium, so that the second and third sets of bottles yielded progressively fewer offspring. All cultures were incubated at 22°C to 26°C. Counts of the number of adult progeny were made only for the first set of bottles.

The collection of male offspring was facilitated because of the following considerations:

1) All male survivors are phenotypically *bb+*, and therefore they develop at a faster rate than their *bb* appearing sisters. (There were only 4 nondisjunctionally produced females.)—2) The frequency of recombinants was higher among the earlier eggs laid (see below).—3) All male survivors were *y+* in contrast to most of their sisters. Only about 6% of the *bb* females that

eclosed were y^+ and these tended to come out later than their *y* sisters.—4) All male survivors have the narrow bar eye characteristic of B^S in contrast to the wide bar of their heterozygous *B* sisters.

Because of the above factors, and despite the extended egg laying period, males were usually among the most developmentally advanced progeny in a culture. Often they were recognized in the late pupal stage and picked off the walls of the bottles or plucked from the surface of the medium before any of the progeny had eclosed. Otherwise, cultures were looked at in the conventional manner in that all progeny were examined after etherization. Each male was initially mated to 3 different virgin females in succession: 1) An attached-*X* female with an ordinary *Y*; 2) sc^8 *B* mal^{12}/*In(1)dl-49* *v* sn^{X2} mal^2; and 3) An attached-*X* female with a B^SY. The third cross established a balanced stock which maintained the recombinant chromosome in the male line. Subsequent tests were made by using individuals drawn from this stock. In addition to the males a number of females that exhibited weaker *bb* phenotypes—i.e., more normal in bristle length and abdominal effects than their sisters, were selected for further tests. Such females were mated to *In(1)dl-49*, *y* sn^{X2} bb^l/B^SY males to establish a balanced stock.

RESULTS

An examination of the offspring in six out of the 124 sets of bottles showed that an error must have been made in the selection of the proper parents. From the remaining 118 bottles of each brood, 60 males were recovered: Brood I–53, Brood II–6, Brood III–1. There were 35 single occurrences, 8 bottles with 2 males and 3 bottles with 3 males. Fifty-five males proved to be fertile. All offspring were counted in the first 69 bottles of the first brood. Of 1707 flies, there were 1585 *y* females, 94 y^+ females and 28 males. All bottles had large numbers of females that died in the late pupal stages; many were unable to emerge from open pupal cases.

Each of the 55 fertile males, upon crossing to the attached-*X* females with an ordinary *Y*, proved to produce only y^+ ac^+ *B* sons, and in numbers at least equal to their sisters. Therefore, each recombinant *X* was lethal-free, and the exchange that produced the males must have taken place at least to the right of the *ac* locus and to the left of all the lethal loci included in mal^{12}. In the tables and text below, recombinant chromosomes will be designated as sc^8 *B* bb^R or simply bb^R.

Classification of recombinant chromosomes: The cross of each original male to sc^8 *B* mal^{12}/*In(1)dl-49*, *v* sn^{X2} mal^2 females (test cross 1, Table 1) was used as a crude screen in a first attempt to differentiate among recombinant chromosomes. The matings were made as soon as each male became available for testing. In each of the subsequent tester crosses in which a number of recombinant lines were compared with one another and/or a parental chromosome, all crosses were done simultaneously so that they were subject to the same temperature fluctuations, usually 22°C to 26°C, during the incubation period. Moreover, single flies from recombinant lines were crossed in vials to one or more tester individuals and the parents were always transferred to fresh vials after three or four days so that the counts of offspring given in the tables represent totals from at least two vials. Often each test was done in duplicate. In a test which yielded a viable *bb* class the following comparisons were made:

1) In each cross the bristle length and degree of etching of abdominal tergites was noted for the *bb* class. In some tests each culture was assigned a letter grade

TABLE 1

Test crosses of recombinant chromosomes

Test cross	Recombinant chromosome parent	Tester stock parent	Tester *bb* mutant	Table
1	$\frac{sc^8\ B\ bb^R}{B^SY}$	$\frac{Df(1)mal^{12},\ sc^8\ B\ mal^{12}}{In(1)dl\text{-}49,\ sn^{X2}\ v\ mal^2}$	mal^{12}	2
2A 2B	$\frac{sc^8\ B\ bb^R}{B^SY}$	$\frac{y\ bb^{l158}}{FM6}$	bb^{l158}	2,3,4
3	$\frac{sc^8\ B\ bb^R}{B^SY}$	$\frac{sc^8\ B\ bb^{l1}}{In(1)dl\text{-}49,\ sn^{X2}\ v\ mal^2}$	bb^{l1}	4
4	$\frac{sc^8\ B\ bb^R}{In(1)dl\text{-}49+B^{M1},\ y\ ac\ pn\ v\ B^{M1*}}$	$\frac{sc^8\ B\ bb^R}{B^SY}$	various recombinant *bb* mutants	3,4
5	$\frac{sc^8\ B\ bb^R}{In(1)dl\text{-}49+B^{M1},\ y\ ac\ pn\ v\ B^{M1*}}$	$\frac{In(1)dl\text{-}49+B^{M1},\ y\ ac\ pn\ v\ B^{M1*}}{Y^{bb-}}$	Y^{bb-}	3,4
6	$\frac{sc^8\ B\ bb^R}{In(1)dl\text{-}49+B^{M1},\ y\ ac\ pn\ v\ B^{M1*}}$	$\frac{Y^SX\cdot Y^L,\ In(1)EN,\ Y^S\ y\ B\cdot Y^L}{0}$	nullo *Y* = 0	3,4

* This chromosome actually carries a weak *bb* mutant which was not used to compare *bb* phenotypes of different recombinant lines.

usually from A to D. The A indicated the weakest *bb* effect, the longest bristles, and D indicated a severe *bb* effect, the shortest bristles. In some cultures there were sufficient differences in the appearance of the flies to assign two letter grades. In general the lines with the more severe bristle effects had the more extreme

abdominal effects. Some lines with bristle sizes that were judged to be about the same, differed in their abdominal effects.—2) For each cross the viability of the *bb* class was determined by taking the ratio of the *bb* offspring to the bb^+ offspring of the same sex.

The assignment of a letter grade to a viable *bb* class was primarily for the purpose of making comparisons between the different recombinant chromosomes or between them and the parental chromosomes that were crossed to the same tester stock and examined at the same time. Where tests were done at different times, particular letter grades are not necessarily equivalent. For test cross 1, slightly different standards were used to assign letter grades. As a consequence, there was some overlapping of classes that complicated comparisons between these results and those of other testers.

Another complicating factor that must be considered when comparing crosses made at different times is the fact that the expressivity and viability of *bb* is temperature dependent. According to BRIDGES and BREHME (1944) "low temperature makes *bb* more extreme but also less viable." Although the incubation temperature in the test crosses usually varied between 22°C and 26°C, the results below were such as to indicate that this temperature spread was usually without significant effect upon the relatively crude distinctions that were being made.

Crosses in which the *bb* class failed to eclose were separated into two groups. In some crosses members of the missing adult class were present in the form of individuals whose development was not arrested until a late pupal stage in which sex, body color and eye shape as well as their very short bristles were clearly discernable under the binocular microscope. In the tables such lethal classes are marked with an asterisk. In other crosses such pupae were absent. Reference to the crosses listed in Table 1 will show how the above non-*bb* characteristics facilitated the discrimination between *bb* and bb^+ pupae.

Test cross 1 (Tables 1 and 2) revealed a wide spectrum of bristle sizes, abdominal effects and viabilities for the bb^R/mal^{12} class. These ranged from lethal to near normal phenotypes. Additional crosses (Table 1) were undertaken in an attempt to establish two points: 1) The phenotypic differences noted in the various lines represented stable transmissible changes. 2a) Among the weak recombinant lines, some had longer bristles than the weaker of the two parental *bb* chromosomes, y^{X2}. 2b) Among the recombinant lines with strong *bb* effects, some were more severe than the stronger of the two parental *bb* chromosomes, mal^{12}. Evidence in favor of both of these propositions was obtained from crosses with mutants at both ends of the spectrum of effects seen by cross 1.

In the test crosses, *bb* lethal *X* chromosomes, Y^{bb-} chromosomes or the absence of a *Y* were used for the most part. These crosses are listed in Table 1 and require some comment. In cross 4, the lethal tester chromosome was one of the recombinant chromosomes obtained from the initial recombination experiment and designated in the tables as bb^{1l}. The chromosome $In(1)dl\text{-}49{+}B^{M1}$, $y\ ac\ pn\ v\ B^{M1}$ used in crosses 4, 5, and 6 actually carries a weak *bb* mutant. Since this chromosome came from a stock which permitted it to exist as a fertile homozygous female, and therefore is subject to the very exchange processes being demonstrated in

this paper, no attempt was made to characterize the *bb* phenotype of the sc^{8} *B* bb^{R}/ *In(1)dl-49*+B^{M1}, *y ac pn v* B^{M1} female offspring in cross 4.

Table 2 lists the results of test crosses 1 and 2B, the only two crosses in which almost all (52 of the original 55) of the recombinant chromosomes were ex-

TABLE 2

Results of test crosses of recombinant lines

Line Number and *bb* Phenotype of bb^{R}/mal^{12}	Cross 1: Viability = bb^{R}/mal^{12} : bb^{R}/+ Tested 12-66	Cross 1: Ratio	Cross 2B: *bb* Phenotype of bb^{R}/bb^{l158}	Cross 2B: Viability = bb^{R}/bb^{l158} : bb^{R}/+ Tested 7-67	Cross 2B: Ratio
1L	0:42	0	L	0:74	0
2L	0:8	0	L	0:144	0
3L	0:34	0	L	0:54	0
4L*	0:36	0	L	0:43	0
5D	4:55	.07	L	0:33	0
6D	3:44	.07	L	0:123	0
7D	1:10	.10	L	0:86	0
9D	7:52	.13	L	0:46	0
10D	16:47	.34	L	0:115	0
11D	15:38	.39	L	0:107	0
12D	14:33	.42	L	0:36	0
13D	25:52	.48	L	0:130	0
14D	30:54	.56	L	0:45	0
15D	42:57	.74	L	0:49	0
21C	29:53	.55	L	0:109	0
22C	19:29	.66	L	0:106	0
32B	44:64	.69	L	0:114	0
8D	2:15	.13	L*	0:41	0
18C	21:46	.46	L*	0:71	0
31B	10:18	.55	L*	0:75	0
30B	11:23	.48	L*	0:78	0
25C	36:36	1.00	L*	0:87	0
36B	23:29	.80	L*	0:101	0
16C	4:29	.14	L*	0:57	0
37B	40:42	.95	D	1:38	.03
28C	54:47	1.15	D	3:114	.03
17C	12:46	.26	D	4:86	.05
24C	32:34	.94	D	5:100	.05
26C	19:19	1.00	D	5:92	.05
20C	17:34	.50	D	4:68	.06
35B	31:41	.76	D	12:106	.11
27C	29:28	1.00	D	8:61	.13
29B	17:39	.44	D	13:59	.22
47A	21:28	.75	D	21:59	.36
23C	38:42	.90	D	33:58	.57
43A	33:50	.66	D	36:35	1.00
19C	36:72	.50	D	27:52	.52†
. .	. . . .	. .	*	0:61	0 †
40AB	45:65	.69	CD	52:98	.57

TABLE 2—Continued

	Cross 1		Cross 2B		
Line Number and *bb* Phenotype of $\frac{bb^R}{mal^{12}}$	Viability = $\frac{bb^R}{mal^{12}} : \frac{bb^R}{+}$ Tested 12-66	Ratio	*bb* Phenotype of $\frac{bb^R}{bb^{l158}}$	Viability = $\frac{bb^R}{bb^{l158}} : \frac{bb^R}{+}$ Tested 7-67	Ratio
42A	39:60	.65	CD	48:94	.51
41A	30:61	.49	CD	56:59	.95
33B	21:30	.70	CD	11:54	.20
49A	15:17	.88	C	17:27	.63
48A	38:50	.76	C	32:48	.67
39AB	26:57	.46	C	82:66	1.24
45A	16:22	.73	C	70:87	.80
44A	36:50	.72	C	31:41	.76
38B	31:30	1.00	C	46:68	.68
50A	21:23	.91	B	33:30	1.00
51A	30:32	.94	AB	36:52	.70
46A	27:37	.73	AB	35:48	.73
34B	30:40	.75	AB	86:85	1.00
52AA	39:52	.75	AB	31:50	.62

See Table 1 for complete genotypes. See text for full meaning of letter grades: L=lethal;
* = late *bb* pupae present; A→D=increasing severity of viable *bb* phenotype;
† Results from duplicate crosses—see text.

amined. From the results of cross 1 the various lines were grouped into eight phenotypic categories indicated by a letter grade. Within each nonlethal category the lines were ranked according to viability and a number was assigned to each line. The combination of number and letter grade is used in column 1 of Tables 2, 3 and 4, and in the text to identify each line. Since test 2B was executed with greater care than test 1, the phenotypic distinctions that were observed are considered to be more reliable. Consequently, the lines in Table 2 are grouped according to the results of cross 2B. The ranking within some of the phenotypic categories (CD, C, and AB) is based upon minor visible differences and, with the one exception noted in the following paragraph, no further use is made of these differences.

The essential observation for both tester crosses is that each produced a broad spectrum of phenotypes. At one end there were lines of complete lethals with no evidence of a *bb* class surviving to a late pupal stage. At the other end there was at least one line, 52AA, in which the *bb* class of test cross 1 consisted of flies with normal abdomens and bristles that appeared to be as long as those of their non-bobbed sisters. However, the bristles of these flies were visibly finer. In cross 2B line 52AA yielded *bb* flies similar to those just described, but there were also *bb* flies with bristles that were slightly shorter than those of their non-*bb* sisters and with slightly etched abdomens. Although the flies of line 52AA in cross 2B were graded AB with respect to bristle phenotype, they differed from some of the flies in the three other lines graded AB which had crossed posterior scutellar bristles and abdomens that were etched to a greater degree.

If we look at the lines that were lethal in cross 2B, and therefore less subject to the reservations associated with the comparisons of viable classes when different test crosses such as these were not done simultaneously, we see thirteen of the seventeen lines that produced lethal cultures without *bb* pupae in cross 2B produced viable offspring in cross 1. One of the remaining 4 lines that yielded lethal cultures in cross 1, as well as cross 2B, had *bb* pupae present in cross 1. All of the seven lines that produced lethal cultures with *bb* pupae in cross 2B produced viable offspring in cross 1. (However, see Table 4 for additional results with line 16C.) This less severe effect, in 21 out of 24 cases, with the weaker of the two testers, is just what was to have been expected on the basis of the known dosage effect of the hypomorphic *bb* alleles (STERN 1929).

The same tendency, a less severe effect in crosses with the weaker of the two testers, is noted among the lines that were viable in both test crosses. But in these cases comparisons are more hazardous. Aside from the absence of cross checks and a less than optimal incubation temperature range, there were differences in the method of classification such that the intermediate grades B and C were not exactly equivalent in both crosses. In cross 1 some of the flies characterized as D would have been classified as C by the standard used in cross 2B, and some of the flies characterized as C would have been classified as B by the standard used in cross 2B. However, an examination of Table 2 will show that when these differences in classification concerning the intermediate grades are taken into account, they actually reinforce the generalization that recombinant lines display weaker *bb* phenotypes with mal^{12} than with bb^{l158}. One possible exception was line 34B in which there was an apparent weaker phenotype in the cross to bb^{l158}. We may conclude from these results that during the interval between the two test crosses, the genetic basis for the *bb* phenotype remained stable while the *X* chromosome was maintained in the male line. Of the 52 lines tested in cross 2B, duplicate matings were made for 31 lines. In only one case was there a discrepant result. For line 19C, the two vials of one cross gave ratios of 14 *bb*:30 bb^+ and 13 *bb*:22 bb^+ at the same time that the two vials of the duplicate cross gave 0 *bb*:29 bb^+ and 0 *bb*:32 bb^+. Both vials in the latter cross had pupae that appeared *bb* but adults failed to emerge even after 21 days of incubation.

Table 3 shows the results obtained from crosses involving a number of lines which had shown the weakest *bb* phenotypes in test cross 1. The most instructive crosses are 2A and its repetition six months later, 2B, which produced substantially similar results. In addition, 2A compared parental chromosomes with recombinant chromosomes. There are 4 lines which appeared to yield weaker *bb* phenotypes than y^{X2}, the weaker of the two parental chromosomes. This visual impression was confirmed by micrometer measurements that were taken of bristle lengths in three representative flies from each of the 5 cultures. The relative sizes of the bristles were expressed as the ratio of the length of the posterior dorsocentral bristles to the distance from the base of the posterior dorsocentrals to the base of the scutellum. The values for each line were as follows: y^{X2}=0.70, 46A=0.92, 50A=0.90, 51A=0.93, 52AA=1.03.

Test crosses 4, 5 and 6 were done simultaneously and the incubation tempera-

TABLE 3

Results of test crosses of weak bb *lines*

Test cross	2A		2B		4		5	6
Line Number and *bb* phenotype of $\frac{bb^R}{mal^{12}}$	*bb* phenotype of $\frac{bb^R}{bb^{l158}}$	Viability = $\frac{bb^R}{bb^{l158}}:\frac{bb^R}{+}$	*bb* phenotype of $\frac{bb^R}{bb^{l158}}$	Viability = $\frac{bb^R}{bb^{l158}}:\frac{bb^R}{+}$	*bb* phenotype of $\frac{bb^R}{bb^R}$	Viability = $\frac{bb^R}{bb^R}:\frac{bb^R}{+}$	Viability = $\frac{bb^R}{Y^{bb-}}:\frac{+}{Y^{bb-}}$	Viability = $\frac{bb^R}{0}:\frac{+}{0}$
Tested 12-66	Tested 1-67		Tested 7-67		Tested 3-67		Tested 3-67	Tested 3-67
43A	C	40:69	D	36:35	..	....	....	21:42
44A	..	....	C	31:41	..	....	58:61	40:37
46A	B	67:72	AB	35:48	C	11:6	51:51	43:5
47A	CD	55:102	D	21:59	A	11:42	15:20	25:52
48A	C	51:61	C	32:48	+	69:78	53:45	29:53
49A	C	68:70	C	17:27	..	....	3:7	....
50A	B	70:61	B	30:30	+	32:40	27:20	54:39
51A	B	80:74	AB	36:52	+	36:42	79:66	32:26
52AA	A	92:71	AB	31:50	..	....	....	....
y^{X2}	C	100:102	..	....	..	....	....	....

See Table 1 for complete genotypes. See text for full meaning of letter grades: + = wild type; A→D increasing severity of *bb* phenotype.

ture was maintained at 25°C. Crosses 5, and 6 showed that these weak lines yielded male viable bb^R/Y^{bb-} and $bb^R/0$ offspring. The high viability of $sc^8B\ bb/0$ males in cross 6 stands in opposition to the report of HESS (1962) that $sc^8/0$ males were relatively inviable because sc^8 was a *Y*-suppressed lethal variegated position effect. In the four stocks tested by HESS the percentage of $sc^8/0$ males as compared to their attached-*X*/*Y* sisters varied from 0.19 to 11%. In cross 4 the phenotype of bb^R/bb^R females in the three lines classified as wild type, and line 47 classified as A, was in accord with the expected additive effects of *bb* mutants. But the classification of line 46 as C was unexpected in view of the weak *bb* phenotype shown in $46A/bb^{l158}$ females (tests 2A and 2B).

Table 4 gives the results from crosses of some of the lines which had shown the most extreme *bb* phenotypes in test cross 1. Once again the results of crosses 2A and 2B done six months apart are very similar. There is little question that at least all but the bottom three recombinant lines are more extreme than mal^{12}.

In crosses 5 and 6 these extreme *bb* lines were lethal or yielded very few bb^R/Y^{bb-} and $bb^R/0$ males. The results are consistent with those from the lethal *bb* testers in females, and may be contrasted with the viable bb^R males of Table 3. Similar results are found among the offspring of cross 4 in which bb^R/bb^R females were usually absent.

Calculation of exchange frequency: In order to determine the frequency with which *bb* locus exchanges take place between y^{X2} and mal^{12} chromosomes, heterozygous females up to 12 hours old in experiment 1 and up to 4 days old in experiment 2 were used in two crosses illustrated in Table 5. Among the female offspring of experiment 1, one out of the two nonrecombinant classes survives

TABLE 4

Test crosses of strong bb *lines*

Line Number and *bb* phenotype of $\frac{bb^R}{mal^{12}}$	2A	2B	3	4	5	6
	Viability = $\frac{bb^R}{bb^{l158}} : \frac{bb^R}{+}$		Viability = $\frac{bb^R}{bb^{l1}} : \frac{bb^R}{+}$	Viability = $\frac{bb^R}{bb^R} : \frac{bb^R}{+}$	Viability = $\frac{bb^R}{Y^{bb-}} : \frac{+}{Y^{bb-}}$	Viability = $\frac{bb^R}{0} : \frac{+}{0}$
	Tested 1-67	Tested 7-67	Tested 5-67	Tested 3-67	Tested 3-67	Tested 3-67
1L	0:139	0:74	0:194	...	...	...
2L	....	0:144	0:147	0:36	...	0:81
3L	0:148	0:54	0:145	...	...	...
4L	0:155	0:43	0:115	0:59	0:32	0:48
5D	0:80	0:33	0:95	...	0:23	0:22
6D	0:147	0:123	0:81	0:48	0:34	0:20
7D	....	0:86	0:137	0:30	0:56	0:76
9D	0:136	0:46	0:69	0:79	0:47	0:4
10D	....	0:115	0:67	2:46	0:78	0:65
11D	....	0:107	0:82	...	0:74	...
12D	....	0:36	0:76	...	0:95	...
13D	....	0:130	0:41	*0:36	0:34	0:47
14D	....	0:45	0:77	0:21	0:27	0:49
21C	....	0:109	0:148	*0:46	0:47	0:35
8D	*0:67	*0:41	*0:165	...	...	...
16C	3:147	*0:57	1:87	...	4:52	...
17C	....	4:86	*0:61	...	3:59	...
mal^{12}	5:160	...	3:274	...	...	...

See Table 1 for complete genotypes. See text for full meaning of letter grades: L = lethal; * = late *bb* pupae present; C→D = increasing severity of *bb* phenotype. In all crosses *bb* survivors are D.

as phenotypically y heterozygous B females and one of the two recombinant classes survives as y^+ heterozygous B females. Among the males, the only survivors belong to this same recombinant class. Since twice as many members of recombinant classes survive as nonrecombinant classes, the nonrecombinants are multiplied by 2 to calculate the percent exchanges. The second experiment takes advantage of the observation that females, heterozygous for *su(f)* and a deficiency such as *mal*^{12} that removes $su(f)^+$, display a distinctive eye, bristle and wing phenotype (SCHALET 1968). Therefore in experiment 2, both of the nonrecombinant and recombinant female classes can be recognized among surviving female progeny. Again the only viable males belong to one of the recombinant classes. Since there are three viable recombinant classes and only two nonrecombinant classes, the nonrecombinants are multiplied by 3/2 to calculate the percent exchanges. The results confirm the impression gained from the original experiment that recombination is higher among the earliest eggs laid.

Note that the frequency of exchanges found in the sc^8 inversion is at least one order of magnitude greater than in an uninverted chromosome where the *bb* locus is located proximally and close to the centromere. A maximum estimate for these

TABLE 5

Frequency of exchanges within bb *locus of* In(1)sc⁸ *chromosome*

Experiment 1	$\frac{Df(1)mal^{12}, sc^8\ B\ mal^{12}}{Df(1)y^{X2}, y^{X2}\ sc^8\ B} \times \frac{y\ v\ f\ mal^2\ l(1)20}{B^SY}$					
Days eggs laid	1–4			5–7		
	Parental female	Exchange male	Exchange female	Parental female	Exchange male	Exchange female
F_1*	733	6	4	831	0	0
Percent exchanges	$\frac{10}{(733 \times 2) + 10} \times 100 = 0.68$			$\frac{0}{(831 \times 2)} \times 100 = 0.0$		

Experiment 2	$\frac{Df(1)mal^{12}, sc^8\ B\ mal^{12}}{Df(1)y^{X2}, y^{X2}\ sc^8\ B} \times \frac{y\ v\ f\ mal^1\ su(f)}{Y}$									
Days eggs laid	1–3					4–7				
	Parental female		Exchange male	Exchange female		Parental female		Exchange male	Exchange female	
	(y)	(y^+)		(y)	(y^+)	(y)	(y^+)		(y)	(y^+)
F_1*	242	204	2	3	2	266	248	0	0	2
Percent exchanges	$\frac{7}{(446 \times 3/2) + 7} \times 100 = 1.04$					$\frac{2}{(514 \times 3/2) + 2} \times 100 = 0.26$				
Total percent exchanges	$\frac{17}{2152} \times 100 = 0.79$					$\frac{2}{2435} \times 100 = 0.08$				

* See text for additional description of F_1 classes and explanation for multiplying by 2 or 3/2 to calculate exchange frequency.

exchanges in the normal chromosomal position, but with an indeterminate number of *bb* genes in each parental chromosome, is based on the spontaneous frequency of exchanges between *su(f)* and the y^+ marker of $Dp(1;1)sc^{V1}$ located to the right of the centromere. Single exchanges within *bb* would have been included in this measurement. HERSKOWITZ, SCHALET and REUTER (1962) found 0.026% exchanges, (3/11,720) between *su(f)* and y^+ for eggs laid during the first week of oviposition. The overall frequency for a period that extended up to almost six weeks was about the same (6/24,206). The total number of genes for both *bb* loci in the sc^8 inversion experiment reported in this paper was less than one half the number that would have been present in two wild-type *bb* loci. Therefore, the exchange frequency of 0.4% (Table 5), may represent an underestimate and provides an additional reason to believe there is a greater than 10-fold increase between a distally and proximally located *bb* locus. Another complication lies in the extent to which the frequency of exchanges would be affected by the relative lengths of the parental loci.

Tests of females with weak bb *phenotypes:* In addition to the 60 males that were found in the initial recombination experiment, a number of y^+ and y females which were judged to show a weaker *bb* phenotype than their respective y^+ and y sisters transmitted this phenotype to their daughters. Of 13 y^+ females

that bred well enough to give sufficient offspring for an evaluation, 8 proved to be male-lethal while the remaining 5 were male-viable and, therefore, must have been recombinants. However, the *bb* phenotype of the female offspring in each line, while weaker than that of the mal^{12}/bb^{l} heterozygote in comparable crosses, was at best no weaker than the y^{X2}/bb^{l} heterozygote.

There were 6 *y* females that transmitted a weak *bb* phenotype to their daughters. Five of the alleles appeared to be about equal in strength and in one case the *bb* was unquestionably weaker than in the others. In each case the weak *bb* phenotype was transmitted to all the daughters and the chromosome carrying the weak *bb* mutant proved to be lethal in the male offspring. Subsequent testing of the six chromosomes demonstrated that: 1) the male lethality was covered by a $y^{+}Y$ chromosome, 2) $su(f)^{+}$ was still present, 3) the weak *bb* phenotype was generally maintained in stock cultures and in outcrosses to tester chromosomes. The single exception involved one outcross of the chromosome with the *bb* allele that was weaker than the others. Here one of two duplicate vials produced a weak *bb* phenotype like that of the five other lines.

DISCUSSION

We have assumed that the *bb* phenotype of the parental chromosomes came about in each case as the result of a partial loss of the *bb* locus induced as part of a larger deficiency that included non-*bb* chromosomal material to the right or to the left of *bb* locus. Although this can be regarded as the most probable explanation for the *bb* phenotype, we might also consider the possibility that the *bb* mutant was present prior to the irradiation or even that it was only coincidentally induced at the same time as the y^{X2} or mal^{12} deficiencies. On this basis an intact segment of the heterochromatic region to one side of the *bb* locus that lacked conventional genetic markers would separate the *bb* lesion from an outside deficiency. Reference to COOPER's (1959; Figure 56) cytogenetic map of the heterochromatic region of the *X* shows a considerable chromosomal (mitotic or polytene *X*) segment between *bb* and the position of the closest conventional genetic markers which have been used here to define the extent of the outside deficiencies. This is seen in Figure 1 where the significant elements of COOPER's cytogenetic map are redrawn for a sc^{8} inversion chromosome. The pertinent markers can be no closer than the following positions: *ac* has to be placed distal to the sc^{8} breakpoint and *su(f)* has to be proximal to the sc^{4} breakpoint.

Even if we concede the unlikely proposition that each parental chromosome contains two discontiguous deficiencies, regular or oblique exchanges that are limited to the heterochromatic segments on either side of *bb* fail to account for a large proportion of the recombinant chromosomes observed. Regular exchanges outside of *bb* would generate recombinants with *bb* phenotypes identical to y^{X2} or mal^{12}. Oblique exchanges would generate recombinants completely deficient for the *bb* locus or with two mutant loci in tandem. The phenotype of the latter would be weaker than y^{X2}. An examination of Tables 2 and 4, crosses 2A and 2B, shows that at the least, the first 17 listed in cross 2B, are more extreme than mal^{12}.

If all of these were completely deficient for *bb*, then they all would be expected to give substantially identical results in test cross 1 and this is clearly not the case. For certain, at least lines 1L and 3L differ from the others, and if these are completely deficient for *bb*, the remainder cannot be completely deficient. The results of cross 4 in Table 4, in which the recombinant chromosomes are rendered homozygous, show that the *bb* locus retains some activity in lines 10D, 13D, and 21C. Consequently, at least 14 of the lines that are more extreme than mal^{12} cannot be explained as an oblique exchange outside of *bb*. An examination of the last five lines in crosses 1 and 2B (Table 2), all of which are weaker than y^{X2}, reveals that line 52AA may be distinguished from the other four. Only line 52AA could be explained as an oblique exchange outside of *bb*. Furthermore, no exchange outside of *bb* would lead to any phenotype intermediate between y^{X2} and mal^{12}. We made no direct attempt to distinguish clearly intermediate classes because a nonquantitative visual separation of such classes on the basis of bristle size was not readily apparent. However, at least the CD lines found in the middle of the distribution shown by cross 2B suggest the existence of intermediate classes. Therefore, at least 1/3 of all recombinants would have to be exchanges within *bb*. If we propose that only one of the parental chromosomes contains the two nearby but discontiguous deficiencies and the other chromosome contains a single deficiency that includes a partial loss of *bb*, then exchanges outside of *bb* produce only two different phenotypic classes. The alternative possibilities are a class completely deficient for *bb* and a y^{X2} class, or a class completely deficient for *bb* and a mal^{12} class. Under these circumstances, the majority of recombinants, perhaps as many as 3/4, would have to be exchanges within *bb*.

Since the genetic tests strongly indicate that each parental *bb* mutant is associated with a lesion that simultaneously eliminated non-*bb* chromosomal material, and since, on an even more complex but less likely interpretation of the structural changes present in and adjoining the *bb* locus, many of the recombinant chromosomes display *bb* phenotypes that can only be accounted for by exchanges within *bb*, the data are consistent with the hypothesis that all recombinants are derived from exchanges within a tandemly-redundant *bb* locus, and that the severity of the *bb* phenotype is more or less related to the level of redundancy. Furthermore, the use of the term, gene, for the *bb* locus should be understood to mean an alternating pair of genes for 28S and 18S r-RNA components (QUAGLIAROTTI and RITOSSA 1968). Exchanges within *bb* produce chromosomes with a variety of altered expressions of the *bb* phenotype and these phenotypes are usually transmitted in a stable fashion. In view of the relatively crude and limited number of tests used to distinguish differences among the recombinant chromosomes, it is not clear how many phenotypically distinct classes were produced. By a conservative reckoning there were at least seven classes that were separable from one another. These included at least two different classes that were more extreme than mal^{12} and at least two different classes that were weaker than y^{X2}. Given the particular function ascribed to the *bb* locus, there seems little doubt that by conventional procedures some of the more extreme lethal lines could be further separated into lines in which development is arrested in egg or larval stages.

Among the nonlethal lines a more rigorous quantitative measurement of viability, bristle length or developmental time could be used to differentiate lines not readily separable upon visual inspection. By the use of such techniques the ostensibly much larger number of distinct alleles apparently indicated by both test crosses of Table 2 could be confirmed. Since the total number of *bb* genes in the parental females was probably at least 100, it is theoretically possible that each recombinant line has a different number of genes and, therefore, each line might be distinguishable by the proper tests.

Observed distribution of bb *phenotypes in recombinant chromosomes:* The *bb* phenotype of each of the 52 recombinant chromosomes tested in cross 2B can be compared with the *bb* phenotypes shown by the parental chromosomes in cross 2A. If a comparison is made of the number of lines that simulate the phenotype of each parent, and the number of lines that appear weaker than y^{X2} is compared to the number of lines that appear more extreme than mal^{12}, then there appears to be an excess of the more severe phenotype in each case. Test crosses 2A and 2B provide a minimum estimate of the number of lines weaker than y^{X2}, (5), and the number of lines more extreme than mal^{12}, (17). The remaining lines in cross 2B of Table 2 approximated one or the other of the parental classes or appeared to be intermediate in phenotype. For those that simulated one of the parental types, the tests were not sufficiently sensitive to determine whether a particular line was identical to it or fell to one or the other side of it in phenotypic grade. Except for the 4 lines in which bristles of both grades C and D were observed, it was clear which parental type a particular line more closely resembled. The 7 lethal lines with late *bb* pupae can be combined with at least the next 9 lines, if not all 12 lines, that only yielded D grade offspring. The total of at least 16 lines that resembled the mal^{12} parental class exceeds the total of 6 grade C lines that resembled the y^{X2} parental class and provides additional evidence for a preponderance of the relatively more severe phenotypes.

Coming back to the two broad classes at the ends of the spectrum of phenotypes, it may be recollected that two different aspects of the *bb* phenotype were used to distinguish the more extreme and weaker lines. The criterion for placing a line in the extreme category was the absence of late pupae belonging to the *bb* class. This appraisal of a phenotypic change was somewhat less subjective than the visual comparison of bristle sizes usually used to separate the weaker lines from the parental y^{X2}. Neither method of measurement indicates the actual change in gene number but only the direction of the change in each case. Whatever the actual change in number, the amplitude for a decrease below the number in mal^{12} is equal to the amplitude for an increase above the number in y^{X2}. Suppose mal^{12} contained about 40 genes and y^{X2} contained about 70 genes. From a single interhomologue exchange a more extreme allele can have from 1–39 genes fewer than mal^{12} and a weaker allele can have from 1–39 genes more than y^{X2}, i.e. the total number of genes in a recombinant can vary from 1 to 109. If the chance of losing a particular number of genes were equal to the chance of gaining the same number of genes, then the total probability of losing genes would be equal to the total probability of gaining genes. Therefore, what is seen as a more extreme phenotype

could have been the loss of at least a certain number of genes. What is seen as a weaker phenotype could have required a gain in the number of genes that was larger than the number needed to be lost in order to produce an extreme phenotype. Under these circumstances there would be a larger number of lines with phenotypes more extreme than mal^{12} in comparison to the number of lines with phenotypes weaker than y^{X2}. But those recombinants with a phenotypically undetectable gain in the number of genes would appear among the y^{X2} parental-like class in numbers approximately equal to the difference between the more extreme lines and the weaker lines. This difference was 12. However, there were only 6 y^{X2} parental-like lines and these could have included recombinants with an unchanged number of genes and recombinants that had lost a number of genes too small to produce a phenotype detectably more extreme than y^{X2} by these tests. Consequently, the apparent excess of the more severe phenotypes, in this comparison of the two general classes at the ends of the spectrum of phenotypes, is not due to the fact that changes in gene dosage from a higher quantitative level can be less effective in producing a phenotypic change than equivalent absolute changes in gene dosage from a lower quantitative level (STERN 1929; see also MULLER 1932). The distribution of phenotypes may be related to the mechanism of pairing and exchange within the *bb* locus itself and/or peculiarities imposed upon the *bb* locus by the abnormal structural features of the parental chromosomes used in this system.

Since the parental chromosomes were *bb* to begin with, each must have lacked a normal complement of *bb* genes. The y^{X2}/mal^{12} heterozygote was also phenotypically *bb*, so that the total number of genes for both chromosomes was also less than 130 genes. Therefore, no interchromosomal exchange unaccompanied by an intrachromosomal exchange would have been capable of reconstituting a normal complement of *bb* genes. In fact, none of the 52 recombinant chromosomes was able to restore a bb^+ phenotype when tested against bb^{l158} which itself probably carried a small number of active genes.

Germ cell stage for interchromosomal exchanges: There is no evidence from these experiments to indicate whether *bb* exchanges were premeiotic or meiotic events. Of the 11 original cases of possible clustering, because of sterility, only 9 were available for testing. On the basis of the phenotypes exhibited in two different test crosses these were reduced to 3 possible clusters of 2. If gonial exchanges had taken place, the use of mass matings and the limited number of gametes sampled per parental females probably precluded the detection of nonrandomly distributed clusters of recombinants usually used as an index of premeiotic events. It can be estimated, that for the first egg laying period which yielded most of the non-recombinant and recombinant offspring, each female produced on the average about 1.7 eggs destined to become male zygotes carrying the y^+ marker. Spontaneous exchanges involving the major heterochromatic region of the *X* chromosome have been attributed to gonial events. Examples include detachment of attached-*X* chromosomes (COOPER 1945 and earlier references cited by him), and *X* chromosome exchanges in males (LINDSLEY 1955, 1958). At least some, but not all, of the exchanges studied by LINDSLEY that involved recombinations be-

tween $In(1)sc^8$ and the short arm of the Y or between the distal heterochromatic region of $In(1)sc^{8L}$ and the second heterochromatic arm of $In(1)EN^R$ in an $In(1)sc^{8L},EN^R$ chromosome are compatible with the concept that the exchanges took place within the *bb* loci known to be present in all three regions concerned.

Of interest too, is the experiment of WHITTINGHILL and HINTON (1950) that detected relatively rare spontaneous recombinants in the centromeric region of the second chromosome. These recombinants, attributed to oogonial events, showed a sharp decrease in frequency during the first week of egg laying. Since such a decrease had long been known for meiotic exchanges (BRIDGES 1927), the similar drop in frequency of *bb* exchanges noted in Table 5 does not provide any clue to the germinal stage at which exchanges occurred.

Origin of females with weak bb *phenotype:* The appearance of a transmissible weak *bb* phenotype in the 6 *y* females from the initial recombination experiment was not accompanied by an exchange of outside markers. These females came from five different bottle cultures. The two females that came from a single bottle showed different phenotypes. Since each parental female produced so few progeny, it is not possible to rule out the premeiotic origin or even the origin in an earlier generation of any or all of the chromosomes that yielded a *bb* phenotype weaker than y^{X2}. Slight phenotypic differences among the parental mal^{12}/y^{X2} heterozygotes were attributed to the effects of uncontrolled fluctuations in culture conditions. In any case it is probable that the altered *bb* loci arose by intrachromosomal exchanges in the manner proposed by PETERSON and LAUGHNAN (1963) for the Bar locus.

If the exceptional chromosomes were present at the start of the experiment, the question can be raised concerning the extent to which they would have been involved in exchanges between parental chromosomes. Since the six detected females were found among the offspring of about 3,500 female parents, they would have been present among the parents at a rate of about 1/580. There could have been present among the parental chromosomes of both types additional *bb* loci which had been otherwise altered and whose intermediate or severe phenotypic effect would have been ignored among the surviving offspring of the experiment. If there had been ten times as many parental chromosomes of both types that had changed, then about 1 in 30 parents would have carried a *bb* locus different from one of the original mutants. Therefore, on the average less than 2 of 52 recombinants listed in Table 2 would have a phenotype effected by any change in gene number that had occurred prior to the exchange between homologues in the recombination experiment.

Bearing of bb *experiments on "master-slave" model of gene structure:* Each of the parental *bb* mutants represents an example of a mutant in which one of the ends of the locus has been deleted. In both cases the locus has retained some degree of functional activity. Since the phenotype of the mal^{12}/y^{X2} heterozygote is *bb*, the deficiencies overlap one another. Consequently, it would appear that for the *bb* locus there is no unique point at which transcription is initiated. The retention of some activity by each mutant also has some bearing upon the "master-slave" interpretation of gene structure. CALLAN (1967) has made a proposal con-

cerning the organization of genetic units in the chromosomes of higher organisms in order to account for apparent contradictions between mutation and recombination data on the one hand and cytological and molecular evidence on the other. The former studies require only a single functional unit in a chromatid while the latter studies indicate that at least some functional units are serially repeated. As pertinent features of CALLAN's scheme and its elaboration by WHITEHOUSE (1967), each unit of genetic function would consist of a terminal unit or "master" gene sequence followed in the chromosome by serially repeated "slave" sequences. Interchromosomal recombination events are restricted to the "master" gene and only mutations in the "master" gene are of significance in germinal or somatic transmission. Accordingly, if an alteration in the specificity of a "master" gene by mutation or recombination occurs, the "slaves", or functional genes, must be corrected by matching with the "master" prior to transcription. It is this matching process that is supposed to produce the lateral lampbrush loops in the meiotic prophase chromosomes of amphibian oocytes. Since a typical loop occurs at the nucleolar locus, the structure of the locus is considered to be the same as other loci. The serial redundancy in the nucleolus organizer region of Drosophila (RITOSSA and SPIEGELMAN 1965) is cited by WHITEHOUSE as providing part of the evidence which is in direct conflict with recombination data that indicate genes are singular. However, the recombination data referred to by both CALLAN and WHITEHOUSE did not involve the nucleolus organizer region of any organism.

Identification of the *bb* locus with the nucleolus organizer region together with the data presented in this paper show no inconsistency between serial repetition and recombination. With respect to the magnitude of recombination and the products of the recombination process, the *bb* locus is not typical of other gene loci. The frequency of recombination at the *bb* locus, 4×10^{-3}, is about 45 times the frequency of recombination at rosy, 8.9×10^{-5} (CHOVNICK 1966), a locus which is a more typical example of a functional unit in Drosophila. That exchanges at *bb* occur throughout the length of the locus is indicated by the following consideration. If, in the sc^8 inversion chromosome, interhomologue exchange at *bb* is in principle equivalent to euchromatic crossing over, then the size of the locus in terms of its DNA content can be estimated in a crude fashion. RUDKIN (1965) has calculated that the *X* chromosome euchromatin contains 3×10^7 nucleotide pairs of DNA. Since the standard length of the euchromatic *X* is 66 map units for a normally ordered chromosome and roughly the same for a sc^8 chromosome, on the average there would be 4.5×10^5 nucleotide pairs/map unit and 0.4 map units (Table 5) would yield a value of 1.8×10^5 nucleotide pairs for *bb*. For the average value of 600/nucleotide pair, this is equivalent to a molecular weight of 1.1×10^8. This figure may be compared to the calculation given by RITOSSA and SPIEGELMAN (1965) that the wild-type haploid genome of Drosophila contains 1.6×10^8 daltons of DNA complementary to ribosomal RNA and therefore the DNA content of both strands would be 3.2×10^8. These figures, 1.1×10^8 and 3.2×10^8, show a remarkable agreement, especially since recombination at *bb* in our system would be limited by the less than wild-type length of the locus. On the "master-slave" model, recombination would have been restricted to the

length of the "master" gene alone. Because the mutation in the "master" gene of each parental chromosome is a deficiency this length would have to be less than the length of a single member of the 130-fold redundancy, that is less than 2.5×10^6. There is a more direct demonstration that, within this unit of genetic function represented by *bb*, recombination is not limited to exchanges between two parental "master" genes. In our initial recombination experiment such a limitation would have produced only a single non-parental type among recombinants instead of the variety of non-parental phenotypes that were regularly found as products of the recombination process. Finally, it is argued that the basis for a transmissible mutational change in a genetic unit of function resides exclusively in a single terminal sequence at one end of a repeated series. A deficiency that involved the partial loss of a terminal unit can account for one of the two parental mutants used in our experiments. Since the other parental mutant was produced by a deficiency that extended into the locus from the opposite end, it would have had to eliminate not only part of a terminal unit but also all of the "slaves." This should have completely inactivated the locus. Yet, both loci maintained some activity. In summary, both recombination and genetic data reject a "master-slave" structure. They are consonant with the model derived from molecular studies that the basis for dissimilar *bb* mutants is usually a difference in the number of genes in a serially repeated sequence and such differences can originate by interhomologue exchanges throughout the length of the locus.

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SUMMARY

A scheme is described that permits the selection of interhomologue exchanges within the distally located *bb* locus of a sc^8 inversion chromosome. Each recombinant chromosome carries a mutant allele of *bb* that generally remains stable when maintained in the male line. Some alleles are readily distinguishable from one another and the parental *bb* phenotypes. The wide range of phenotypes is consonant with the structure proposed for the *bb* locus by RITOSSA, ATWOOD and SPIEGELMAN. Accordingly, the *bb* locus consists of a linear array of tandemly repeated genes; and the various alleles, with different degrees of hypomorphism, have different numbers of genes generated by asymmetrical pairing and interhomologue exchange throughout the locus. The distribution of phenotypes among 52 recombinants suggests an excess of the more extreme alleles, but this distribution may not be primarily related to the process of pairing and exchange within *bb*. The frequency of exchanges is estimated at 0.8% for the first 3 or 4 days of oviposition but declines rapidly during the latter half of the first week so that the total for the first week is 0.4%. Since the total number of genes for both parental *bb* loci, and the influence of the relative lengths of both loci is not known, this frequency may be an underestimate. Among the progeny of a recombination experiment, female offspring appear that differ in their *bb* phenotype from the

parental chromosomes but are nonrecombinant for outside markers. The *bb* alleles of these chromosomes generally remain stable when maintained heterozygously in females that have *X* chromosome crossing over suppressed. These nonrecombinant *bb* alleles *may* have originated by intrachromosomal exchange within the germ cells of parental females. The deficient nature of *bb* in the parental chromosomes, the variety of *bb* phenotypes generated by interhomologue exchanges and the frequency of exchanges provide evidence against a "master-slave" structure for the *bb* locus.

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