GENETIC ANALYSIS OF THE ABERRANT BEHAVIOR OF AN *X*-CHROMOSOME DUPLICATION IN THE GERM LINE OF *DROSOPHILA MELANOGASTER* MALES[1]

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THE *X*-chromosome duplication *Dp(1;1)NBB-8*, a modified form of which is involved in these studies, was encountered in the course of investigations (PETERSON and LAUGHNAN 1961, 1963) on intrachromosomal recombination at the Bar locus in *Drosophila melanogaster* females. It was first identified in an exceptional f^+ *BB* os^+ son of a f^+ *B* os^+/f *B os* mother whose male and female progeny were being searched for exceptions that might indicate the occurrence of intrachromosomal crossing over. Subsequent genetic and cytological analyses indicated that the exceptional *BB* male carried a tandem, serial duplication of salivary regions 16A through 17E (one-tenth) of the *X* chromosome, and that portions of both *X* chromosomes of the mother are represented in the duplication.

It was established (PETERSON 1961) that crossing over in females carrying this duplication *X* chromosome, and a normal *X* chromosome, may be of the intrachromosomal type, involving left and right members of the duplication, or may be conventional in nature, with exchanges occurring between one or the other member of the duplication and the corresponding homologous segment of the normal *X* chromosome.

In a subsequent study (PETERSON and LAUGHNAN 1964), it was noted that males carrying the *X* chromosome duplication in slightly modified form, when mated with attached-*X* females, not infrequently produce exceptional patroclinous sons that are most readily attributed to intrachromosomal exchange between adjacent members of the duplication. Moreover, the distribution of these exceptional male offspring among the progeny of duplication-bearing males is not random and suggests that the exchange event is predominantly premeiotic.

While it was appreciated that the explanation based on premeiotic crossing over between members of the duplication was subject to cytological verification, further investigation of the phenomenon was rendered difficult because of the tendency of the duplication-bearing stocks to stabilize in regard to the production of exceptional offspring. More recently, however, a number of lines have been identified in which a relatively high proportion of duplication-carrying males produce exceptional patroclinous sons. This instability has persisted for a sufficient number of generations to allow detailed genetic and cytological analyses of the unusual events in males carrying the duplication. The investigations reported

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here deal with genetic studies on the occurrence of exceptional offspring from these lines. An ensuing report deals with the corresponding cytological analyses of exceptional derivatives.

MATERIALS AND METHODS

Origin and Characteristics of the *X*-Chromosome Duplication

As shown in Figure 1, the duplication event occurred in a female that was homozygous for *B* (Bar eye), and heterozygous for *f* (forked bristle), *os* (outstretched wing, small eye), and *car* (carnation eye). One break occurred to the right of the 17E1-2 doublet in the *f*+ chromosome, and the other probably to the left of the distal 16A1-2 doublet of the *f* chromosome; broken ends were joined to form the *f*+ (*B os*+) (*B os*) *car* (duplicated members in parentheses) duplication *X* chromosome that was delivered to the exceptional *BB* (double-Bar) son (Figure 1). On the basis of cytological evidence to be detailed in the ensuing report, *os* is assignable to the 17A subdivision; more specifically it lies to the right of 17A1-2 and to the left of 17A9-10. On similar grounds, it has been established that the break in the *f* chromosome occurred either immediately to the left of 16A1-2 of the distal member of the bar duplication, or between 16A1-2 and 16A4 of that member. Thus the duplication known as *Dp(1;1)NBB-8* carries a Bar position effect at the left extremity of each duplication member, in this way accounting for the double-Bar phenotype of the exceptional son.

The original duplication with double-Bar phenotype gave rise, by crossing over, to a new version of the duplication which produces a Bar, rather than double-Bar phenotype. The derived form, which is designated *Dp(1;1)NB-8*, is routinely obtained from females carrying the original duplication *X* chromosome and a nonduplicated *f B*+ *X* chromosome, in which single crossovers, involving the left member of the duplication and occurring to the right of *B*, produce *X* chromosomes with the constitution *f* (*B*+ *os*+) (*B os*) *car*. Males carrying this duplication have a single 16A section (*B*+ effect) at the left end of the distal duplication member, and two 16A sections (*B* position effect) at the left end of the proximal member; they therefore have a Bar, rather than double-Bar phenotype. This version of the *X*-chromosome duplication is referred to hereafter as the standard long duplication or SLD.

The standard long duplication has been held in stock by mating with *y f* attached-*X* females. Cytological analysis reveals that in one of the SLD strains so maintained there was a deletion of a portion of the left member of the duplication; the left break occurred far to the right in the 16E subdivision, and the right break was placed between 17A1-2 and 17A3-4, so that the deletion includes the segment from 16F1-2 through 17A1-2. This modified form of the duplication, designated *Dp(1;1)MNB-8*, was employed in all the studies reported here. It produces a *B os*+ phenotype and is thus indistinguishable in effect from the standard long duplication from which it was derived. It is referred to hereafter as the modified long duplication and, where appropriate, this is abbreviated to MLD to describe males in the duplication pedigree that have the *B os*+ phenotype. According to this description, males carrying cytological changes which do not affect the *B os*+ phenotype would also, but inadvertantly, be designated MLD.

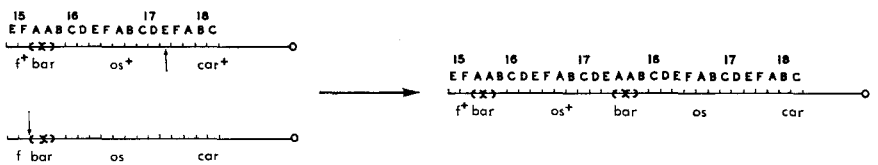

FIGURE 1.—Origin of *Dp(1;1)NBB-8*. Spontaneous breaks occurred at indicated sites in *X* chromosomes of female, as shown at left. Joining of broken ends produced duplicated *X* chromosome, shown at right, as recovered in single male offspring.

Figure 2 shows several photomicrographs of the original long duplication and of its SLD and MLD derivatives as seen in the salivary gland *X* chromosomes of the male. Invariably, the duplicated members are involved in a pairing configuration, though the details of the pairing vary somewhat from cell to cell and from individual to individual. Most typically, the 16A sections of the proximal duplication member are seen in an attenuated condition as they pull out from behind the 17E section to pair with the leading 16A section of the distal member of the duplication.

Derivation of Lines

Since the viability and productivity of SLD and MLD males are much higher than that of homozygous females, the duplications have been maintained by mating SLD or MLD males with attached-*X* sibling females in each generation. In this manner a number of duplication strains have been maintained for many generations. The studies reported here deal with derivative lines of one such strain of the MLD duplication designated d12. It was noted that d12 males, like those of other duplication-bearing strains, so rarely produced exceptional offspring that a genetic and cytological study of the latter would not be feasible. Therefore, an attempt was made to increase the frequency of exceptions by carrying out a series of backcrosses in which MLD males of strain d12 were initially mated with a variety of attached-*X* and non-attached-*X* strains. In the former case, patroclinous sons in each generation were mated with virgin females from the attached-*X* strain. This procedure leads to replacement of the autosomes, but of course, not of the *X* chromosome, in the resulting MLD lines. In the latter case, MLD males of strain d12 were mated initially with virgin females from the nonattached-*X* strain and heterozygous MLD daughters from this mating were crossed with males from the recurrent nonattached-*X* strain. This mating procedure, which was repeated in ensuing generations, leads not only to substitution of autosomes of the d12 strain but, through crossing over, may also bring about replacement of portions of the *X* chromosome.

Most of the backcrossing programs described above were ineffective in enhancing the rate of occurrence of exceptions, but a number of lines recovered from these matings did produce exceptional offspring with a far higher frequency than that encountered in the original d12 source strain. The derivation of these lines, designated A through F, is given below.

Lines A and B. MLD males from the d12 strain were mated with *y f* attached-*X* females from a stock in which the females had been repeatedly mated with *f B+ os car* males. MLD sons were chosen and again mated with females from the *y f* attached-*X* stock. Eight successive cycles of such matings were carried out, with occasional testing of individual MLD sons in matings with *y f* attached-*X* females to determine whether there had been an increase in yield of exceptional offspring. The results were negative and it was decided to discontinue the backcrossing program, and to propagate the backcrossed stock thereafter by sibling matings involving attached-*X* females and MLD males. After only one generation of such matings, however, it was noted that one or more stock cultures carried exceptional sons. Sibling MLD males (*f B os+ car*) from these cultures were thereupon mated individually with *y f* attached-*X* females. Twelve of 43 such cultures gave one or more exceptional male offspring. Line A, characterized by a high rate of exceptions, derives from MLD males among the 12 exceptional cultures, while Line B, which typically exhibits a low rate of exceptions, originates from MLD males among the 31 cultures that failed to yield exceptions. This study deals with 14 successive generations of matings involving Lines A and B.

Line C. MLD males from the d12 strain were mated with females from a homozygous *f B+* stock designated D13, and heterozygous MLD daughters were in turn mated with males from the D13 stock. MLD sons from this cross were mated individually with *y f* attached-*X* females to determine the frequency of exceptional offspring. Seven of these cultures had only MLD sons, but the eighth, culture 126, had 23 MLD and six exceptional male offspring. Line C derives from MLD males among these eight cultures and has been carried through 13 generations.

Line D. In this case, as with Line C, MLD males from strain d12 were mated initially with

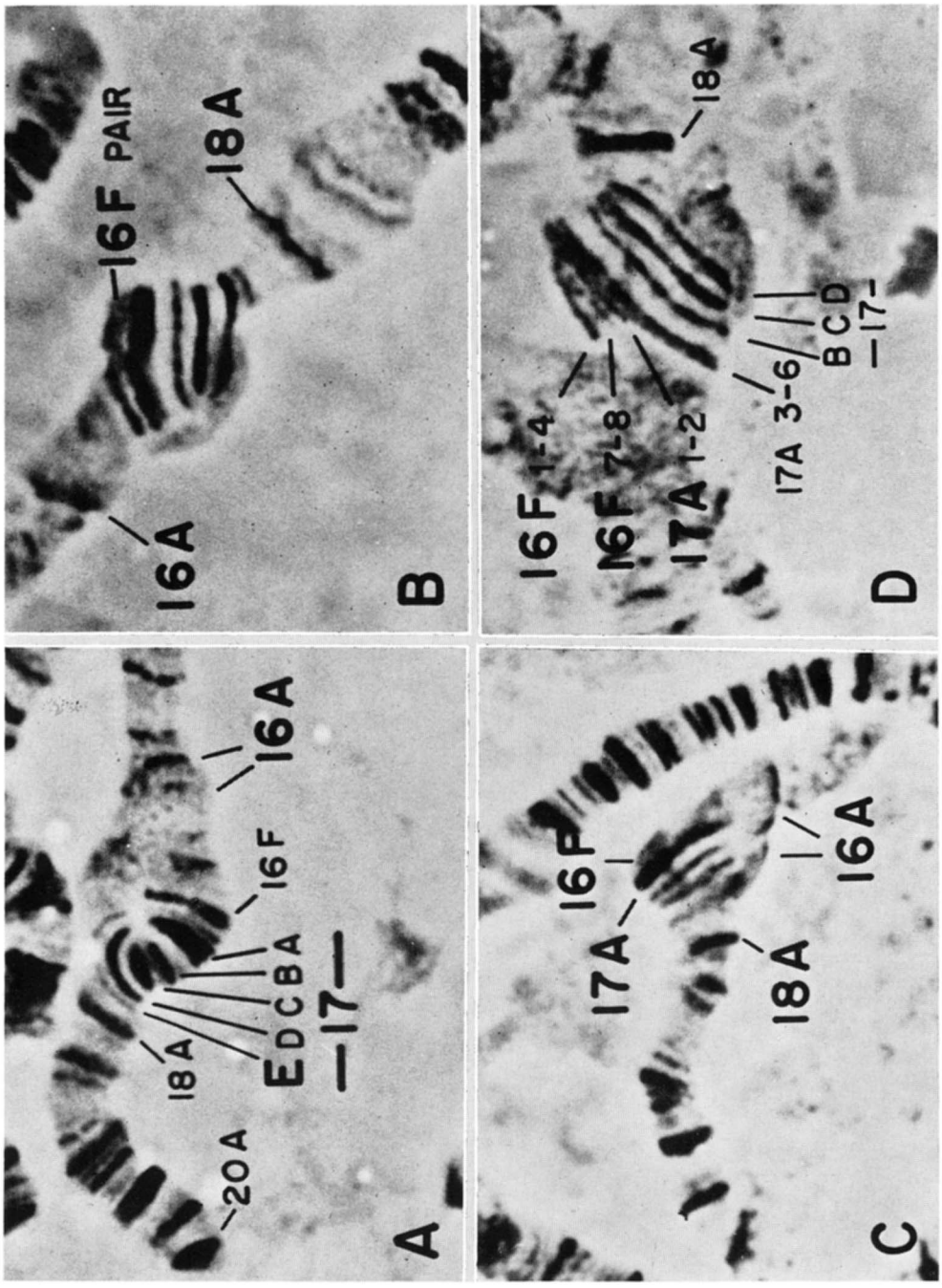

FIGURE 2.—*X*-chromosome duplications as seen in salivary gland preparations of the male. A. The original duplication, *Dp(1;1)NBB-8*, whose origin is detailed in Figure 1; each duplication member carries two 16A subdivisions, the proximal ones seen here in attenuated condition as they pull out from behind the 17E1-2 doublet to pair with the 16A subdivisions of

females from the D13 stock. Heterozygous MLD daughters in this and the ensuing four generations were backcrossed with males from the D13 source, after which the offspring of this backcrossing program were propagated by sibling matings. After two generations of the latter, six MLD males were secured and mated individually with *y f* attached-*X* females. None of these cultures yielded exceptional offspring. Nevertheless, MLD males from one of these, culture 1255, were used to establish Line D which turned out, in later generations, to yield significant numbers of exceptional offspring. Line D has been carried through 9 generations.

Line E. MLD males from the d12 strain were mated with females from a homozygous *f B*+ stock designated D3. Heterozygous MLD daughters were mated with males from the D3 stock and MLD sons from this cross were mated individually with *y f* attached-*X* females to determine whether exceptional offspring were produced. Fourteen of these cultures had only MLD sons, but the fifteenth, designated 127, had 14 MLD and two exceptional male offspring. Line E derives from MLD males among these fifteen cultures and has been carried through 13 generations.

Line F. MLD males from the original d12 strain were mated with females from a *B*+; *se* stock. Heterozygous MLD daughters were mated with males from the *B*+; *se* stock, and this procedure was repeated for four more generations. Progeny of the fifth backcross were thereafter propagated by sibling matings. MLD males from the third such cycle of brother-sister matings were testcrossed individually with *y f* attached-*X* females to determine whether exceptional progeny would be produced. Two of eight such cultures gave exceptional male offspring. Line F derives from MLD sibling males from these two cultures. It has been carried through 15 successive mating cycles.

Mating Procedures

A uniform mating procedure was adopted for all of the experiments reported here. Individual MLD males of genotype *y*+ *f* (*B*+ *os*+) (*B os*) *car* were mated in successive generations with two virgin females carrying the attached-*X* compound designated *C(1)DX*, which has *In(1)dl-49* in one *X* chromosome, and *In(1)sc*^{8} in the other. Females with this compound are homozygous for *y* and *f* and also carry the *Y* chromosome. The particular females employed in these matings came from a stock maintained by mating the *y f* attached-*X* females with wild-type males. Male offspring resulting from detachment occur rarely but are easily distinguishable from patroclinous sons by their yellow body color, and any illegitimate sons of an occasional nonvirgin female were, by reason of their *f*+ phenotype, distinguishable from legitimate patroclinous sons of both the MLD and exceptional classes.

Cultures were reared at 23.3°C. Parents were removed on the seventh day, and on the fourteenth day cultures were scored for the number of *y f* female offspring and for the number of unchanged (MLD) and exceptional patroclinous sons. MLD males were secured from selected cultures and again mated individually with two virgin females from the *y f* attached-*X* stock to produce the next generation.

Exceptional Offspring

MLD males, having the genotype *f* (*B*+ *os*+) (*B os*) *car*, and the phenotype *f B os*+, when

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the distal member. The bands identified by letter are the leading and prominent ones of their respective subdivisions.

B. *Dp(1;1)NB-8*, derived from *Dp(1;1)NBB-8* by crossing over, and identified in text as SLD (standard long duplication); note presence of 16F subdivisions in both members of the duplication.

C. *Dp(1;1)MNB-8*, a spontaneous derivative from *Dp(1;1)NB-8*, and identified in text as MLD (modified long duplication); note side-by-side pairing alignment of the proximal and distal 16A subdivisions, and the absence of the 16F subdivision in the distal member of the duplication.

D. Same as C, providing detail on deletion of bands 16F1-4 through 17A1-2. MLD was employed in genetic studies reported here.

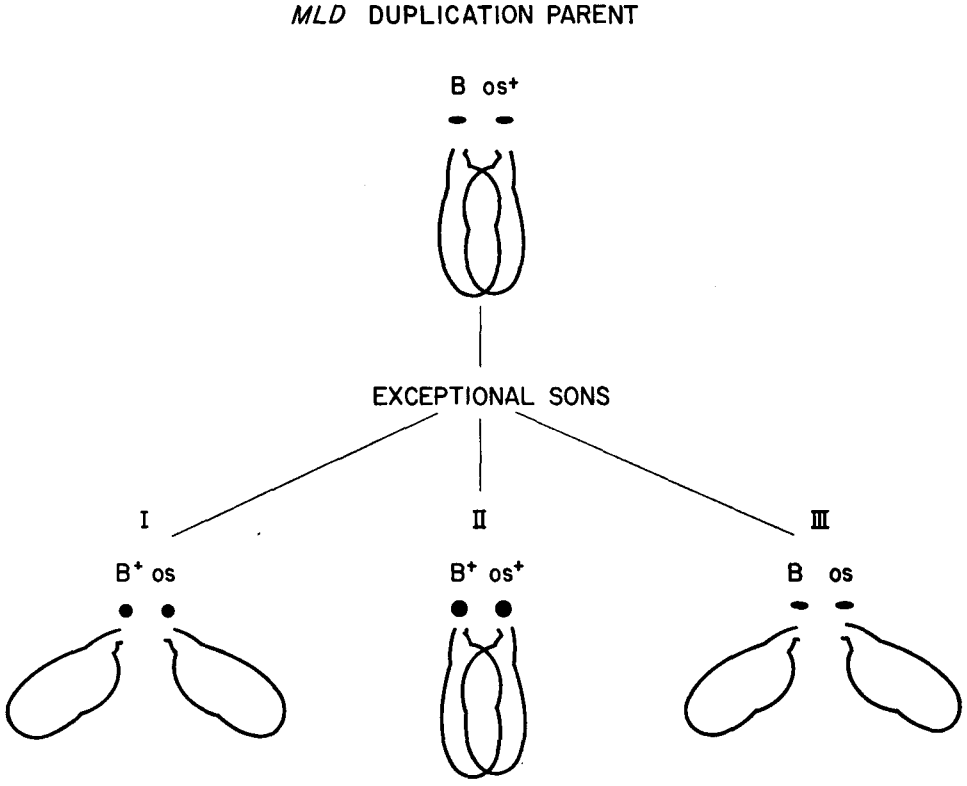

FIGURE 3.—Diagrammatic illustration of eyes and wings of MLD male parent and of the three kinds of exceptional patroclinous sons. The *os* symbol indicates outstretched wings and small eyes; the effect of *os* on facet number is evident in *B*+ (non-Bar) individuals but is not apparent in *B* (Bar) males.

mated individually with *y f* attached-*X* females, produce mainly *B os*+ patroclinous sons. Occasionally, however, they give rise to exceptional male offspring which, as illustrated in Figure 3, may be any one of three types. The most frequently occurring exception, designated Type I, has the phenotype *B*+ *os*; these males have "reverted" from *B* to *B*+, and have outstretched rather than normal wings. Type II exceptions have the phenotype *B*+ *os*+; these males have "reverted" from *B* to *B*+, but retain the *os*+ parental phenotype. Type III exceptions, the least frequent in occurrence, have a *B os* phenotype; these males retain the parental *B* phenotype but have changed from normal to outstretched wing.

In the cases of Type I and Type II exceptions, where the change from Bar to round eye is easily discernible and classication is not subject to error due to phenotypic overlap, validation of exceptional status does not require progeny tests; this is apparent from the observation that all but one of the many Type I and Type II presumptive exceptions that were chosen for the purpose of establishing stock cultures turned out, in fact, to represent valid exceptions. The occurrence of progeny males with Bar eyes and outstretched wings, however, could, at the time of original encounter, be considered only as presumptive cases of Type III exceptions, and such cases were considered valid only upon confirmation based on progeny tests. Many such progeny tests failed due to the low viability and poor productivity of these individuals and for this reason it is considered that frequencies of Type III exceptional events are greatly underestimated.

Some cultures were found to contain one or more exceptional male progeny, and occasionally,

all of the male offspring of a culture were exceptions. In any one culture, males of a particular exceptional type were considered to have arisen from a single occurrence, hereafter referred to as an exceptional event. This leads to an underestimation of the frequency of the event, however, since the occurrence of a number of exceptional males in a single culture could well result from more than one exceptional event. In fact, there were instances of cultures that carried more than one type of exception, and it seems logical to attribute these to separate exceptional events.

Stocks of the three kinds of exceptional males described above were established and maintained by mating with *y f* attached-*X* females from the inversion stock previously described. In the earlier generations of these experiments, most of the exceptions were established in stock cultures, but it soon became impractical to stock all of the exceptional cases, and thereafter only a representative sample was maintained. However, for reasons that will be apparent later, a special attempt was made to progeny test each male that occurred as the sole exceptional individual in a culture.

RESULTS

Frequencies of Exceptional Events. The frequencies of the three kinds of exceptional events among the progeny of MLD males in the six lines derived from the original d12 strain are given in Table 1. It is apparent that most MLD males produce cultures having no exceptional male offspring. However, the frequencies of exceptional events recorded in Table 1 are considered to be minimal estimates since (1) more than one exceptional event might occasionally have been involved in the case of cultures carrying more than a single exceptional male; in Table 1, such cultures are credited with only a single exceptional event; (2) Type III exceptional events are greatly underestimated because progeny tests, required in these instances to validate exceptional status, quite often failed to produce offspring; and (3) MLD males themselves are low in viability and

TABLE 1

Frequencies of exceptional events among progeny of MLD males in the six lines derived from the original d12 strain

Source line	Number of cultures	Exceptional events*							
		Type I		Type II		Type III		Totals	
		No.	%	No.	%	No.§	%	No.‡	%
A	714†	333	46.6	11	1.5	1	0.1	345	48.3
B	1127	71	6.3	4	0.4	4	0.4	79	7.0
C	503	40	8.0	11	2.2	3	0.6	54	10.7
D	61	13	21.3	0	0.0	0	0.0	13	21.3
E	300	22	7.3	18	6.0	1	0.3	41	13.7
F	582	132	22.7	26	4.5	3	0.5	161	27.7

* This refers not to the number of exceptional offspring but to the number of original exceptional occurrences, each leading to the production of one or more exceptional male progeny in a culture.

† Not included here are 104 cultures in two stabilized sublines that produced no exceptions.

‡ These figures are not exactly equivalent to the number of exceptional cultures, as there were instances of paired exceptional events in which a single culture exhibited more than one type of exception.

§ As noted in MATERIALS AND METHODS, progeny tests are required to establish the validity of the Type III exceptions. Due to the lower viability and poorer productivity of these males, many of these progeny tests failed; for this reason it is considered that these figures underestimate the frequencies of Type III exceptions.

have poor productivity; the average number of $y\ f$ attached-X female offspring per culture was 20, while that of the patroclinous male offspring, among which the exceptions were sought, was only 10; it seems likely, therefore, that quite a number of cultures that were accorded nonexceptional status really belonged in the exceptional class but failed to exhibit exceptions because of the small number of males.

The data in Table 1 indicate that there are striking differences among the six lines in regard to the overall frequencies of exceptional events. Thus, almost half of the MLD males in Line A produce exceptional offspring while in Line B, which is otherwise essentially isogenic with Line A, only seven percent of MLD males produce exceptional progeny. Lines C and E also exhibit low frequencies of exceptional events, while Lines D and F are obviously intermediate in this regard.

While it is apparent from Table 1 that Type I exceptions are the most frequent, Type II the next most frequent, and Type III the least frequent, there are, among the six lines, obvious differences in the relative frequencies of these exceptional types. If, for reasons already discussed, the Type III exceptions are excluded for the purposes of this comparison, it may be noted that the ratio of Type I : Type II exceptional events is 30:1 for Line A, 18:1 for Line B, 4:1 for Line C, and 5:1 for Line F. Surprisingly, in Line E these two exceptional types occur with about the same frequency, and in fact, as detailed later, there exist sublines of Line E in which Type II exceptions predominate. There were no Type II exceptions in Line D which, incidentally, is represented by far fewer cultures than are the other lines. It is instructive to note that Type I exceptions occur with a much higher frequency in Line A than in Line E (ratio of 6:1), while the reverse is true for Type II exceptions (ratio of 1:4).

These differences in overall frequencies of exceptional events among the six lines, and in the relative frequencies of the different types of exceptions within the lines, are significant for a consideration of the hypothesis which holds that the exceptional event has the characteristics of a crossover; they are considered in this light in the discussion section.

Time of occurrence of the exceptional event. It is anticipated that the number or proportion of exceptional male offspring in exceptional cultures may provide some clue concerning the time of occurrence of the exceptional event. If, consistently, a high proportion of male offspring is found to be exceptional it would not be unreasonable to consider that the critical event occurs at an early stage in the development of germinal elements in the MLD male parent, and that stem (apical) cells of the testes may carry the changed condition. If, on the other hand, there were only one, or occasionally two, exceptional offspring per culture, it could be concluded that the exceptional event occurs in the gonial stages, or during spermatogenesis, at the earliest, or, at the latest, sometime during the early development of the exceptional individual itself. Neither of these extremes appears to predominate; as noted in a foregoing section, an exceptional culture may produce one or a number of exceptional males and, not infrequently, all the males in such a culture are exceptional.

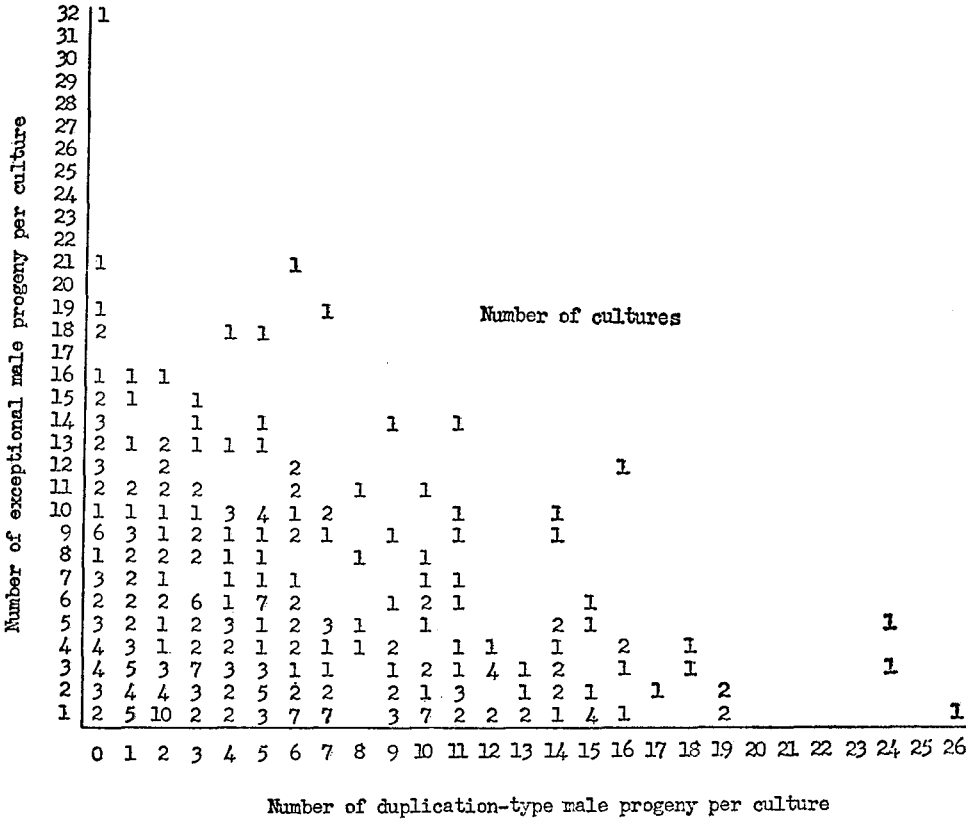

FIGURE 4.—Distribution of 326 exceptional Type I cultures of Line A with respect to the numbers of duplication-type (parental) males and exceptional males per culture.

Since the distribution of numbers of exceptional offspring in exceptional cultures appears to be similar among the six lines treated here, we have limited consideration of this aspect of the problem to Line A, which is characterized by a high proportion of exceptional cultures. Figure 4 summarizes the distribution of numbers of exceptional (Type I) and nonexceptional male progeny among 326 exceptional cultures of Line A that carried Type I, but not Type II or Type III exceptions. Surprisingly, these cultures produced more exceptional males (1866) than parental type males (1793), the average numbers of exceptional and parental type males per culture being 5.7 and 5.5, respectively. It is obvious from Figure 4, however, that the number and proportion of exceptional males vary greatly among these cultures. For example, 63, or 19.3%, of the 326 cultures had only a single exceptional male, while, at the other extreme, 47, or 14.4% of the progenies produced exceptional males exclusively. While there were very few males in some of the latter cultures, there were 34 such cultures with five or more males represented, and in one of these there were 32 exceptional males. These data suggest that, in at least some instances, the exceptional event occurs so early as to involve all of the functional germinal elements of the MLD male

parent. Moreover, in view of the large number of additional cultures with predominately exceptional males, it appears likely that the exceptional event often occurs at a relatively early stage in the development of germinal elements of the male parent, and that in many of the latter the changed condition is carried in stem cells of the testis. This interpretation is supported by the results of preliminary analyses of broods of individual MLD males.

The data presented in Figure 4 also indicate that the exceptional event may occur at a relatively late stage in the development of germinal elements of the male parent. As noted above, 63 (19.3%) of the exceptional cultures carried only a single exceptional male, and 42 of these cultures had five or more males of the parental type. These data suggest that the exceptional event may occur at the gonial (cyst) stage or even during meiosis, though it must be emphasized that the evidence now available is not sufficient to distinguish between, or even to support, either of these possibilities. Taking note of the relatively small numbers of male offspring in these cultures we are obliged to conclude that the data are not inconsistent with the hypothesis that all of the exceptional events occur prior to or during the divisions leading to the establishment of the stem cells, though there is no compelling evidence to adopt this view.

On the other hand, in view of the relatively high frequency of exceptional cultures with only a single exceptional male, we must consider the further possibility that these are cases in which the functional sperm is unchanged (parental type) and in which the exceptional phenotype of the male offspring is the result of an event occurring at an early stage in the development of the exceptional individual. If the latter is to register as a phenotypic exception (change in *B* or in os^+), the critical event must involve the soma, but it could be exclusively somatic, or it might involve both somatic and germinal tissues. To distinguish between these possibilities, a special effort was made to test the progeny of males that occurred as single exceptions in their cultures. In all, 55 such single exceptional males were tested successfully; 41 of these were Type I exceptions, ten were Type II exceptions, and four were in the Type III category. Fifty-four of these exceptional males produced exclusively exceptional progeny, indicating that the change was propagated in both the somatic and germinal tissues of the exceptional individual and, most likely, that the exceptional event had occurred in the male parent rather than in the offspring. Only one of the tested individuals, a Type II exception, failed to breed true for exceptional status in the progeny test, and this individual produced both Type II and parental type progeny. The bulk of evidence thus supports the view that the vast majority of exceptions, including single occurrences in a culture, result from an exceptional event in the male parent producing one or more sperms with the changed condition, and suggests that the exceptional event very rarely occurs in the somatic tissues.

There is additional, and perhaps more convincing, evidence that the exceptional event occurs almost exclusively in germinal tissues. If such events occurred during the development of somatic tissues, it is expected that male offspring that register as mosaics involving both the parental and exceptional phenotypes would be produced. The outstretched wing phenotype associated with *os* is not suffi-

ciently reliable to be diagnostic for this analysis, but, as indicated earlier, the Bar and round-eye phenotypes do not overlap and no difficulty would be anticipated in identifying male offspring with one Bar and one round eye. In the studies reported here, well over 30,000 male offspring have been scored, and among these only one such mosaic individual was encountered; this male had one Bar eye and one round (*os* type) eye, but the wings were normal. Evidently, the exceptional event we deal with here very rarely occurs in somatic tissue; on the other hand, its occurrence in the germ line is frequent and, as we have seen in some cases, such as Line A, almost 50 percent of MLD males carry the changed condition in their germ cells.

The Effect of Selection on Frequency of the Exceptional Event. No concerted effort was made to determine the effect of selection upon the rate of occurrence of the exceptional event. The opportunity to observe the effect of selection was no doubt greatly diminished as a result of the open-pedigree mating procedure adopted here in which MLD males, and their patroclinous sons were mated, in successive generations, with females from the $y\,f$ attached-X inversion stock. Moreover, there is the technical difficulty, noted in the preceding section, that exceptional events occur almost exclusively in the germinal elements of the male, thus necessitating the use of progeny tests to identify those individuals in which an exceptional event has occurred. In spite of these difficulties, it is possible to make some limited observations concerning the effectiveness of selection in these experiments. As noted earlier, the procedure followed in producing the test cultures was to choose a number of sibling MLD males from a culture and mate them individually with $y\,f$ attached-X females, thus establishing a group of cultures whose male parents are siblings. One or more of such sibling cultures may carry exceptional male offspring, in which case the sibling cultures are designated as a case group; or none of the cultures in such a sibling group carry exceptions, in which case it is called a noncase group. The vast majority of duplication-bearing males that were chosen for propagation came from cultures in case groups; thus these cultures either carried exceptions, in which case the MLD males chosen for mating were sibs of exceptions, or they did not carry exceptions, in which case the MLD males that were propagated were not sibs of exceptions. It is reasonable to inquire whether, in the course of these experiments, the MLD males in the first category produced a higher frequency of exceptional cultures than did the males in the second category.

Among the cultures in Line A (see Table 1) there were two sublines, numbers 57 and 67, that were carried for the full 14 generations. In subline 57, there were 234 matings that involved MLD males which were sibs of exceptional males, and among the resulting cultures, 128, or 54.7% carried exceptions; 29 of the matings in this subline involved MLD males that came from case group cultures that carried no exceptions and 17, or 58.6%, of these gave exceptional offspring. In subline 67 of Line A, 303 of the male parents were in the first category, as defined above, and 139, or 45.9%, of these produced exceptional offspring, while, of the 39 matings involving male parents in the second category, 22, or 56.4%, had exceptional offspring. In Line B, subline number 94 was carried through 8

generations. Among the 125 cultures from male parents with exceptional siblings, 13, or 10.4%, carried exceptions, while, of the 170 cultures derived from males from case group cultures that carried no exceptions, 20, or 11.8%, had exceptions. None of these differences is significant and there is therefore no evidence that the frequency of exceptional events is enhanced among progenies of MLD males that derive from exceptional cultures, as compared with progenies of MLD males selected from nonexceptional cultures. As noted and documented elsewhere in this report, it appears, rather, that a change in frequency of occurrence of the exceptional event is more likely to result from sudden, inherited change than from small cumulative effects attributable to selection.

Genetic Behavior of the Exceptions. As indicated earlier, attempts were made to propagate many of the exceptions obtained in these studies. Large numbers of stock cultures of the three kinds of exceptions were established and maintained until detailed cytological studies were completed. In the course of routine propagation of these stock cultures it was noted that some of the exceptions not infrequently undergo further changes. Thus, 14 of the Type II, and six of the Type III exceptions, have, in one or more instances, given rise to true-breeding Type I segregates. It seems likely that all Type II and Type III exceptions are capable of yielding Type I derivatives, but so far no changes of Type II exceptions to Type III, or *vice versa*, have been identified. And, in spite of the large numbers of stocks of Type I exceptions that have been maintained, none of these has given rise to either Type II or Type III exceptions. Hence, Type I exceptions appear to be extraordinarily stable, while both Type II and Type III exceptions are relatively unstable.

Among the 3,287 cultures represented in Lines A through F, 693 carried exceptions, and 19 of the latter were mixed in that they carried more than one type of exception. In view of the fact that stocks of Type II and Type III exceptions not infrequently give rise to Type I exceptions, the question arises whether the Type I exceptions that occur in test cultures with Type II or Type III exceptions, arise from either of the latter as secondary derivatives, or whether they occur independently. The evidence available at this time does not permit an answer to this question, but it is clear that the occurrence of any one type of exception is not dependent on the prior occurrence of another, since the vast majority of exceptional cultures carried only one of the three possible kinds of exception.

Heritable Changes in Frequency of the Exceptional Event. While the frequency of the exceptional event varies widely among Lines A through F (see Table 1), it appears in general to be maintained at a characteristic level among MLD males in any particular pedigree. Nevertheless, several notable departures from this behavior have been identified. Two of these occurred among pedigrees originating from subline 67 of Line A, in the latter of which, it will be remembered, the average frequency of exceptional events is close to 50%. The first such case originated among cultures numbered 531 through 541. The male parents of these 11 cultures were MLD siblings that came from an exceptional culture, number 336. Seven of these cultures carried exceptional male offspring and four

had only parental type male offspring. One of the latter, culture 537, apparently became stable in regard to the occurrence of the exceptional event, since, among 79 cultures that traced their male parentage through three successive generations to MLD males in culture 537, there were no exceptions, while among 103 cultures involving parallel pedigrees in the same three generations, but deriving initially from cultures 534, 535, 539, 540 and 541, there were 37 exceptional events.

Another striking instance of stabilization occurred in later generations of the 541 pedigree. Male propagates from this culture, carried through four successive generations, produced 18 cultures, among which there were 14 exceptional events. In the succeeding generation, only one culture, number 2185, was represented in the pedigree, and it carried no exceptions. Over the next four generations, MLD males in this pedigree produced 26 cultures, among which there were no exceptions.

While the instances of stabilization described above are the most striking ones encountered in these studies, there are reasons for considering that many additional cases of the same phenomenon have gone unrecognized. For example, a number of the lines investigated here are characterized by a relatively low frequency of exceptional events and it is immediately apparent that the lower the frequency of these events, the greater is the number of nonexceptional progenies in a pedigree required to establish the stabilization phenomenon. Even in Line A, where approximately 50% of the males are expected to produce exceptional offspring, a minimum of seven or eight nonexceptional sibling cultures is required to justify the conclusion that there has been stabilization, let alone that there has been a change of lesser extent in frequency of exceptional events. Moreover, for obvious reasons, the nonexceptional MLD males that were chosen for propagation in successive generations came primarily from exceptional cultures, so that there was a conscious effort to avoid, for this purpose, MLD males that occurred in cultures, or groups of sibling cultures, that exhibited no exceptions.

Finally, it should be noted that some of the pedigrees within Line A suggest the occurrence of inherited changes leading to a partial reduction in the frequency of exceptional events. In any case, there is no doubt that whatever governs the occurrence of exceptional events, it not infrequently undergoes a change leading either to a loss of the capacity to produce the exceptional event, or to a lower incidence of the latter. For the time being, this unidentified entity that appears to govern the occurrence of the exceptional event, and to be subject to inherited changes of state, will be referred to as the controlling element.

There are also several well established cases in which the controlling element has undergone changes in the other direction, that is, from a stable or low-rate state to a condition associated with a relatively high frequency of exceptional events. One example of the latter involves Line A itself, which, as described in MATERIALS AND METHODS, was established originally by matings involving MLD males from exceptional cultures which suddenly appeared in a strain that was generally characterized by a low rate of exceptions. Line A, in the studies reported here, has been carried through 14 generations, and various representative sublines of Line A continue to be propagated. Except for the cases of stabilization

already noted, these sublines have retained the characteristic high rate of exceptional events, approaching fifty percent.

Another instance of this kind of change in the controlling element is found among certain pedigrees of Line B, the latter of which is characterized by a relatively low frequency of exceptions (see Table 1). Male propagates of subline number 100 produced only four exceptional cultures among a total of 152 cultures involved in generations three through seven. In the eighth generation, culture number 1803 carried exceptions. Over the next six generations, duplication-carrying male propagates tracing to culture 1803 produced a total of 49 cultures, and of these, six carried exceptions. In contrast, among 238 cultures involving parallel pedigrees over the same six generations, there were no exceptions. Apparently duplication males in the 1803 pedigree carry a changed controlling element associated with a higher incidence of exceptional events.

Changes in the controlling element that have been considered heretofore have involved Type I exceptions. One of the cases to be considered now is unique in that the increase in frequency of the exceptional event occurs principally in the Type II exceptional class, and suggests therefore that the determination of the relative frequencies of Type I and Type II exceptions, and probably also of those in the Type III category, is a function of the same mutational process that governs the overall rate of exceptions. The case under consideration involves all the cultures in the eleventh, twelfth, and thirteenth generations of Line C, and all these are a part of a single pedigree stemming from culture 1181 in the fourth generation of this line. Duplication males originating from culture 2681 in the tenth generation produced a total of 27 cultures over the next three generations; of these, two carried Type I, eight carried Type II, and one gave Type III exceptions. A quite different result was obtained among a parallel series of 25 cultures originating from culture 2687 in the tenth generation; here there were ten Type I exceptions, and no Type II or Type III exceptions. Control frequencies are provided by a parallel series of cultures that originated from duplication males taken from all other cultures in the tenth generation; here there was a total of 125 cultures, among which there were nine Type I, no Type II, and one Type III exceptional cultures. Clearly, males in the 2681 pedigree have an enhanced frequency of Type II exceptions, while those in the 2687 pedigree exhibit a higher incidence of Type I exceptional events, as compared with the control cultures.

It must be concluded that heritable changes or mutations in the element which governs the occurrence of the exceptional event occur rather frequently. It is also apparent that these changes affect not only the overall frequencies of exceptional events, but that they also play a role in the determination of the relative frequencies of the different types of exceptions.

Genetic Control Over the Exceptional Event. In a foregoing section of this report it was concluded, from a consideration of the data presented in Table 1, that duplication-carrying males in the various lines differ in regard to both the overall frequency of exceptional events and the relative frequencies of the several different kinds of exceptions. This conclusion is supported by evidence, given in the preceding section, on heritable changes in the controlling element. A con-

sideration of the mating procedures employed in these studies would suggest that if the differences associated with the several lines are due to a controlling element that has genic characteristics, such a hypothetical element resides in the X chromosome rather than in any one of the autosomes. In each generation, and in each of the six lines, MLD males that were chosen as male parents of the next generation offspring, were mated with virgin females from the same $y\ f$ attached-X stock. This procedure leads to the eventual substitution of the autosomes, and of the Y chromosome, of the male-parent line, by the autosomes and Y chromosome from the $y\ f$ attached-X stock. If the hypothetical gene which controls the occurrence of exceptional events were autosomal or were carried in the Y chromosome, the mating procedures used here would invariably lead to a loss of the differences in frequencies of exceptional events which characterize the various lines. This is clearly not the case since the lines have retained these characteristic differences over many generations.

On the other hand, the attached-X mating system employed here does not lead to substitution of all, or any part of, the X chromosome in the MLD male parent. Except for mutational events, this X chromosome is passed unchanged from father to son in each mating cycle, duplication-bearing sons in each generation being chosen as parents of the next generation. If exceptional events are under the control of a chromosomal gene, it would be predicted that the latter is located in the X chromosome of the male parent.

Investigations designed to test this hypothesis are now being undertaken. These involve initially the use of marked females that derive one X chromosome from a stock characterized by a relatively high frequency of exceptional events, and another X chromosome from a source that is stable in this regard. The two types of sons derived from these females are mated individually with $y\ f$ attached-X females and the resulting cultures are searched for exceptions. Preliminary results of these experiments indicate that the exceptional event is indeed under the control of a gene, and they suggest that this gene is carried in the X chromosome at a site in, or not far removed from, the duplication itself.

DISCUSSION

The data presented here indicate that most of the exceptional events occur prior to spermatogenesis in the duplication-carrying male parent, and they are not inconsistent with the view that all the exceptional events are premeiotic. It is established that exceptions only rarely originate from a primary event occurring in somatic tissue, so that the exceptional event most typically occurs in nonmeiotic, germinal cells.

In searching for a mechanism to explain the various kinds of exceptions encountered here, primary emphasis should be accorded the possibility that they are the result of intrachromosomal crossing over occurring in connection with mitosis. This mechanism was invoked, at the meiotic level, to explain the origin of nonrecombinant B^+ and BB derivatives from females homozygous for the standard Bar duplication (PETERSON and LAUGHNAN 1963). According to such

a model, pairing of homologous members of the modified long duplication, *Dp(1;1)MNB-8*, dealt with here, followed by crossing over, could lead, depending on the crossover site, to any one of the three types of exceptions we have identified. In general, however, the genetic evidence considered in this report can not be regarded as favorable to the crossover hypothesis. The six lines studied here show striking differences in overall frequency of the exceptional event, and there are also wide variations among these lines in the relative frequencies of Type I and Type II exceptions. Moreover, several instances have been recorded in which sublines have undergone sudden changes, as in the cases of the two sublines of Line A which were stabilized and no longer produce exceptional progeny. On the crossover hypothesis these differences must be assigned to correspondingly sharp changes in frequency of crossovers within the duplication. While it is conceivable that a genic element, such as that which has here been identified in the *X* chromosome, might control relative frequencies of crossing over within the duplication, in view of the quite large differences in these frequencies that have been encountered, a crossover model appears not to be the most satisfying explanation.

It is apparent that cytological analyses of the exceptional offspring afford a crucial test of the crossover hypothesis. These studies, which will be reported in a subsequent paper, render the crossover model untenable since they indicate that the exceptions encountered in this system are the result of a break-and-join mechanism that appears not to have all of the characteristics of a crossover event.

SUMMARY

Males carrying *Dp(1;1)MNB-8*, designated the modified long duplication (MLD), and having the genotype *f* (B^+ os^+) (*B os*) *car* (duplicated members in parentheses), are *B* os^+ in phenotype. When mated with attached-*X* females, these males produce mainly *B* os^+ (parental) patroclinous sons; occasionally they give rise to exceptional male offspring that may be any one of three types: B^+ *os* (Type I), B^+ os^+ (Type II), or *B os* (Type III).—Genetic analyses of progenies involving six strains of MLD males mated in successive generations with attached-*X* females reveal the following: (1) There is a striking variation in frequencies of exceptional events among the six lines, and in one of the lines almost 50% of MLD males produce exceptional cultures. (2) The lines exhibit pronounced differences in the relative frequencies of Type I and Type II exceptions. (3) Two instances of stabilization have been identified in sublines of Line A, which is otherwise characterized by a high frequency of exceptional events; there is independent evidence that stabilization occurs frequently. (4) A number of sublines have undergone changes in the other direction, from a relatively stable condition to one characterized by a relatively high frequency of exceptional events. (5) Changes in frequency of the exceptional event apparently result from sudden inherited changes, as yet unidentified, rather than from cumulative effects attributable to selection. (6) Type II and Type III exceptions have, upon further analysis, been shown to produce Type I exceptions, but Type I exceptions are

extraordinarily stable; moreover, no instances of changes from Type II to Type III exceptions, or *vice versa,* have been encountered. (7) The exceptional event occurs almost exclusively in germinal tissues of the MLD male parents involved in these studies and, although the evidence does not exclude a meiotic occurrence, it suggests that the crucial event often takes place at a relatively early stage in the development of germinal elements. (8) Preliminary experiments indicate that the exceptional event is controlled by a genic element carried in the X chromosome at a site in, or not far removed from, the duplication itself.—It is conceivable that the exceptional events encountered here coincide with an intrachromosomal crossover occurring within the confines of the X-chromosome duplication, but the genetic evidence is not in full accord with this explanation. Cytological analyses of exceptional offspring afford a crucial test of the crossover hypothesis. They are the subject of a separate report which indicates that the exceptions encountered in this system are the result of a break-and-join mechanism that does not have the precision expected of a crossover event.

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