A GENETIC ANALYSIS OF SOME REARRANGEMENTS INDUCED IN OOCYTES OF DROSOPHILA¹

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THE difficulty in obtaining rearrangements by treatment of *Drosophila* females has long been recognized (GLASS 1940, 1955). This difficulty might result from a relatively large volume of the oocyte nucleus causing breaks in non-homologous chromosomes to be more widely separated than in sperm. Furthermore, meiotic segregation would limit the recovery of those translocations induced. The techniques which have been employed may have been improper for the detection of the types of rearrangements which are possible in female germ cells. Finally, due to the considerable differences in sensitivity of oocytes in different stages (PATTERSON, BREWSTER and WINCHESTER 1932; PARKER 1955; KING, DARROW and KAYE 1957) the dosages used may have been high enough to guarantee nearly complete killing of the more sensitive cells which might give rise to a greater variety of rearrangements.

The technique of detachment of attached-X chromosomes has given a means for the study of translocation processes in *Drosophila* females. Though the frequencies obtained are relatively low when compared to translocation rates in male germ cells, rearrangements may be detected in the F₁, which makes possible the obtaining of as large numbers as one wishes.

The recovery of a detachment requires breakage of the attached-X in the proximal region, giving rise to separated X's, one having and the other lacking the centromere. Another break must occur near the tip of an X, Y, or autosome. Two products may result: the "centric X" with the tip of the other broken chromosome joining its broken end, or the "acentric X" which must join the broken end of the centric fragment of another chromosome to be recovered.

Detachments have been induced in newly emerged females and in those aged three or more days. Using the designations of KING, RUBINSON and SMITH (1956), these are stage 7 and stage 14 oocytes, respectively. PARKER (1954a,b) showed by means of fertility tests and tests for linkage of X and fourth chromosomes that nearly all detachments induced in stage 7 are translocations either with Y or with chromosome 4. The increased sensitivity of stage 14 oocytes, found in females three or more days old, together with the differences from stage 7 in sex ratio and phenotype of flies carrying a rearrangement, show that rearrangements induced in these older cells differ qualitatively as well as quantitatively from

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those in younger cells (PARKER and HAMMOND 1957, 1958). There must be a high frequency of induced lethality in this stage, since there is relatively poor recovery of males carrying detached X chromosomes. This may result from changes in the types of rearrangements being induced in aged cells. The work here reported deals with the identification of rearrangements picked up in the experiments of PARKER and HAMMOND as well as in some earlier ones, showing some of the differences in types of rearrangements induced in the two stages treated. An attempt is made to draw conclusions as to the types of exchanges which occur, based on a comparison of the frequency of recovery of complementary exchange products.

EXPERIMENTAL PROCEDURE

All of the detachments studied here were of the attached-X chromosome marked with $\gamma^2 su-w^a u^a bb$. In earlier work, various Y chromosomes had been used in the irradiated females to see any possible effect of the Y chromosome on detachment frequency (PARKER 1954a,b). PARKER and HAMMOND (1958) used the sc^s Y, since the yellow⁺ marker on the long arm of the Y gives a method for recognition of some X-Y translocations. In addition, the segregation of this marked Y might allow some inferences as to the translocation product recovered.

The testing procedure consisted of the following steps, in the order listed: (1) scoring phenotype and sex of the F_1 of treated females, (2) breeding of F_1 detachment females from some irradiations to see if males carrying the detached X chromosome were viable, (3) testing for X-4 translocations of all detachments viable in the male, (4) testing for male fertility to show attachment of a Y arm to the detached X, (5) testing for gross deletion of most of an X chromosome resulting in a "duplication" of the left tip of X, (6) testing for involvement of chromosome 2.

Tests for X-4 translocations were initiated in the original cross, where the irradiated attached-X females were mated to *Muller-5*; *ci ey^R* males. Males carrying a detached X were then crossed to attached-X females, γ ; *ci ey^R*. If the euchromatic part of 4 is linked with X, in the F_2 all males will be of *ci⁺ ey⁺* phenotype; their attached-X sisters would usually be expected to be divided between *ci ey* and *ci⁺ ey⁺* since usually we expect the male being tested to be hyperploid, and therefore to carry a free fourth chromosome with the normal alleles of the markers being used. Recovery of the acentric detached X chromosome with the centromere of chromosome 4, or appropriate nondisjunction of fourth chromosomes when the centric X is recovered with linkage of chromosome 4, may give rise to individuals which are not hyperploid. In this case, there will be only the *ci ey* class of attached-X females.

Where detachments recovered in females were being tested for X-4 translocations, females carrying a detached X balanced by *Muller-5* were backcrossed to *Muller-5*; *ci ey^R* males. In most cases it was possible to recognize the X-4 translocation without further tests, but recognition of the presence of a free fourth carrying the wild type alleles of *ci* and *ey* was difficult as it required distinguish-

ing between heterozygous and homozygous Bar in eyeless flies. For this reason, stock cultures were established by crossing the appropriate males to γ ; $ci\ ey^R$ females.

Male fertility in *Drosophila melanogaster* requires presence of both Y chromosome arms. It is possible to test for the presence of Y arms attached to the X chromosome by testing the fertility of males carrying the detached X and Y chromosome fragment having the fertility region of only one arm of the Y. The Y chromosome fragments used were marked with yellow⁺ enabling one to follow their segregation and to eliminate the possibility of any tested males having a complete Y as a result of nondisjunction. Some types of detachments, e.g. X-4 translocations, give fairly high (ten percent) frequencies of primary nondisjunction of the Y, making it extremely likely that one would get false positive fertility tests, were this procedure not used. The presence of nondisjunctional individuals in the progeny of the tested males required repetition of the tests, using individual males, rather than mass matings. Detachments were recorded as having a given Y arm attached to the X chromosome only if the fertile males produced the expected regular classes with no nondisjunctional offspring. Males carrying the detached X to be tested were crossed to attached-X females which carried either the $sc^{VL}Y^S$ as the short arm of the Y, or scY^L or sc^sY^L "CY-9" (LINDSLEY 1955) as the long arm of the Y. The F_1 males were then crossed to yellow attached-X females to see if they were fertile.

Gross deletion of most of one of the X's would give rise to an apparent detachment of the attached-X. This would leave the centric X with a small duplication of the tip of XL. Tests for this duplication were made by examining the crossover products of females heterozygous for the detached X and another X carrying yellow¹ and a duplication yellow⁺ to the right of the centromere. Since deletional "detachments" would have yellow² both in the normal position and in the duplicated section, crossovers must be either yellow⁺ or yellow². Presence of the yellow¹ crossovers is evidence of no X duplication including the yellow locus.

Detectable translocations of attached-X with major autosomes must involve breakage near the tips of the latter; otherwise the segregation of X chromosomes which would allow detection of the rearrangement would also create severe aneuploidy which would be lethal. Chromosome 2 was chosen for these tests because of the availability of good stocks for testing. The two products of this type of translocation would be the centric X joined with the tip of one arm of chromosome 2 (hyperploid for tip of 2L or 2R), or the acentric X joined to the broken end of one arm of chromosome 2 (hypoploid for tip of 2L or 2R). Males carrying detachments unidentified in previous steps were crossed to attached-X females carrying four recessive markers on chromosome 2, γ ; $al\ b\ c\ sp^2$. The male offspring were backcrossed to females like their mothers. A small duplication of a tip of chromosome 2 including al^+ or sp^+ would be recognized by production of F_2 males showing three of the four recessives as well as those being normal for all four, while their sisters would either show all four recessives or none. The attachment of an entire X near the tip of chromosome 2 might be recognized by deficiency of one of

the end markers, the F_1 males showing this one recessive. In the F_2 , all males should be similar to the F_1 males and their attached-X sisters should be homozygous for the four recessives.

RESULTS

Table 1 summarizes the results of all tests described below.

Types of rearrangements produced in stage 7 oocytes

PARKER (1954a,b) has reported on some types of rearrangements which resulted in detachment of attached-X chromosomes. These results are included here, together with additional tests on the stocks which had not been identified at that time. In the case of detachments induced in the presence of Y fragments, tests for X-4 translocations and fertility tests have been carried out. With the $sc^s \cdot Y$ and the no-Y series, all of the appropriate tests described above have been carried out.

Detachments in no-Y females. It was reported previously that 61 out of 102 detachments induced in the absence of a Y were X-4 translocations. Tests of the remaining 41 failed to show any case of X deletion. Three cases of involvement of chromosome 2 were found, with two being hyperploid for 2L (al^+) and one hyperploid for 2R (sp^+).

Detachments with Y^S . Y^S #2. Tests for X-4 translocations showed nine such rearrangements out of 100 tested chromosomes. Of 23 of the remaining 90 tested, 18 were found fertile and were therefore $X \cdot Y^S$, while five were sterile in combination with $sc \cdot Y^L$.

Detachments with $sc^{V1} \cdot Y^S$. The acrocentric Y-short, $sc^{V1} \cdot Y^S$, gave a ratio of 68 detached X chromosome carrying the yellow⁺ locus to 42 which did not. Fifty of the former and 40 of the latter were tested for X-4 translocations and of these, eight of yellow² phenotype were found to have X and 4 linked. Fertility tests were run on 28 of the 32 not found to have X-4 translocations; 20 were found to be $X \cdot Y^S$. Forty of the 50 yellow⁺ stocks were tested for linkage of Y-short, and all proved to be sterile in combination with $sc \cdot Y^L$.

Detachments with $sc^{S1} \cdot Y^L$ #2. This is a nearly rod-shaped Y-long arising by inverted exchange with the distal end of the Muller-5 chromosome. Thirty-eight detachments, 22 of which were yellow² and 16 yellow⁺, were recovered. None of the yellow⁺ group had Y-long linked with the X, while two of the yellow² group where $X \cdot Y^L$, two were X-4, and 12 were unidentifiable by these two tests. The remainder were not tested.

Detachments with $sc \cdot Y^L$. In this case, of 67 detachments, 43 were yellow² and 24 yellow⁺. Ten of the yellow² class were X-4 translocations; 20 of the remainder were tested and three were found to be $X \cdot Y^L$. Nineteen of the yellow⁺ were tested for Y-linkage, and all were found to be sterile in combination with $sc^{V1} \cdot Y^S$.

Detachments with $sc^s \cdot Y$. PARKER (1954a) tested 68 detachments induced in females carrying $sc^s \cdot Y$ and found the following. There were 30 cases where yellow⁺ was linked with the X; nine of these proved to be $X \cdot Y^L$ while the remaining 21 were sterile in combination with either the Y-long or Y-short fragment. Thirty-eight were of yellow² phenotype; nine proved to be X-4 translocations, 19

TABLE 1
Estimated proportions of various types of rearrangements involved in detachment of attached-X chromosomes

Y Chromosome present	Stage treated	Rearrangement Types :									
		Translocations with Y					Translocations with autosomes				
		X ^s .Y ⁺	XY ^L .Y ^S	X.Y ^L .Y ⁺	X.Y ^S	X.Y ^L	XY ^S .Y ^L .Y ⁺	X Deletions	X-4	2L Hyper-ploids	2L or 2R Hyper-ploids
sc ^s .Y	7*	.31	.06	.13	.28	0	0	0	.13	0	0
sc ^s .Y	14	.16	.03	.02	.16	0	.02	.06	.10	.09	.04
no Y	7*	..	..	..	..	..	..	0	.60	.02	0
Y ^S .Y ^S # 2	7	..	..	..	.70	..	..	..	.09	..	..
sc ^{V1} .Y ^S	7	.61	..	..	.22	..	..	..	.08	..	..
sc ^{S1} .Y ^L # 2	7	.42	..	0	..	.08	..	..	.08	..	..
sc.Y ^L	7	.36	..	0	..	.07	..	..	.15	..	..

* Data on stage 7 with sc^s.Y or with no Y from PARKER (1954a), except for tests for X-deletion and chromosome 2 involvement, which are from current data.

were $X \cdot Y^s$, while four were $XY^L \cdot Y^s$ as the males were fertile in the absence of a free Y. The remaining six were not accounted for by the preceding tests.

Subsequently, tests have been run on these six. No cases of gross deletion of the X, nor of exchange with chromosome 2 were found. We can account for about 90 percent of detachments as being products of translocation between X and Y or X and fourth chromosomes.

Recent experiments have given a large number of detachments which were not analyzed, except to record linkage of yellow⁺ and linkage of X and fourth chromosomes. Of 1706 detachments obtained, 976 were recovered in females, 393 were yellow² males and 337 yellow⁺ males. Two hundred and six of the yellow² stocks were tested for X-4 translocations, and 30 cases of this type of rearrangement were found, which makes about eight percent of all male-viable detachments this kind of rearrangement.

Types of rearrangements induced in stage 14 oocytes

All detachments studied here had been obtained by irradiation of aged females carrying $sc^s \cdot Y$. Of 2116 detachments induced in stage 14 oocytes, 1449 were recovered in females, 553 were yellow² males, and 114 were yellow⁺ males. In a random group of 391 stocks which were male-viable and recovered in either sex, there was a ratio of 315 yellow² to 76 yellow⁺ stocks, i.e., about 20 percent carried at least a part of Y-long from $sc^s \cdot Y$.

Three hundred and seventy-three of the yellow² stocks were tested for X-4 translocations, and 48 such cases were found. This means about ten percent of all male-viable detachments are X-4 translocations.

A group of 138 detachments, all negative for X-4 translocations, were chosen for analysis. Thirty-three which carried yellow⁺ were tested with both Y arms for male fertility; three proved to be $X \cdot Y^L$, while three were fertile with no free Y, hence were probably $XY^s \cdot Y^L sc^s$. Otherwise, there must have been some reorganization of the $sc^s \cdot Y$ in these three cases so that the sc^s piece was no longer on the tip of Y-long, as in the case of $sc^s \cdot Y:bw^+$, as shown by BAKER (1955). The remaining 27 were sterile in combination with either Y-short or Y-long fragments.

One hundred and five of the yellow² stocks were tested with both Y fragments for fertility. Twenty-three were found to be $X \cdot Y^s$, and four were $XY^L \cdot Y^s$ since these gave fertile males with no added Y fragment. This makes about 19 percent of all detachments X-Y translocations of these two types.

The exchanges between chromosome X and 4 or Y, which accounted for 90 percent of all detachments in stage 7 can account for about 50 percent in stage 14. The great excess of yellow² males suggested the occurrence of types of rearrangement where the preferred detachment (the centric X) would segregate from the $sc^s \cdot Y$ at the first meiotic division, hence no free $sc^s \cdot Y$ would be recovered. This would mean recovery of a centric X with a duplication of the tip of an X or of an autosome.

Seventy-four of the 77 yellow² stocks which were negative in fertility tests were tested for duplication of the tip of X; nine such cases were found, with a dupli-

tion carrying the mutant, γ^2 . These account for about six percent of all detachments.

Sixty-three of the remaining 65 stocks were tested for involvement of chromosome 2; 12 had duplications of 2L (al^+) linked with X, four had 2R (sp^+) linked with X, and five had the entire second chromosome linked, including normal alleles of all of the four markers used. In addition, these flies are not Minute, hence they are not deficient for the $M(2)33a$ locus. The break in chromosome 2 in each case must lie outside the genetic map. ABRAHAMSON, HERSKOWITZ and MULLER (1956), who recovered one case of this same type, likewise did not find it deficient for any known locus on chromosome 2. Identifiable exchanges with chromosome 2 are found to account for about 16 percent of all recovered male-viable detachments.

Without testing for ends of chromosomes 3 or one end of chromosome 4, we are able to account for over 70 percent of all detachments as being translocations or deletions. It is likely that there are also exchange products involving breaks in the chromosomes distal to the markers being used in these tests, so that hyperploidy is not detectable. The indirect evidence for a significant number of these will be discussed below.

Non randomness of recovery of complementary exchange products

One of the questions of significance is the nature of the exchanges induced: that is, do these involve chromatids or whole chromosomes? There are two lines of evidence on this. In experiments with individual females, it is possible to recover a detached X with part of the Y chromosome linked to an X, and to recover an entire Y chromosome at the same time, where the original treated female had only one Y chromosome. Two cases have been found in which an $X \cdot Y^s$ chromosome was recovered along with an unaltered Y; in one case the Y was $sc^s \cdot Y$, in the other $sc^{V1} \cdot Y^s$. Both of these recovered Y's were subjected to male-fertility tests to show their completeness. In both cases, all of their attached-X sisters were yellow² as would be expected if the mother had only one Y chromosome.

Another basis for the conclusion that exchanges involve individual chromatids is the non randomness of recovery of complementary exchange products. In stage 7, detachment in the absence of the Y gives about 60 percent X-4 translocations; the complementary type, having the heterochromatic portion of chromosome 4 linked with X, can make up no more than 38 out of the 102 cases tested. In the $sc^s \cdot Y$ series, there were 21 cases where only the yellow⁺ was linked with X, while the complementary product, $XY^L \cdot Y^s$, was represented by four cases. The ratio of $X \cdot Y^L$: $X \cdot Y^s$ was 9:19. Similar figures are found in the case of each Y chromosome fragment used, where complementary types may be recognized. Detachments in stage 14 oocytes give similar figures. There were 23 $X \cdot Y^s$: 3 $X \cdot Y^L$ and 27 yellow⁺ (without Y-long fertility): 4 $XY^L \cdot Y^s$. Since the sequence is $sc^s \cdot Y$ is γ^+ $Y^L \cdot bb^+$ Y^s , breakage of this chromosome must be about equally divided between the long and short arms, with breakage in the short arm usually being proximal and in the long arm distal to fertility factors.

The basis for interpretation of this is the finding by NOVITSKI (1951) that there is non random segregation of unlike chromatids in the second meiotic division, with the shorter chromatid being more likely to be included in the egg nucleus. The cases studied at that time gave about a 2:1 recovery of the shorter chromatid. More recently, NOVITSKI and SANDLER (1956) have been able to extend the applicability of the principle of non randomness to include the tandem acrocentric compound X, where they found coefficients of non randomness as high as 0.92.

There was one difficulty in making the interpretation that non randomness implied chromatid exchanges. The results obtained with use of $sc^{V1} \cdot Y^S$, where the majority of detachments carried the yellow⁺ marker and about one third as many were $X \cdot Y^S$, required the assumption of a high frequency of breakage in the Y between the centromere and yellow⁺. Since this Y is derived by crossing over of a normal Y chromosome with the pericentric sc^{V1} inversion, this piece would be expected to be very small in relation to the short arm of the Y.

Irradiation of reversed acrocentric compound X's with $sc^{V1} \cdot Y^S$ gave a total of ten detachments, all the proximal (centric) X. In four of these cases, the yellow⁺ was linked with the detached X; the remainder were not tested. Since the recovery of the centric X requires the recovery of the acentric fragment of the other broken chromosome, this shows a high breakage frequency between the centromere and yellow⁺, as expected. The reversed acrocentric used was $\gamma ac sc pn-sc^s$, supplied by LINDSLEY. These irradiated females, also carrying $sc^{V1} \cdot Y^S$, were crossed to yellow *Muller-5* males carrying the $\gamma sc^s \cdot Y$ of LÜNING, thus allowing males with the detached proximal X deficient for the yellow and achaete loci to survive.

Chromatid translocations involving autosomes should allow recovery of the centric X more often than the acentric for another reason. Figure 1 shows the sixteen possible products of a primary oocyte in which such an exchange has occurred. It can be seen that the centric X should be detected in three and the acentric in only two of the sixteen products. In case of translocations with the Y chromosome, since the Y normally should segregate from the X at the first division, the difference in recovery of centric and acentric X's should depend primarily on non random second division segregation.

An alternative interpretation of non randomness has been suggested by ABRAHAMSON, HERSKOWITZ and MULLER (1956), where they conclude that the higher rate of recovery of "capping" than of "capturing" is due to the relative inviability of the two classes of aneuploids, with hyperploids being more viable than hypoploids. This argument might apply with more force in stage 14 than in stage 7 oocytes, and in the case of X-autosomal translocations than with X-Y translocations, where the hypoploidy of the X in many cases would be covered by the hyperploidy of a segment of the Y. In every case the hypoploid X would be recovered either with a complete X or a complete Y chromosome, and if breaks are confined to the proximal heterochromatin, there should be little effect on viability. However, there is non random recovery of complementary products with all testable types of rearrangement.

FIGURE 1.—Diagram of a translocation between attached-X and an autosome, showing products of first and second divisions, to show inequality of chromatids which will separate at Anaphase II. A, autosome; Y, sc⁸-Y; X·X, attached X; X_c, “centric” X; X_a, “acentric” X; X_a-A, “acentric” X attached to tip of autosome.

* This product dies.

** This product cannot be distinguished from attached-X without further testing.

The argument of aneuploidy can hardly apply in the case of detachment of reversed acrocentric compound X's involving exchanges with a Y chromosome, as X hypoploidy should be as likely in the proximal (centric) as in the distal (acentric) X, since breaks anywhere in the interstitial heterochromatin (or perhaps even in adjacent euchromatic regions) may give rise to centric-X detachments. The number of cases of this type reported here is small, but in each of the ten cases the proximal X was the one recovered.

The interpretation of non randomness as the result of chromatid translocations means that where breaks occur to give rise to recoverable detachments, the centric X must be the one more often recovered. The centric X will rejoin with the acentric tip of another chromosome, so that the single X will be smaller than the complete attached-X it will separate from at the second division. The acentric X must be joined near the tip of another chromosome, Y or autosome, and this rearranged chromatid must therefore be longer than the normal chromatid it separates from at the second division. We may expect the coefficients of non randomness to vary considerably for the different types of exchange, and it would be difficult to estimate the frequencies of unidentifiable exchange products.

Another argument for the recovery of the centric X in most cases is the relatively infrequent recovery of the unaltered sc^8 ·Y along with the detached X. If normal first division segregation occurs, the centric X is separated from the unbroken sc^8 ·Y; the acentric X, joined to the centric part of another chromosome, can be recovered separately only if it separates from the centric X at the first division. Hence, recovery of the acentric X should also mean recovery of sc^8 ·Y unless nondisjunction occurs. There are some reasons for believing this may not always be the case.

The recovery of the centric X should require that the individual be hyperploid for the tip of the Y or autosome involved in the translocation, while the recovery of the acentric X implies a corresponding hypoploidy. In the case of a major autosome, chromosome 2, it is possible to distinguish between these two types, while with rearrangements involving Y or fourth chromosomes there are difficulties in distinguishing directly between the two products. Recovery of the centric X with 4 linked should always be in an individual hyperploid for the euchromatic part of chromosome 4: i. e., the egg should have a free fourth chromosome in addition to the four attached to X. Breakage of chromosome 4 in the heterochromatic arm likewise can give rise to a chromosome showing X-4 linkage. In this case, it would be expected to be recovered along with sc^8 ·Y. In stage 7 oocytes, out of 30 X-4 translocations, 26 carried two free fourth chromosomes, while four cases had only one. In stage 14, out of 48 X-4 translocations, 36 carried two free fourth chromosomes and 12 had only one. In only two cases, X-4 translocations induced in stage 7 oocytes were recovered with sc^8 ·Y, while none of those induced in stage 14 were recovered with the sc^8 ·Y.

Another difficulty lies in the failure to recover sc^8 ·Y in any of the five cases where the acentric X was linked to chromosome 2. One would have to assume a

high rate of nondisjunction of Y and centric X to account for the repeated failure to realize the expectation with this type of exchange.

We have been able to identify the chromosomes involved in detachments in stage 7 oocytes in about 90 percent of the cases, and in stage 14 oocytes in over 70 percent of the cases. The high frequency of involvement of chromosome 2 in the latter case might lead us to expect some increase in involvement of chromosome 3. However, there are several possibilities involving chromosomes tested for, which cannot be excluded. If breakage in the heterochromatic arm of chromosome 4 is high, non randomness would favor the recovery of the centric X with the unmarked chromosome 4 arm. This, too, could account for a significant number of the unidentified cases. The finding of five cases of linkage of X with the entire chromosome 2, with no evidence of breakage within the genetic map, i.e., between *aristales* and *Minute* (2) 33a, means that the complementary type, hyperploid for the unmarked tip of chromosome 2 which has joined the centric X, could not be detected by these tests. This product should be greatly favored in the second division because of the great inequalities in sizes of separating elements. These five cases of apparent translocation of an entire X to the end of the entire second chromosome are of the kind which WHARTON (1943) found to have occurred in the case of three *Drosophila* species: *spinoformosa*, *testacea*, and *tranquilla*.

Lethality

The problem of the origin of lethality in detachments of attached X has been discussed (PARKER 1953, 1954a) and the conclusion reached that recessive lethals may accumulate in the proximal region of attached-X chromosome, and perhaps ultimately become balanced by other very closely linked spontaneous lethals. The basis for this interpretation is the difference in behavior of several different attached-X lines. There is very little induced lethality resulting from rearrangement. These data were obtained by the irradiation of stage 7 oocytes; when stage 14 cells are treated, a different situation is found. This difference may be seen in the various tables of PARKER and HAMMOND (1958), where relatively about twice as many females per male carrying the detachments are found resulting from treatment of stage 14 as compared to stage 7. In the stage 7 results, there is a ratio of 976 females to 730 males in the detachment classes, giving 1.34 females per male. In stage 14, there were 1449 females to 667 males, or 2.17 females per male.

Tests for viability of males carrying the detached X recovered in females were carried out on 267 females derived from treated stage 14 oocytes; 65 were sterile (24 percent), and 68 of the remaining 202 X chromosomes were lethal in the male (34 percent of fertile females). When stage 7 cells were treated, a total of nine out of 110 tested chromosomes were found to be lethal in the male (eight percent of fertile females).

The difference in sex ratio may be accounted for by the increase in induced lethality. It is evident that many changes are sublethal, as many of the females derived from treated stage 14 show very low fertility and poor recovery of males

carrying the detached X. The high frequency of sterility, low fertility, and the general appearance of the detachment females recovered suggest aneuploidy.

That the increased lethality is due to the rearrangement and not to independently induced recessive lethals is shown by data obtained by irradiation of Oregon-R females of similar ages with similar doses. Oregon-R females were irradiated in each stage, and lethal rates measured by the Muller-5 method. Stage 7 oocytes given 2000r of X-ray gave an F_1 ratio of 1298 females to 1346 males. Tests were made of 1209 F_1 females; 20 were sterile, and 39 of the remaining 1189 chromosomes were lethal in the male. When stage 14 was treated with 500r of X-ray, the F_1 count gave 1437 females and 1398 males. Tests of 1129 F_1 females showed 20 to be sterile, and 13 of the remaining 1109 chromosomes were lethal in the male. The types of changes which may be induced in the free X chromosome cannot account for the induced lethality in the detachments. The greater variety of rearrangements possible in stage 14 has already been seen. Perhaps a higher frequency of types of rearrangement which can give rise to more severe aneuploidy is the basis for the increased frequency of lethal detachments in stage 14 oocytes.

GENERAL DISCUSSION

Various interpretations have been advanced to account for differences in response to irradiation of cells of different types. KAUFMANN and WILSON (1949) suggest that failure of breaks to rejoin in *Drosophila* sperm until after sperm entrance is due to restriction of movement in the sperm nucleus. CROUSE (1950) similarly accounts for failure to obtain translocations by irradiation of *Sciara coprophila* oocytes as due to the "failure of non-homologous chromosomes to contact each other before restitution or intra-chromosomal rearrangement has occurred."

GLASS (1955) has pointed out some difficulties in making comparisons of relative frequencies of different types of rearrangements in oocytes and in mature sperm of *Drosophila*, particularly in the case of complete recovery of a chromatid translocation in oocytes. In addition to this difference, one should recognize the effect of non random disjunction on the recovery of each of the kinds of aberrations. If the exchanged segments in translocations are grossly unequal, one might conceivably lower the probability of recovery of the complete translocation to less than one percent. Deficiencies induced in females should be favored in recovery while inversions presumably would behave as normal sized chromosomes. Some inversions would be lost through anaphase bridge formation if appropriate chiasmata occurred, but a similar number of cases might be expected where potential radiation-induced bridges would be resolved by the same chiasma configuration. In the absence of sister-strand crossing over, breaks at different loci in two sister chromatids could give rise to deficiency (or duplication) but not to inversion.

WHITING (1945) has interpreted the differences in sensitivity of different meiotic stages in the *Habrobracon* oocyte in terms of the condition of the chromosomes: "Tension is the main factor in determining the permanence of breaks

caused by ionizations, chromosome form and movement in determining the nature of the new combinations of broken ends." BOZEMAN and METZ (1949) correlate the change in sensitivity in *Sciara ocellaris* oocytes with the breakdown of the nuclear membrane, postulating intervention of a cytoplasmic agent which is radiation-induced or which sensitizes the chromosomes to radiation. They suggest alternatively that some healing process ("formation of matrix material") may intervene in the less sensitive stages before rearrangement may occur.

It is possible to distinguish at least three levels of sensitivity in the *Drosophila* oocyte. PARKER (1953, 1954a) was able to show that detachments of attached-X chromosomes were obtained almost exclusively from the oldest 60–90 oocytes in newly emerged females. PARKER (1955) and PARKER and HAMMOND (1957, 1958) obtained a still higher level of sensitivity by aging females three or more days before treatment, and suggested the possibility of differences in oxidative metabolism as a basis for differences in sensitivity of stages 7 and 14 oocytes. If this is true, then failure of rejoining of many breaks in mature sperm might be due to nonavailability of energy required for rejoining rather than limitations of space or distribution within the sperm nucleus. SPARROW and MALDAWER (1950) found a difference in ratio of acentric fragments to dicentrics and rings in Metaphase I and postmeiotic interphase cells in *Trillium*, and concluded that the more sensitive Metaphase I cells showed less rejoining of breaks, but concluded that differences in sensitivity must in part be due to differences in the number of primary breaks induced. KING, DARROW and KAYE (1956) likewise can account for at least three levels of sensitivity in the *Drosophila* oocyte. KING (1957) accounts for these differences in part at least by differences in diffuseness of the chromatin.

Table 1 shows the considerable difference between stages 7 and 14 in kinds of rearrangements found. On the basis of the evidence available it is not possible to account for the difference in behavior of the two stages. As has been suggested by various authors, the changes could be due to changes in nuclear structure, with greater coiling and with closer association of non-homologous chromosomes in stage 14; this would be consistent with the description given by KING, RUBINSON and SMITH (1956). This would account for the increase both in X deletions and in autosomal translocations, relative to X-Y translocations. BOZEMAN and METZ (1949) found comparable changes in types of rearrangements in *Sciara*. On the other hand, since breaks rejoin more slowly in stage 14, it is possible that restitution is much less likely than in stage 7, and much more widely separated breaks are able to join together to give a greater variety of rearrangements.

These results may also suggest a reason for the difficulty in inducing interchromosomal rearrangements heretofore. Stage 7 oocytes give very few detachments involving the major autosomes, and it seems unlikely that many translocations between two major autosomes or between a free X and a major autosome could be induced. Stage 14 would be much more likely to give recoverable translocations by the usual procedures. However, the dosages most probably employed would guarantee recovery almost exclusively of stage 7 products. At a dose of

about 2500r, 75 percent of stage 7 would survive, while less than 1.5 percent of stage 14 oocytes would remain viable (PARKER 1955; SONNENBLICK 1940). It is possible that a dose of about 500r given to stage 14 might give rise to a variety of rearrangements which would be recoverable with suitable screening methods, such as the bithorax method of LEWIS (1954).

SUMMARY

Tests were made on a number of detachments of attached-X chromosomes induced either in young females (stage 7 oocytes) or aged females (stage 14 oocytes). These were tested for translocations with Y, second, or fourth chromosomes and for gross deletion of an X chromosome. It is possible to account for about 90 percent of detachments in stage 7 and 70 percent in stage 14 by these types of rearrangement. Aging results in an increase in frequency of autosomal translocations relative to X-Y translocations, with the relative increase in involvement of chromosome 2 being greater than in chromosome 4. There is also a significant number of X deletions in stage 14, while none were found in stage 7.

It is concluded that the rearrangements involve individual chromatids, since complementary translocation products are recovered in unequal numbers. It is suggested that a large number of these rearrangements may be hyperploid for small unmarked tips of the various chromosomes involved since one class of rearrangement involved breakage of chromosome 2 distal to the terminal markers.

The increased lethality of detachments induced in stage 14 is attributed to the increased variety of rearrangements involved, with greater possibility of more severe aneuploidy. The induced lethality cannot be accounted for by the independent induction of recessive lethals or sublethals in the detached X.

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