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CURRENT PROBLEMS IN RESEARCH

Genetics of Mammalian Sex Chromosomes

Mouse studies throw light on the functions and on the occasionally aberrant behavior of sex chromosomes.

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In the past two years or less there has been a veritable explosion of knowledge in the field of mammalian cytogenetics, and a great many of the important findings concern the sex chromosomes, particularly in the mouse and in man. Much of the sudden progress can be attributed to recent advances in cytological techniques, and this is particularly true in man. In the mouse, however, it is the combination of cytological analysis with genetic experimentation, and, in many cases, even the latter by itself, that has yielded several exciting results. The genetic work would not, of course, have been possible before the discovery of useful sex-linked marker genes and of other genetic tools which have become available only in relatively recent years.

Discussions in this article focus primarily on the results of experimental work in the mouse, with special emphasis on new and hitherto unpublished findings. No attempt is made to review the voluminous literature on sex-chromosome anomalies in man, but evidence from this literature is cited wherever it helps in the presentation of a well-rounded picture. The findings discussed shed light on the functions of the sex chromosomes, both with respect to sex determination and with respect to other actions, and on the mechanisms by which anomalies in chromosome number come about.

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Sex Determination

With the exception of a few species (discussed below), mammals have an XX-XY type of sex-chromosome mechanism, the male being the heterogametic sex. *Drosophila melanogaster*, too, has such an XX-XY mechanism, and in that species it has been known for over 30 years that sex determination is the result of a balance of female determiners on the X chromosome and of male determiners on the autosomes, the Y chromosome being inert (although necessary for male fertility). Only two years ago it was shown that mammalian sex determination is not of the *Drosophila* type. Specifically, the Y chromosome, instead of being inert, is strongly male-determining. In this respect, therefore, the mammalian system resembles that of the plant *Melandrium*.

The male-determining property of the Y chromosome was demonstrated in the mouse by combined genetic and cytological evidence which indicated that animals with the XO sex-chromosome constitution were normal and fertile females (*1*, *2*). These females were detected by virtue of the fact that they exhibited the phenotypes normally produced by certain sex-linked marker genes in males only. Breeding experiments (which, in cases where the phenotype was sublethal, involved ovarian transplantation) ruled out several of a number of possible alternative hypotheses and pointed strongly to the XO explanation. Final evidence for the XO hypothesis was provided by the cytological data, which indicated that the exceptional females had 39, instead of 40, chromosomes.

Almost simultaneously with our reports of the mouse experiments, there appeared in the literature reports of parallel findings in the human species. Thus, Ford *et al.* (*3*) found the XO sex-chromosome constitution in a female suffering from gonadal dysgenesis, and Jacobs and Strong (*4*) demonstrated the association between the XXY constitution and the Klinefelter syndrome (small testes, variable endocrine disorders). Since then, a great many more cases of both of these conditions have been reported. Chromatin-positive Klinefelter males, presumably XXY, occur relatively frequently in human populations (*5*). On the other hand, the XXY type had not been found in the course of the XO experiments in the mouse, even though the markers used in crosses for the detection of XO would, in most cases, also have led to detection of XXY. When, for some time thereafter, subsequent experiments also failed to yield this type, we concluded that either XXY mice occurred very rarely or that they were, for one of several reasons, undetectable. Thus, XXY might be female, or a female-like intersex; or XXY might be inviable; or the expression of the sex-linked markers used might be altered by the presence of the Y.

Doubts concerning detectability have now been dispelled by our recent finding of an XXY mouse in crosses marked by sex-linked genes. The XXY constitution, detected by genetic means, has been verified by cytological studies (*6*) (Fig. 1). The exceptional animal is

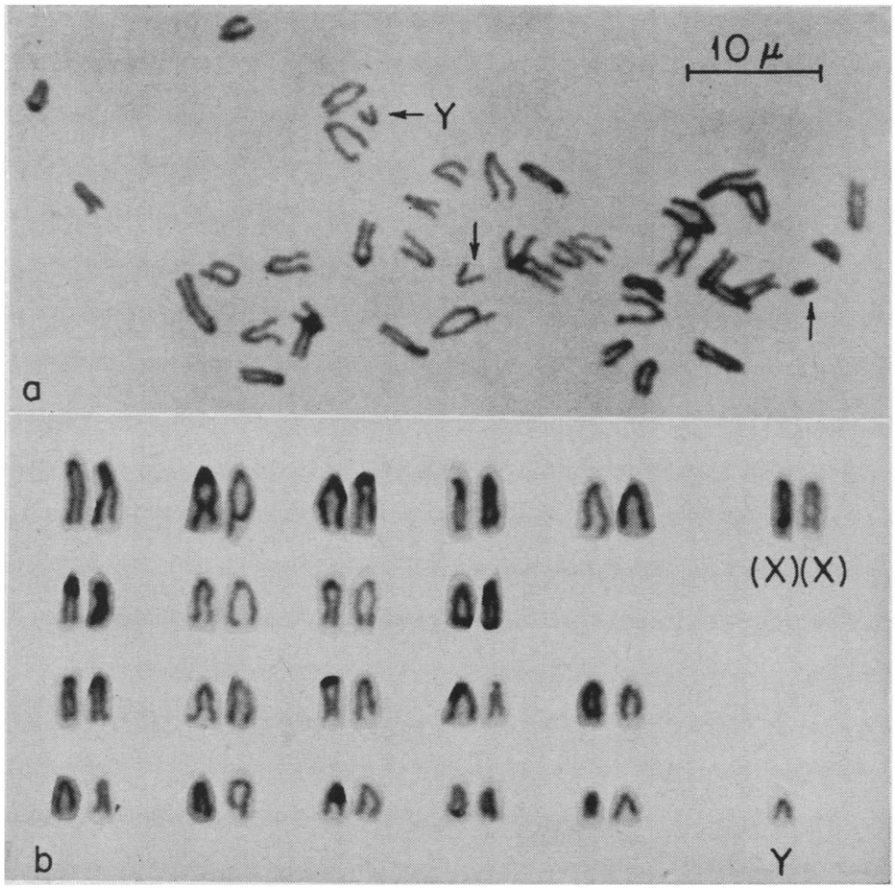

Fig. 1. Chromosomes of a mouse presumed, on genetic evidence, to be XXY. The cytology shows that this is, indeed, the case. (*a*) A metaphase cell in tissue culture derived from tail biopsy. There are 41 chromosomes. The Y chromosome is labeled. The unlabeled arrows indicate the smallest autosome pair. (*b*) The chromosomes of the same cell arranged in pairs, showing the presumed X-chromosome pair and the Y chromosome. [From Russell and Chu (*6*)]

viable, of normal size and male phenotype. It shows normal male mating behavior, but is apparently sterile. Its genotype is $+/Ta/Y$, and the expression of the marker *Ta* (= tabby) is not affected by the Y. Evidence derived from this animal strengthens an earlier report of a presumed XXY mouse (*7*) for which no cytological proof could be obtained because of the animal's untimely death.

In summary, then, it can be said that, both in the mouse and in man, XO is female or female-like, and XXY, male or male-like—clearly the opposite from *Drosophila*, where XO is male and XXY is female. In man, the deviation of the chromosomally aberrant individuals from sexual normality may be somewhat greater than in the mouse. On the other hand, this difference between the species may be only an apparent one, since, as has been pointed out (*8*), the more extreme cases among human XO and XXY individuals would selectively come to the attention of medical investigators. In any case, it is quite clear that, far from being inert, the Y chromosome in mammals is strongly male-determining, a conclusion that has not been altered by the discovery, during the past year, of several aberrant sex-chromosome types in man, in addition to XO and XXY (for example, XXX, XXXY, XXXX, and XXYY).

Not all mammals have the classical XX-XY sex-chromosome mechanism. The few exceptional species (among the well over 200 that have been studied cytologically) have recently been discussed by White (*9*). In three or four species, the male is XY_1Y_2. Five other species had been reported to have XO males. If true, this would have indicated that male determination by the Y chromosome, as demonstrated in mouse and man, did not occur in all mammals. However, White gives reasons for suggesting that, in at least some of the exceptional species, the Y is not really lacking but is present in a fused state. His general conclusion is "that the Y is an indispensable part of the mammalian sex-determining mechanism . . . and that it has probably not been lost on any occasion in mammalian evolution."

The demonstration that the Y is strongly male-determining has given us a basic insight into mechanisms of mammalian sex determination, but many questions remain. Are there male determiners also on the autosomes? How are the female determiners distributed? It seems improbable, from the available evidence that the X is strongly female-determining—that is, that female determiners are preferentially located on the X; for, if this were the case, both XO and XXY should be much more in the direction of intersexuality than they actually are. In the mouse, at least, it is not necessary to assume any female determiners at all on X, since the slight difference between XO and XX can be explained in terms of general vigor rather than of amount of "femaleness"; and the sterility of XXY could also be due to dosage phenomena of genes not necessarily directly concerned with sex determination. Thus, it is conceivable that in XXY males there are disturbances due to the presence of gene products of *two* sets of certain sex-linked genes in a Y-determined male developmental system which is normally in harmony with a smaller quantity of such gene products (*10*). It is also possible that X and Y have a short homologous region and that sterility may be the result of imbalance due to trisomy for this region.

Genetic Content of Y and X

The fact that the Y chromosome in mammals is so strongly male-determining indicates that it is not genetically empty. Yet, no other genes are definitely *known* to be located on the Y, although some are suspected. In the mouse, the so-called Eichwald and Silmser effect—that is, the rejection of within-strain tissue grafts when the donor is male and the recipient female—has been explained on the basis of Y-linked genes controlling antigenic differences (*11*). No other Y-linked genes, however, are known, in spite of

the fact that the conditions for both the induction and the detection of such mutations have been most favorable. In man, the formerly classical case of completely Y-linked inheritance, the "porcupine man," has recently been shown to be false, and the evidence for Y-linkage is considered incomplete in the case of other traits often assumed to show this kind of inheritance (*12*).

Partially sex-linked genes (presumably carried on portions of X and Y which are homologous to each other) have been reported in man, but, recently, doubt has been thrown on the reliability of the evidence (*13*). In the mouse, too, earlier reports of partial sex linkage have not been verified by more recent results. It may be that the homologous portions of X and Y are extremely short, as seems also indicated by cytological observations which reveal end-to-end pairing of the X and Y in the first meiotic division of the male mouse (*14*). The Y is the shortest of all chromosomes in the mouse (*6*) and in most primates (*15*). In man it is among the three shortest (*16*).

In contrast to the Y, the X chromosome bears a number of genetic factors. In the mouse, about 20 sex-linked mutations have occurred, more than half of them at this laboratory. A curious finding, however, is that the majority of the X-linked mutations are associated with a particular phenotype—namely, a dominant mottling of the fur—and are usually lethal in the male. Several of these "mottling" mutations have been located at the same, or approximately the same, spot on the X chromosome, and it is not inconceivable that they represent some type of chromosomal change rather than true gene mutations.

V-type Position Effect

One interesting discovery made at this laboratory in the last few years is that the X chromosome of the mouse, when it is involved in certain chromosomal rearrangements, has the power to produce so-called variegated-type (or "V-type") position effects (*17*). V-type position effects have been known for years in *Drosophila* (*18*) but had never been demonstrated in any other animal. At least four of them are now known in the mouse (*19, 20*).

The best-studied case of V-type position effect—that involving the brown locus—is used as an example in the discussion that follows (see Fig. 2). Experimental findings indicate that this position effect is probably the result of a reciprocal translocation between the X chromosome and chromosome 8, which bears the brown locus. That is, it is assumed that these two chromosomes have exchanged pieces, so that the brown locus—which carries the normal, or "wild-type," allele (+)—has come to lie in the vicinity of a piece of X chromosome. Normally, this + allele is completely dominant over the gene *b* (brown), so that the combination of + in one chromosome and *b* in the homolog gives a wild-type animal. However, when the + gene has been transposed to its new position near a piece of X chromosome, its action is impaired, or made uncertain, in some of the somatic cells. Consequently, with *b* present in the homologous chromosome, certain regions of the body are brown, even though the animal is only heterozygous for the recessive *b* gene. The random mingling of brown and wild-type regions produces what is called, in accordance with *Drosophila* terminology, a variegated phenotype. Rigid proof of the genetic basis of this system has been sought in a number of ways that are too complex to go into here (*19*).

Two points are of particular interest for the purposes of this discussion. It has been shown in *Drosophila* that the basis for the great bulk of V-type position effects is change in location of a euchromatic gene to the vicinity of heterochromatin. Four cases of V-type position effect are known in the mouse, and in all four of them the gene in question has been brought to lie in the vicinity of a piece of X chromosome. Although the number of rearrangements of autosomes with other autosomes is presumably a large multiple of the number of rearrangements of autosomes with the X chromosome, no V-type position effect has yet been found that does *not* involve the X. The dispropor-

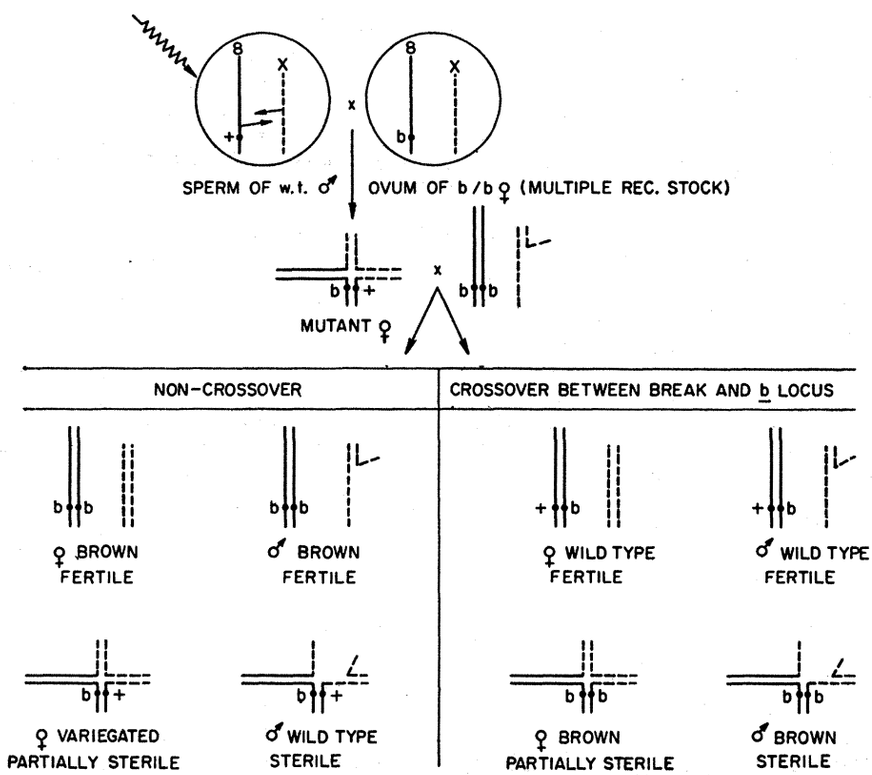

Fig. 2. Interpretation for the origin and transmission of position effect at the *b* locus. A reciprocal translocation between chromosome 8 (solid line) and the X chromosome (dashed line) has placed the wild-type allele of *b* (+ symbol) in the vicinity of a portion of the X chromosome. As a result of this new position, the action of the wild-type allele (+) is made "uncertain." Thus, + does not exert its normal dominance over *b* in all cells of the body, and the animal is variegated with brown. Note that this applies to females only (see text). A cross of such a variegated female and a normal brown (*b*/*b*) male produces eight types of progeny, as a result of segregation and crossing over. [From Russell and Bangham (*19*)]

Table 1. Dependence of the expression of the V-type position effect in the mouse on the number of X chromosomes present.

Constitution of chromosomes affected by rearrangement*	Sex chromosomes	Phenotype
8^X, X^8, 8, X	XX	Variegated
8^X, X^8, 8, Y	XY	Wild-type
8^X, X^8, 8	XO	Wild-type
†	XXY	Variegated

* The symbol 8^X denotes a translocated chromosome composed of the centromere and the proximal portion of chromosome 8 plus the distal portion of the X chromosome. The composition of X^8 is the converse. The gene for brown (*b*) is present on the intact chromosome 8, while the rearrangement carries the wild-type allele. † Rearrangement involving chromosome 1 and the X chromosome; also, intact chromosomes 1, X, and Y (*20*).

tionately great power of the X to produce such effects in the mouse would seem to indicate that there may be very little heterochromatin on the autosomes, while the X is strongly heterochromatic. The work of Ohno and his collaborators (*21*) brings cytological corroboration of this conclusion, which we drew on genetic grounds.

A second point of interest is derived from the finding that the rearranged position of the wild-type gene produces variegation in females only. Males bearing the rearrangement are nonvariegated—that is, wild-type (see Fig. 2, noncrossover progeny). In other words, in males the gene acts as though it were in its normal position.

In the early stages of our investigations, the most likely explanation for this sex difference seemed to lie in some suppressing action of the Y chromosome. In *Drosophila,* supernumerary Y's are known to suppress position effects, and it was, therefore, considered not impossible that, in the mouse, the single Y normally present in the male might be sufficient to suppress variegation. Recent results, however, lead to a different interpretation.

Certain females have occurred in our stocks which, although they carried the X-8 translocation, were wild-type rather than variegated. Breeding tests revealed that these animals lacked the intact X chromosome—that is, they were essentially XO (*22*). Thus, females with the translocation and an intact X (that is, carrying a total of two X's) are variegated; males with the translocation and the Y (carrying a total of one X) are wild-type; and females with the translocation but lacking the intact X (carrying a total of one X) are also wild-type (Table 1). We therefore conclude that the presence of *two* X chromosomes is necessary for the expression of the V-type position effect. This conclusion has recently been confirmed by Cattanach's finding of XXY males carrying an X-autosome rearrangement. As expected on the basis of our hypothesis, these animals were variegated (*20*).

A particularly interesting aspect of the foregoing conclusion—that the presence of two X chromosomes is necessary for the expression of what is presumably a heterochromatic effect—is that it can be related to a number of independent findings. In the hamster, Yerganian (*23*) has noted that females have one "predominant" X (which duplicates at the same time as the autosomes and has a euchromatic short arm) and one "retarded" X (which duplicates later in the cycle and has a heterochromatic short arm). The single X of the male is of the "predominant" type. A possible interpretation of this "triheterosomic scheme of sex determination" is that the X may assume temporary states that are determined by the company in which it finds itself. In man, cytological investigations on normal individuals, as well as on patients with an abnormal number of sex chromosomes, have indicated that the number of heteropyknotic chromosomes at somatic prophase, or of "sex-chromatin bodies" at interphase, is, in general, always one less than the number of X chromosomes present (*24*). These various findings permit the hypothesis that, in mammals, genic balance requires the action of *one* X in a manner which precludes realization of its heterochromatic potentialities, so that only *additional* X's present assume the properties characteristic of heterochromatin.

Obviously, the results described for the V-type position effect fit into the framework of this hypothesis. When two X's are present, heterochromatic action of one is possible and produces the expression of the position effect, since the other X chromosome, inherited from the father, fulfills the requirement of nonheterochromatic action. When, however, no paternal X chromosome is present—that is, when the father provides a Y or no sex chromosome at all, as in the case of the XO—then the rearranged X must fulfill the nonheterochromatic functions and will not be able to produce the position effect.

Aberrant Behavior of Sex Chromosomes

It is true in almost all fields of science that accidental or abnormal processes can shed much light on the nature of the normal mechanisms, which would otherwise remain hidden. In the case of the sex chromosomes it has already been shown how the abnormal sex-chromosome types have elucidated the manner of sex determination, and how, in conjunction with position effect studies, they have thrown light on gene action in the X chromosome. Naturally, the problem of how the abnormal sex-chromosome types are produced is of the greatest interest.

Abnormal sex-chromosome types may involve rearrangements of *portions* of sex chromosomes, and the study of these may turn out to be very profitable at a future time. The present discussion, however, is limited to the simpler types of aberrations on which some evidence is already available—namely, those involving changes in *number* of sex chromosomes, specifically the presence of a supernumerary sex chromosome or the absence of one of the sex chromosomes. The mechanisms that can be postulated as leading to these aberrations are grouped, for the purposes of this discussion, under the headings of nondisjunction and of chromosome loss other than that resulting from nondisjunction (which, for the sake of brevity, is referred to below merely as "chromosome loss"). Nondisjunction may broadly be defined as the failure—for one of several possible reasons—of two members of a chromosome pair to separate to the two daughter cells. It results in one daughter cell's having both members of the pair and in the other cell's having neither. In discussing frequency of individuals with an abnormal number of sex chromosomes, "nondisjunction" and "chromosome loss" must therefore be considered separately, since nondisjunctional events are expected to produce, with equal probability cells with a supernumerary sex chromosome and cells lacking a sex chromosome, while chromosome loss (as a result, for example, of anaphase lagging) leads only to production of cells of the latter class.

Both nondisjunction and chromosome loss can, theoretically, occur in the meiotic divisions of either the father or the mother of the affected individual, or they can occur during

the cleavage divisions of the affected individual himself. A knowledge of the relative frequencies with which these various events take place is of considerable basic interest. The acquisition of this knowledge, however, is beset with practical difficulties. In the first place, the total frequency of abnormal sex-chromosome types is low. More important still is the fact that in only a few of them can there be any certainty about the causation of the abnormality. In human beings there is the added difficulty that the frequencies are distorted through the selection of patients. Nevertheless, it is already possible to draw certain conclusions, mainly from our data on the mouse, gathered during studies in which, using five sex-linked markers, we have made dozens of different types of crosses for the elucidation of various problems.

Theoretical Expectations

Figure 3 shows schematically the various possible divisions at which nondisjunction of the sex chromosomes can occur in meiosis, and the gametes that are produced. The subscripts *1* and *2* distinguish the centromeres of the two maternal X chromosomes. It should be noted that any sex-linked markers present may have changed places as a result of crossing over (for example, X_1 X_2 may be homozygous, rather than heterozygous, for a sex-linked marker). The four different types of abnormal sperm, on fertilizing X-bearing eggs, would produce X^MX^PY, X^MO, $X^MX^PX^P$ and X^MYY zygotes (where the superscripts *M* and *P* designate maternal and paternal derivation, respectively, of a given X chromosome). Abnormal eggs can be fertilized by either X- or Y-bearing sperm, and thus there is a larger number of possible combinations: OX^P, OY; three of the XXY type ($X_1^MX_2^MY$, $X_1^MX_1^MY$, $X_2^MX_2^MY$); and three trisomic for X ($X_1^MX_2^MX^P$, $X_1^MX_1^MX^P$, $X_2^MX_2^MX^P$).

Even though the diagram is elementary, it serves to illustrate a number of points that might otherwise be lost sight of in a discussion of relative frequencies. First, among the various types of abnormal zygotes expected, the XO type is, theoretically, the most frequent, since it can result from nondisjunction at either of the meiotic divisions and in either parent. There is the additional possibility that the relative frequency of meiotically caused XO can be greatly increased by the occurrence of chromosome loss other than that resulting from nondisjunction. Second, if one compares the relative frequencies of X^MO and OX^P, it is obvious that, even if equal frequency of nondisjunction in the two sexes is assumed, the ratio will be 2:1, since the O gamete from the mother always has a half chance of combining with a Y chromosome, and this, as has been shown in the mouse (*1*), leads to a lethal combination. Third, it should be noted that all but a very few of the types of offspring resulting from the abnormal gametes listed in Fig. 3 can also result from events other than meiotic nondisjunction. Meiotic chromosome loss as a possible source of XO has already been mentioned. In addition, cleavage events, as is shown below, can result in XO's as well as in several of the other types of abnormal individuals.

Various possible abnormal sex-chromosome types resulting from events associated with the first cleavage are summarized in Fig. 4. The first of these events, chromosome loss, would again lead to the simple XO condition; and, again, OX^P is expected only half as frequently as X^MO, since half the cases of loss of X^M produce the inviable OY type. Both chromatid loss and nondisjunction would basically lead to mosaics. However, where one of the daughter cells is OY, this cell would probably fail to yield viable cell progeny, so that the resultant individual would be nonmosaic XY or XXY. Cell selection is, of course, possible in other cases as well and can lead to normalcy, or at least to nonmosaicism, after a mosaic phase in early development. A similar result would be achieved by separation of the cell lines to form embryonic and extra-embryonic tissue, respectively.

Conversely, it is possible that *normal* tissue of a mosaic may be the secondary derivative in an individual having a basically abnormal number of sex chromosomes. Thus, in the case of a human XX/XO mosaic it has been postulated that the zygote was XO, and that XX cells were the result of nondisjunction in cleavage (*25*); and a human XXY/XX mosaic has been explained on the basis of loss of the Y in cleavage (*26*).

Although Fig. 4 shows only expectations concerning abnormal events that occur at the *first* cleavage, it can be used to predict the results of similar events occurring at later cleavages. Such later-cleavage events would always yield potential mosaics, with usually more than half the cells normal at the time of inception of the cell lines. Thus, chromosome or chromatid losses at a later cleavage would produce the same types of mosaics as those resulting from chromatid loss at the first cleavage (Fig. 4), except for differences in the proportion of cells establishing the cell lines. Nondisjunction at a later cleavage would lead to production of individuals with three types of cells. Naturally, the remarks made above, concerning the possibilities of cell selection and of separation of cell lines to form embryonic and extra-embryonic

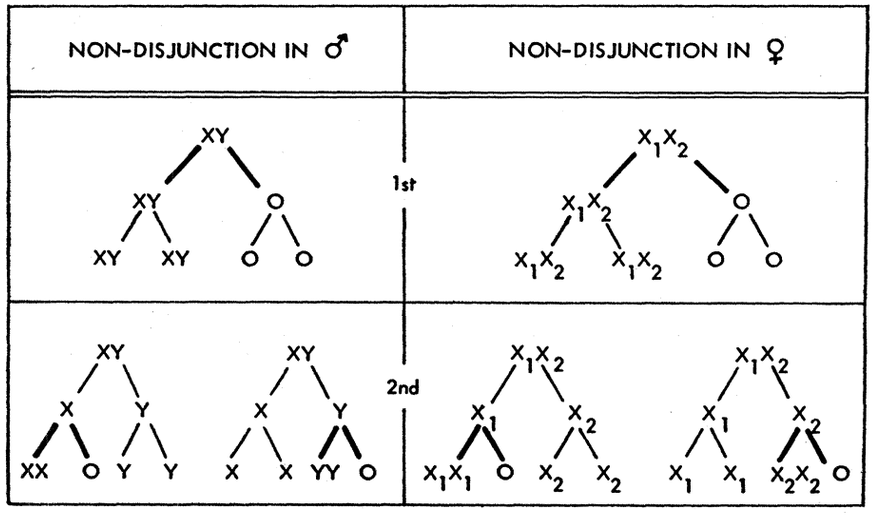

Fig. 3. Theoretical expectation concerning nondisjunction in meiosis. The cell division in which nondisjunction occurs is indicated by heavy lines.

tissue in initially mosaic individuals, apply to later-cleavage as well as to first-cleavage mosaics.

Spontaneous Incidence of Abnormal Sex-chromosome Number

Of the several abnormal sex-chromosome types expected on theoretical grounds (Figs. 3 and 4), a large number have already been discovered in mammals. In the mouse, the X^MO and X^MX^PY chromosome constitutions have been demonstrated genetically and cytologically (*1*, *2*, *6*, *7*, *20*), and OY has been shown to be prenatally lethal (*1*). Animals of the OX^P type have been reported (*7*), but it is not certain whether these were of primary occurrence or the offspring of a mating of undetected primary X^MO. In man, also, several cases of X^MO but only a very doubtful one of OX^P are on record (*5*). Human XXX's have been reported, but it is not known whether these are $X^MX^PX^P$ or $X^MX^MX^P$. All cases of XXY analyzed in man have been of the X^MX^MY type (*5*). In addition to these simple types of sex-chromosome anomalies, many others (such as XXXY, XXXX, and XXYY) and many mosaics, including some of those outlined in Fig. 4, have been reported in man. No sex-linked markers were available in these cases, and they cannot be reviewed in detail at this time.

The only condition which gives proof that a particular instance of nondisjunction has occurred in meiosis rather than in cleavage is the presence of both of the sex chromosomes of one parent in the offspring. This condition exists in X^MX^PY. It also exists in X^MX^MY and $X^MX^MX^P$, provided the two X^M chromosomes differ in their genetic markers. Actually, it is doubtful whether $X^MX^MX^P$ would be detectable with markers available in the mouse at this time. The XXY anomaly was expected to be detectable, but only if the animal was viable and male (or male-like) and if the presence of the Y did not affect the expression of X-linked markers. Our recent discovery of an $X^+X^{Ta}Y$ male (*6*), mentioned above, has now shown that these conditions are met. Since both X^{Ta} and Y are known to have been contributed by the father, this exceptional animal is actually the only mammal reported to date in which the stage of nondisjunction can have been no other than the first meiotic division in a presumably normal male. In man, cases of Klinefelter's syndrome in which the derivation of the X's is marked by the gene for color blindness are of the X^MX^MY type (*5*), with both X^M chromosomes bearing the same marker. Such individuals can be the result of nondisjunction at any one of three stages: in the second meiotic division of the mother; in the first meiotic division combined with a crossover (*27*); and in the first cleavage (see Fig. 4).

Comparisons of the frequencies of the various spontaneously occurring sex-chromosome anomalies in the mouse lead to a number of interesting conclusions. Data for these comparisons, some of which are summarized in Tables 2 and 3, come from a large number of crosses in which five sex-linked markers were used in various combinations and in XX × XY as well as XO × XY matings. Two comparisons that have been made are between the frequency of animals in which a paternal sex chromosome is lacking (P−) and those in which there is an extra paternal sex chromosome (P+), and between the frequency of animals in which a maternal sex chromosome is lacking (M−) and those in which a paternal sex chromosome is lacking (P−).

Table 2 summarizes results from 16 crosses in which *simultaneous* detection of P− and P+ events was possible. The estimated frequency of occurrence (columns 6 and 7) has been calculated by taking account of the fact that detectability of one or the other event is only ½ in some crosses (columns 1 and 2). The single P+ event is the $X^+X^{Ta}Y$ male mentioned above. Unfortunately for the clarity of Table 2, this animal occurred in an experiment in which postfertilization stages were irradiated (see below). However, its

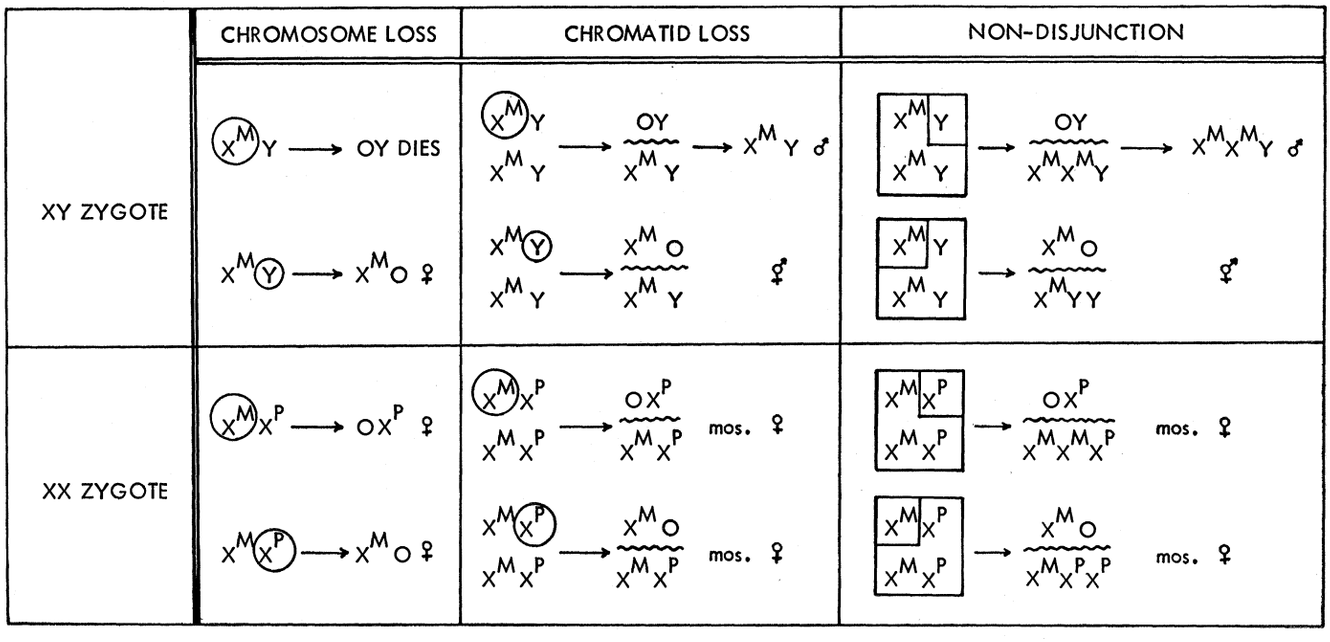

Fig. 4. Theoretical expectation concerning first-cleavage events that can result in sex-chromosome anomalies. Lost chromosomes or chromatids are encircled. In the case of nondisjunction, the possible groupings of one and three chromosomes, respectively, are indicated by divisions of the squares. Mosaic, *mos.*; hermaphrodite, combined symbols for male and female. A wavy line separates the two genotypes in mosaics.

Table 2. Frequencies of mice lacking a paternal sex chromosome (P—) and of mice having an extra paternal sex chromosome (P+) (combined data from 16 different types of crosses in which simultaneous detection of these events was possible).

Detectability		Animals observed (*N*)			Corrected frequency (%)*	
P—	P+	Total	X^MO	X^MX^PY	P—	P+
1	1	1819	17	0	0.93	0
½	½	3660	11	0	0.60	0
½	1	350	1	0	0.57	0
		Radiation experiment†				
1	1	539‡	17§	1		
	Totals	5829	29		0.76	
		6368		1		0.02

* Estimated frequency of occurrence calculated by taking account of detectability, which, in some crosses, is only ½. † Irradiation on day 0 after fertilization (see Table 4). ‡ Excluded in calculating total frequency of spontaneous P— but included in calculating frequency for spontaneous P+ (see text). § Excluded in calculating total frequency of P—.

chromosome constitution rules out radiation as the cause of the XXY anomaly. [Naturally, the irradiated group with its 17 P— events, most or all of which could have been the result of irradiation, was not included in calculating the frequency of spontaneous occurrence of P— (Table 2, totals)]. While it cannot be stated with any degree of certainty that the ratio of P— to P+ is actually 38 (a value based on only one occurrence of P+), it is quite clear that the ratio is, indeed, quite high.

Table 3 summarizes data from eight crosses in which genetic markers were present for the detection of both M— and P— events. In a population in which 13 spontaneous occurrences of P— could be detected, there were no cases of M—. For comparison, data from the radiation experiment are also shown in Table 3.

Not only spontaneous M— but M+ cases also are lacking among anomalies discovered to date. They could have been found among 4711 offspring from nine types of crosses in which M+ events resulting from nondisjunction in the first meiotic division are theoretically detectable with a probability of up to 50 percent. [Half of the M+ events would presumably go unrecognized as a result of the $X^MX^MX^P$ type being nondetectable with the markers available at this time. In addition, if, as a result of crossing over, X^M becomes homozygous for the markers carried by the mother, some of the X^MX^MY animals, too, would escape detection. Altogether, in our crosses, the detectability of M+ from first-meiotic nondisjunction is ½ (1 — ½ *c.o.*), where *c.o.* is the frequency of crossing over between the X centromere and the marker used, an as yet unknown quantity.] Thus, although the present indications are that M+ events are very infrequent (if, indeed, they occur at all), it cannot yet be stated with certainty that M+ is even rarer than P+.

Before discussing the implications of the frequency comparisons, it is necessary to present the results of investigations designed to test whether the frequency of XO mice could be altered by artificial means.

Induction of Abnormal Sex-Chromosome Number

Evidences of nonrandomness in the original XO data in the mouse (*1, 2*) (for example, the finding of XO litter mates) suggested that cleavage events might be very important in the spontaneous origin of the XO anomaly and led us to attempt to increase the frequency of XO individuals by irradiation during cleavage stages. The results of this experiment are summarized in Table 4. A preliminary report was presented earlier (*28*).

With appropriate sex-linked markers, instances of both maternal and paternal sex-chromosome loss could be detected. We were able to show that the frequency of XO individuals could be significantly increased by irradiation on the day of fertilization (day 0 in Table 4). Since, in the mouse, almost a full day elapses between fertilization and the first cleavage, it was possible to test the effect of irradiation at various times during that day. The frequency of XO mice was highest after irradiation at the earliest time tested (11:00 A.M.) and somewhat lower when animals were exposed later in the day. Irradiation during subsequent cleavages (1½ to 4½ days after fertilization) or at postcleavage stages was ineffective in increasing the frequency of XO individuals. One point of considerable interest was that exposure at 11:00 A.M., the stage at which irradiation yields the highest frequency of induced XO mice, produced some animals lacking the *maternal* X, an event which has not yet been observed to occur spontaneously (see the discussion above and Table 3) or from irradiation at other stages (Table 4).

None of the XO mice obtained in this experiment gave the appearance of mosaicism. Moreover, when progeny was obtained from the XO animals as well as from many phenotypically nonexceptional animals, in an attempt to detect possible gonadic mosaicism, the results were negative. We thus conclude tentatively that we have induced chromosome loss prior to the first cleavage. Although four, and in some cases five, autosomes were also marked, no autosome losses were detected. It may be assumed that such losses, if they occur, are cell-lethal.

Mechanisms That Produce Abnormal Numbers of Sex Chromosomes

That nondisjunction of sex chromosomes *can* occur in meiotic divisions was shown by our discovery of an $X^+X^{Ta}Y$ animal from an $X^+X^+ \times X^{Ta}Y$ mating. However, the absence, to date, of both M+ and M— would seem to indicate that nondisjunction in the first meiotic division of the female is very rare, if not nonexistent; and the low frequency of X^MX^PY, as compared with X^MO, suggests that, in the male too, nondisjunction in the first meiotic division is a rare occurrence. It is obviously

Table 3. Frequencies of mice lacking a paternal sex chromosome (P—) and of mice lacking a maternal sex chromosome (M—) (combined data from eight different types of crosses in which simultaneous detection of these events was possible).

Detectability			Animals observed (*N*)			Corrected frequency (%)*	
P—	M—		Total	X^MO	OX^P	P—	M—
1	½		1271	13	0	1.02	0
½	½		67	0	0	0	0
		Total	1338	13	0	0.99	0
			Radiation experiment†				
1	½		425	14	4	3.29	1.88

* Estimated frequency of occurrence calculated by taking account of detectability, which, in some crosses, is only ½. † Irradiation at 11:00 A.M. on day 0 after fertilization (see Table 4).

Table 4. Frequencies of sex-chromosome loss after irradiation at various postfertilization stages.

Stage irradiated		Animals observed (*N*)			Corrected frequency (%)* of sex-chromosome loss	
Day	Hour	Total	X^MO	OX^P		
0†	11 A.M.	425	14	4	5.2	4.6
0	12:30–2 P.M.	44	2	0	4.5	
0	3:30 P.M.	70	1	0	1.4	
1½ to 13½		402	2	0	0.5	1.0
Control		420	6	0	1.4	

* Estimated frequency of occurrence calculated by taking account of the fact that only half of all losses of maternal X are detectable. † Day 0 designates the day on which the vaginal plug is found.

necessary to look for other events that might account for the relatively high frequency of XO, specifically X^MO.

Events yielding X^MO but not X^MX^PY can occur during meiosis (for example, anaphase lagging, or nondisjunction in the second division, which would also produce presumably nondetectable XX and YY gametes); or they can occur following fertilization. Cleavage as a relatively frequent source of XO's in the mouse had already been suggested by the nonrandomness of the original XO data—for example, the finding of XO litter mates—and has now been implicated even more by the demonstration that the frequency of XO animals can be increased by irradiation shortly after fertilization. On the basis of cytological evidence, Ohno, Kaplan, and Kinosita also believe nondisjunction to be more frequent at cleavage, since they found either one X or one Y in each of 1460 second-meiotic nuclei examined (*29*).

As was shown above, not only is X^MO much more frequent than X^MX^PY, but X^MO is also much more frequent than OX^P. A hypothesis that would fit *both* of these results is that there exists a relatively high probability of loss of the paternally contributed sex chromosome some time between fertilization and completion of the first cleavage. Such a hypothesis is not unreasonable in the light of the observation (*30*) that, during that interval, the nuclear material contributed by the male undergoes considerably more changes than that contributed by the female, with respect to such factors as size of the pronucleus, number of nucleoli, and total nucleolar volume. If the paternally contributed nuclear material, in general, is indeed vulnerable at this stage, it is not inconceivable that there is increased probability that individual paternal autosomes, as well as X^P or Y, will be lost. Such an event, however, would probably lead to the death of the individual, unless the maternally contributed homolog were present (or came to be present) in double dose—an explanation which has been proposed to account for a certain class of spontaneous mutants in the mouse (*31*).

The hypothesis that it is the paternally contributed sex chromosome which is more easily lost at, or prior to, the first cleavage does not exclude the possibility that this event may be influenced by factors traceable either to the mother or to the circumstances of fertilization. For example, properties of the zygote cytoplasm that could be under maternal control, or that could be influenced by the interval between ovulation and fertilization, might in turn affect the behavior of chromosomes contributed by the male pronucleus. Explanations of this nature could account for the occurrence of XO litter mates (*2*), the nonrandom distribution of spontaneous primary XO mice among sibships (*1*), and the possibility that there may be "high XO" lines (*1*).

Although the foregoing discussion has applied to presumably nonmosaic types, it should be noted that sex-chromosome mosaics of various kinds have been reported in man, and that a few have been found (although not yet well studied) in the mouse. Such mosaics would, of course, result from postfertilization events, but not necessarily from the simple loss of a paternally contributed sex chromosome that has been discussed here.

Summary

The great strides made during the past two years in the whole field of mammalian cytogenetics have, in particular, enlarged our knowledge of the role of the mammalian sex chromosomes. The following summary briefly lists the most recent discoveries in the mouse, where genetic findings have played a relatively greater role than in the other species of mammals.

The male-determining property of the mammalian Y chromosome, established earlier in mouse and man, has been further confirmed by the finding of an XXY mouse, which was detected by genetic means and has been studied cytologically. This animal is a fully viable, phenotypically normal, though sterile, male. Since various doubts concerning detectability of the XXY type have been removed by the discovery of this animal, it can be concluded that the occurrence of XXY in the mouse is extremely rare.

It has been shown that the X chromosome of the mouse, when it is involved in certain chromosomal rearrangements, has the power to produce variegated-type position effects, a phenomenon formerly not observed in any animal except *Drosophila*. The fact that the X chromosome is involved in all four of the known cases of V-type position effect in the mouse indicates that it is strongly heterochromatic, while there may be little heterochromatin on the autosomes. Recent findings have shown that the presence of *two* X chromosomes is necessary for the expression of the position effect in one of them. This fact, when related to various cytological findings in other species, permits the hypothesis that, in mammals, genic balance requires the action of *one* X in a manner which precludes realization of its heterochromatic potentialities, so that only any *additional* X's present assume the properties characteristic of heterochromatin.

A variety of different findings sheds light on the mechanisms that may lead to the occurrence of individuals with abnormal numbers of sex chromosomes. The XXY mouse proves, by virtue of its sex-linked marker genes, that nondisjunction *can* occur in the first meiotic division of a normal male (a proof not previously provided by human cases of XXY, which could have been of different origin). However, first-meiotic nondisjunction is apparently very rare in males, and there is not yet any evidence that it ever occurs in females. Data from numerous types of crosses involving five sex-linked markers yield the following results: no cases of X^MX^MY or OX^P have occurred to date; $X^MX^PY \ll X^MO$; $OX^P \ll X^MO$ (where the superscripts M and P designate maternal and paternal derivation, respectively, of the X).

The total frequency of XO individuals can be increased by irradiation shortly after fertilization. This treatment has yielded, in addition to X^MO, several animals of the OX^P constitution, a type that has not yet been found to occur spontaneously.

The various findings on spontaneous

and induced frequencies of mice with abnormal numbers of sex chromosomes lead to the conclusion that XO individuals are most often the result of events occurring after fertilization. Specifically, it is suggested that there exists a relatively high probability of loss of the paternally contributed sex chromosome some time between fertilization and the first cleavage (*32*).

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Population and Politics in Europe

Demographic factors help shape the relative power of the communist and noncommunist blocs.

A. F. K. Organski

World population problems have attracted wide attention in recent years, but discussions of demography all too often skip over Europe with only a bare mention. True, Europe is not a demographic trouble spot today. Although the "population explosion" of which we hear so much originated in Europe, its force is spent and the continent has escaped unscathed, indeed enhanced, by the experience. Europe's population today is large and dense, more dense in fact than that of Asia, if population per square kilometer is considered (see Table 1), but population pressure in Europe offers no obstacle to economic development or political tranquillity. Nor is Europe's population growing as rapidly as that of the underdeveloped areas that fill us with concern. The annual rate of increase from 1950 to 1958 was only 0.7 percent in Europe as against 1.8 percent in Asia, 1.9 percent in Africa, 2.1 percent in the Americas, and 2.3 percent in Oceania (*1*, Table 2). Already highly developed, Western Europe can easily absorb any increases that are likely to occur in the future. Those portions of Southern and Eastern Europe that are not yet developed are rapidly increasing the efficiency of their economies, and they, too, should be able to handle future increments in their population.

This does not mean, however, that demographic facts no longer affect the politics of Europe. Population trends, both past and present, have a direct effect upon the power position of European nations in relation to the rest of the world and in relation to each other, and this effect is the greater precisely because the economic and social organization of European nations is such that population growth no longer presents a problem. Because most of the European nations are industrial or are industrializing rapidly, they can use their people for purposes of power, unlike the struggling underdeveloped nations, who may find their population growth a liability.

The relationship between population and politics in Europe is of long standing. In the laissez-faire century before World War I, demographic trends influenced European power, but political developments had little effect on demographic trends. Immense population growth, unplanned and uncontrolled, was crucial in making Europeans first in power in the world. Europe's population explosion provided the working hands to run the new industrial economies at home, the migrants to create European allies outside of Europe, and the administrators and soldiers to run far-flung empires that encompassed half the world's area and one-third of its population.

The population growth that had enabled Europe to reap such handsome political yields from its economic development slacked off in the 20th century. Birth rates dropped first in Western Europe, where the Industrial Revolution had started and where, in consequence, urban values, favoring small families, had had the longest time to become widespread and deeply entrenched, but as the century progressed, birth rates began to fall in Eastern Europe, too (*2*, pp. 12–13). Low birth rates, low death rates, and low or moderate rates of increase are facts of life today in most of Europe,

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