COMPOUND CHROMOSOMES INVOLVING THE X AND Y CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

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Received March 17, 1958

DURING the past several years a large number of new chromosome types involving the X and Y chromosomes of *Drosophila melanogaster* have been constructed. One group, known as the compound X chromosomes, has been described in detail (NOVITSKI 1954). Another group, known as the attached XY chromosomes, contain attached to a single centromere (usually derived from the Y) all the material in the X and the Y chromosomes necessary for male viability and fertility. These chromosomes are extremely useful in manipulating the Y chromosome complement of both males and females. Emphasis will be placed in this discussion on the mode of origin of these new chromosomes and the wide variety of uses to which they have put in genetic experiments.

Construction of attached XY chromosomes

Inversion(1)EN: The X chromosome from which many attached XY's were ultimately derived is known as *In(1)EN* (NOVITSKI 1949). This chromosome was found to be the free X present in a stock that was alleged to carry X^{c1} . This derivative of X^{c1} is considered to be a spontaneous opening out of the ring chromosome. It was first detected in an experiment involving parental females heterozygous for a normal chromosome and a chromosome derived from the X^{c1} stock and thought to be X^{c1} . Their progeny included a number of double crossovers, but the double crossovers were represented with equal frequency by both the normal sequence and the supposed ring instead of in the 3:1 ratio that is to be expected from a ring rod heterozygote (see NOVITSKI 1952). Subsequent experiments in which this chromosome was present in combination with a scute inversion in a heterozygous female showed that single crossovers survived; that is, both this new chromosome and *In(1)sc^s* are in the same sequence. Cytological examination showed that the chromosome in fact is not a ring but is J-shaped with one arm somewhat longer than a normal X and the other a shorter heterochromatic arm. This chromosome is designated *In(1)EN*, the last two letters standing for "entire." The prophase cytology of this chromosome has been described by LINDSLEY (1958). It is not known what steps led to the formation of this particular chromosome. It should be noted, however, that the original ring chromosome was carried in an attached-X stock so that in all probability *In(1)EN* arose in a male, although it has not been possible to demonstrate any factors derived from the Y chromosome in *In(1)EN*. It carries a mutant allele of bobbed both distally and proximally (probably on the short arm), which

¹ Operated by Union Carbide Nuclear Company for the United States Atomic Energy Commission.

when acting together produce a normal phenotype (NOVITSKI 1949; LINDSLEY 1958). It seems quite likely that both *bb* loci could have been derived from the original ring X. It should be further pointed out that stocks now labeled as X^{c1} in a number of genetics laboratories probably were originally derived from this mislabeled stock in Pasadena and consequently are not X^{c1} but instead carry *In(1)EN*.

In addition to its usefulness in the construction of attached XY chromosomes, *In(1)EN* has proved useful in several other ways. *In(1)sc^{sL},EN^R*, a combination of the left end of *In(1)sc^s* and the right end of *In(1)EN*, has been used to facilitate the recovery of spermatogonial recombinants between the distal heterochromatin of *In(1)sc^s* and the Y chromosome or other heterochromatin in the complement. *In(1)EN^R* is useful in this connection because the terminal deficiency for γ^+ and ac^+ produced by such an exchange is covered by the duplication for these loci, which is carried proximally by *In(1)EN* (see LINDSLEY 1955, 1958). Also *In(1)sc^{sL},EN^R* chromosomes have become attached (LINDSLEY 1950) to form attached-X chromosomes with inverted sequence. Subsequent detachment has yielded an array of inverted X chromosomes with varied proximal heterochromatic constitution that have been used in studies of centromere strength (NOVITSKI 1951).

Before the discussion of attached XY chromosomes is continued, some convention should be adopted for the designation of combinations of X and Y. For a complete characterization of such a chromosome, we should like to know (a) the elements (i.e., X, Y^S , and Y^L) present, their order, and the relative position of the centromere; (b) the sequence of the X element; and (c) the markers present and their order. We propose that the complete designation give this information in the order presented, that an abbreviated designation be that portion of complete description required by the context, and that the designation XY be used when no other information is necessary. The XY described in the following section would be fully designated as $Y^S X \cdot Y^L, In(1)EN, K_s \gamma \cdot K_L \gamma^+$, where the centerpoint denotes the position of the centromere and K_s and K_L correspond to K_1 and K_2 of STERN (1929) and are the collective fertility factors of Y^L and Y^S respectively. Abbreviated designations could be, for example, $Y^S X \cdot Y^L$, $Y^S In(1)EN \cdot Y^L$, or $K_s \gamma \cdot K_L \gamma^+$. One further convention is the inclusion within parentheses of any elements whose relative position is unknown; e.g., the case of $X(\cdot Y^L)4$ used by the authors (LINDSLEY and NOVITSKI 1958) in their studies of centromere strength.

$Y^S X \cdot Y^L, In(1)EN$: The first use to which *In(1)EN* was put was the construction of an attached XY chromosome (LINDSLEY and NOVITSKI 1950). This chromosome was constructed by the following steps:

Among the heterochromatic recombinants recovered from *In(1)sc^{sL},EN^R* males were several in which the terminal $\gamma^+ ac^+$ segment derived from *In(1)sc^s* was replaced by part of Y^S to yield an *EN* inversion with the fertility factors of Y^S carried terminally. Males carrying this recombinant and $sc^s \cdot Y$ (MULLER 1948) were crossed to females of constitution γ/Y'' ($Y'' = Y^S \cdot Y^S$; STERN 1929). All regular sons from such a cross are sterile ($Y^S X \cdot Y''$), but exceptional sons

arising from nondisjunction in the male are fertile ($Y^S X \cdot / sc^s \cdot Y / Y''$), as would be any recombinant in which Y^L had been attached proximally to the $Y^S X$ chromosome ($Y^S X \cdot Y^L / Y''$). Since Y^L from $sc^s \cdot Y$ carries the normal allele of yellow, both types of fertile males should be γ^+ in phenotype. The initial matings were made en masse, and after a suitable period of oviposition, all parents were removed from the cultures. The progenies from these cultures were transferred to fresh medium without examination. Larvae appeared in but four of a large number of bottles; each of these bottles contained one γ^+ male. The four γ^+ males were mated individually to $\gamma^s su-w^a w^a bb / 0$ females; three of them produced both γ and γ^+ sons and daughters, indicating that γ^+ and, inferentially, Y^L were not linked to the X, but the fourth produced only γ^+ sons and γ^s daughters. Furthermore, the male descendants of the fourth male were repeatedly backcrossed to XX/0 females and were consistently fertile. The events proposed to account for the origin of this chromosome are diagrammed in Figure 1. Cytologically, this chromosome appears to have one arm of about the same length as one of the large autosomes and one arm about one third as long. Homozygous XY females are somewhat less viable than heterozygotes, or homozygous normal females.

Very soon after the origin of $K_s \gamma \cdot K_L \gamma^+$, a derivative that had lost the normal allele of yellow was recovered. It is not known whether this loss was the result of deletion or mutation. Of course it has also been possible to insert many combinations of markers into the XY chromosome by double exchange with a normal sequence. It was also surprisingly simple to insert $In(1)dl-49$ accompanied by markers, v , f , and car by double exchange with $Ins(1)sc^s, dl-49, car f v sc^s$.

The attached XY chromosomes are ordinarily maintained as the single sex chromosome of the males in a stock in which the females carry an attached X but no Y chromosome. The attached-X chromosome usually carries the recessive alleles of γ and bb , making it simple to detect the presence or absence of a free Y or $sc^s \cdot Y$ chromosome. Occasionally, $Y^S X \cdot Y^L$ chromosomes break down and

FIGURE 1.—The events postulated to account for the origin of $Y^S X \cdot Y^L, In(1)EN$. It is not known whether the exchange in step B took place between Y^S and the base of the long arm of $In(1)EN$ as figured, or between Y^L and EN^S .

produce free heterochromatic elements; this can be recognized by the appearance of bb^+ females and, in those cases in which the XY has the normal allele of yellow on the end of Y^L , by the appearance of y^+ females in the stock. Tests of the y^+ element from these y^+ females reveal that in many instances it is a complete Y chromosome and in others it is an incomplete Y, presumably lacking Y^S . Attachment of two XY chromatids occasionally results in the production of attached X-bearing daughters by XY-bearing males. The origin of the XY derivatives described is diagrammed in Figure 2.

It has also been possible to link chromosome 4 with the XY chromosome to produce an attached XY4. This was done by recovering γ sons from $Y^S X \cdot Y^L$, $In(1)EN$, $K_s \gamma \cdot K_L \gamma^+ / In(1)dl-49$, $\gamma w lz$ females that had been irradiated with 3600r and crossed with M-5 males. Many of these cases of loss of γ^+ from the XY were accompanied by the simultaneous loss of at least some of the fertility factors of Y^L and acquisition of the normal alleles of ci and ey from chromosome

FIGURE 2.—Methods of spontaneous alteration of $Y^S X \cdot Y^L$.

4. There was one case in which all the fertility factors of both arms of the Y had been retained and the fourth chromosome loci also acquired (LINDSLEY and NOVITSKI 1953).

Because the XY chromosome was derived from *In(1)EN*, it is, of course, in inverted sequence. An XY chromosome whose X component is in normal sequence was derived by irradiating the XY chromosome described and screening for cases in which the euchromatic segment had become involved in a long inversion (NOVITSKI 1951). One such case, which was labelled *In(1)24* appears to be an almost complete reinversion since it crosses over freely with a normal sequence. The recombinant class with the left end of a normal chromosome and the right end of *In(1)24* is inviable in the male, however, indicating that some normally proximal genes, located distally in the irradiated XY, were not shifted back to their normal position by the second inversion. Replacement of the deficient proximal region with one derived from an $X \cdot Y^L$ detachment of an attached-X (A-2) has produced a reinverted XY [*In(1)24^L*, A-2^R] with a full complement of proximal euchromatic markers carried proximally; however, a small duplication for the most proximal euchromatic regions is carried distally. Another useful derivative from irradiated $Y^S X \cdot Y^L$, *In(1)EN* is *In(1)26*, which has one break in polytene chromosome section 10A and another in the heterochromatin. This second inversion, which partially overlaps *In(1)EN*, is effective in eliminating recombination between the XY chromosome [$Y^S X \cdot Y^L$, *Ins(1)EN*, 26] and both a normal sequence and *In(1)sc⁸* (NOVITSKI 1951).

Fragment 1: Fragment 1 was originally thought to be the product of the rupture of a bridge formed by a single exchange between $Y^S X \cdot Y^L$, *In(1)EN* and a normal sequence as diagrammed by NOVITSKI (1952). Since that time, however, BRAVER (1955) has demonstrated that FR 1 carries the fertility factors of the short arm of the Y chromosome distally and is consequently $Y^S X \cdot$, in which the X chromosome is in normal sequence. This chromosome, as it arose, was marked with γ , *cv*, *v*, and *f*, and it has proved extremely useful in constructing XY chromosomes in which the X chromosome component is in normal sequence. This was accomplished by replacing the centric region of $Y^S X \cdot$ with that derived from $X \cdot Y^L$ detachments of attached X chromosomes.

A possible pair of events to account for the origin of FR 1 is diagrammed in Figure 3. The expected low frequency of the events postulated in this case cannot be used as an argument against this particular set, since the experiment from which it was recovered was designed to screen for very rare events, and this instance was one of two such rare events out of a total of 20,000 flies examined.

$XY^L \cdot Y^S$ and $XY^S \cdot Y^L$: PARKER has recovered, from a large number of induced detachments derived from $\gamma^{s^2} su-w^a w^a bb/sc^8 \cdot Y$ females, X chromosomes that have essentially an intact Y chromosome proximally; we are indebted to him for permission to include these cases in this discussion. These XY's are of two types: γ^{s^2} and presumably $XY^L \cdot Y^S$ (PARKER 1954), and γ^+ and presumably $XY^S \cdot Y^L$ (PARKER and McCURNE 1958). The mode of origin of these chromosomes is diagrammed in Figure 4. Two cases of formation of attached X chromosomes from $XY^L \cdot Y^S$ have been obtained, one of which has proved to be $XY^L \cdot X$ and the other

FIGURE 4.—Mode of origin of $XY^L \cdot Y^S$ and $XY^S \cdot Y^L$ as derived by PARKER.

the fertility factors of the Y chromosome. It has also proved very useful in recovering and carrying free X chromosome duplications. In all the preceding situations, selection is required to maintain the desired element if the rate of primary nondisjunction is high; methods of obviating this inconvenience are discussed in a later section. An XY chromosome is commonly used, in stocks that carry a compound X chromosome, to eliminate the free Y chromosome and thereby vastly decrease the frequency with which the compound X chromosome detaches, since exchange with a free Y chromosome is the primary cause of detachment of compound X's. Furthermore, the viability of some compound X's is reduced by the presence of a Y; consequently, a stock that lacks a free Y chromosome is advantageous.

The XY chromosome has been used by SANDLER and BRAVER (1954) in studies of the meiotic behavior of univalent chromosomes in *Drosophila*; they showed that $Y^S X \cdot Y^L$ in the absence of a pairing partner is lost during meiosis with a relative frequency of 0.15 and this loss is eliminated by the addition of a free Y to the complement. Furthermore XY chromosomes have been used by LINDSLEY and SANDLER (1958) to study the primary nondisjunction of heterochromatic X chromosome duplications. They found that, in combination with these duplications, the recovery of the XY chromosome could be drastically reduced. LINDSLEY and C. W. EDINGTON (unpublished) are using XY chromosome stocks to enable them to measure the relative sensitivity to X-rays of XY, Y, and nullo X, nullo Y sperm. XY/0 males can be used to synthesize attached-X females without Y chromosomes where such a condition is a prerequisite to a further step, such

as the detachment of the attached-X chromosomes by the fourth chromosome, an event that rarely occurs unless the competitive Y chromosome is removed (PARKER 1954; MULLER and HERSKOWITZ 1954). Also, HERSKOWITZ and MULLER (1954) used an XY stock in experiments that failed to substantiate the notion that the chromosomes are arranged in an end-to-end linear order in the sperm head. They checked for a bimodal distribution of sperm head length in males carrying a sperm population made up of XY-bearing sperm and nullo X, nullo Y sperm, but found a normal distribution.

XY stocks have been useful in physiological studies where it was desirable to check the effect of increased or decreased heterochromatin on a phenomenon under investigation. COOPER (1956) used $Y^S X \cdot Y^L$ in a study of the effects of Y chromosome hyperploidy on the variegation of self-colored eyes and on male fertility and found that the portion of the Y carried on $Y^S X \cdot Y^L$ is less effective than a complete Y either in promoting variegation or in reducing male fertility. BAKER and SPOFFORD (1957) used an XY to study the effect of Y fragments and no Y on the expression of white variegated position effects. BURDETTE (1954) used this chromosome to alter the amounts of heterochromatin in individuals in a study of the effects of altered heterochromatic constitutions on the frequency of induction of tumors in certain tumor susceptible lines. PATTERSON *et al.* (1954) used an XY chromosome to show that, in contrast to the oocyte where an extra Y increases the amount of 2537 A-absorbing material, the pentose nucleic acid and deoxyribonucleic acid content of salivary gland cells is the same for individuals of constitution XY, XO, XXY, and XX. Also GAY (see KAUFMANN *et al.* 1957) has used an attached XY stock to extend this observation in electron microscope studies of salivary gland cells. She finds that alteration of the Y chromosome content of these cells has no detectable effect on structures thought to be associated with RNA.

XY chromosomes for special purposes

$XY^L \cdot Y^S$, $y \text{ l}(1)259 \text{ K}_L \cdot \text{K}_S$: Lethal 259 is a recessive lethal very closely linked to yellow [it has not been tested for allelism with $\text{l}(1)J-1$]. This lethal has been put onto the end of $XY^L \cdot Y^S$ by exchange and the resulting recombinant requires a duplication for the γ region for survival [$\text{l}(1)259$ has been used in preference to $\text{l}(1)J-1$ because it carries a mutant allele of γ]. This chromosome is used for balancing elements duplicated for the tip of the X that would otherwise have to be maintained by arduous selection in each generation. Such elements may equally well be balanced to another chromosome type that has been constructed. This is a reversed acrocentric compound X chromosome in which the proximal chromosome is deficient for the terminal region of the X including the normal allele of $\text{l}(1)J-1$, and the distal chromosome carries $\text{l}(1)J-1$. It was derived as a single crossover product between the distal uninverted chromosome of a reversed acrocentric, which carried $\text{In}(1)sc^s$ proximally, and a free X carrying $\text{l}(1)J-1$ in a triploid female. Both these chromosomes may be used simultaneously in balancing duplications to assure the presence of the duplication in all viable males and females. An advantage of this line is that there will be automatic selection against

any free Y chromosome that does not carry the tip of the X that may persist in the stock. Such Y chromosomes would cause nondisjunction of the X from the duplication and would lead to the production of lethal zygotic types that carry the Y but not the duplication. This selective inviability should lead to the elimination of the free Y from the stock, which is generally desirable since a free Y interferes with many of the tests that are commonly made with such duplications.

$Y^S X \cdot Y^L$, $In(1)EN$, $y^+ K_s y \cdot K_L y^+$: This XY chromosome in which both termini are marked with y^+ was recovered as a detachment of a reversed acrocentric compound X chromosome. The proximal element of the compound X was derived from a $Y^S X \cdot Y^L$ that had Y^L marked with a normal allele of y , and was consequently $XX \cdot Y^L y^+$. This chromosome was detached with $sc^s \cdot Y: bw^+$, which according to BAKER (1955) is probably $y^+ K_s bw^+ K_L$ in constitution. The fact that a fertile detachment that could be shown to carry y^+ distally as well as proximally by recombination was recovered confirms BAKER's contention that y^+ is carried on Y^S in $sc^s \cdot Y: bw^+$. This XY chromosome was constructed specifically for the subsequent synthesis of a closed XY chromosome (LINDSLEY and EDINGTON 1957). Simultaneous loss of both y^+ alleles from this compound, i.e., an apparent double terminal deficiency, would be strong presumptive evidence for ring formation; however, it has not yet been possible to induce loss of both y^+ alleles without also deleting some of the Y chromosome fertility factors.

$Y^S XX \cdot Y^L$: This is a reversed compound acrocentric compound carrying two X chromosomes and a Y chromosome attached to a single centromere. It was constructed by replacing the centromere region of a reversed acrocentric with one derived from $Y^S X \cdot Y^L, In(1)EN$ and replacing the tip with that of $Y^S X \cdot$ (i.e., FR 1). This was accomplished by exchange in triploid females. Such a chromosome has an X in normal sequence attached terminally to an X in inverted sequence and has the long arm of the Y attached proximally and the short arm of the Y attached distally. This chromosome has been used in an unsuccessful attempt to recover fertile tetraploid males.

SUMMARY

This paper is primarily a discussion of the origin and utility of attached XY chromosomes; i.e., chromosomes that have attached to a single centromere all of the material of X and Y chromosomes necessary for male fertility and viability. Chromosomes are described that have the entire euchromatic portion of the X chromosome, singly inverted, multiply inverted or in normal sequence, essentially inserted into a normal Y between the fertility factors of Y^S and the centromere. The evidence suggests that some Y chromosome material is missing from these XY chromosomes. The counterparts of these chromosomes with the X chromosome inserted into the long arm of the Y do not yet exist. Also described are compounds produced by PARKER in which at least the euchromatic portion of the X is appended to the long or short arm of the Y chromosome distal to the appropriate fertility factors. Finally, rod shaped XY chromosomes are mentioned that are $Y^S XY^L$ in constitution.

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