luminosity to period.   The second is that in this region of chaotic diffuse nebulosity, irregular variation in the light of deeply red stars is prevalent. And, of most·significance, the absolute photographic luminosities of giant eclipsing binary stars are determined with relatively high accuracy, and a preference for absolute magnitude −2 is indicated.

¹ *Astrophys. J.*, **89**, 340–342 (1937); *Harvard.Reprint* 141; see also *Harv. Circ.* 271, 8, 1925.

² *Harv. Ann.*, **60**, No. 4, 87–108 (1908).

³ *Ibid.*, **90**, No. 1, 1–25 (1933).

⁴ A star only suspected of variability by Miss Leavitt now proves to be conspicuously variable and has been assigned the number HV 11088.

⁵ *Harv. Ann.*, **90**, No. 10, 253–261 (1940); These PROCEEDINGS, **26**, 326–332 (1940); *Harvard Reprint* 202.

⁶ These PROCEEDINGS, **26**, 541–548 (1940); *Harvard Reprint* 207; for HV 2749 see D. Hoffleit, *Harv. Bull.* 905, 25, 1937.

⁷ *Harv. Ann.*, **90**, 171 (1934); *Harv. Bull.* 917, 1–5 (1943).

⁸ *Harv. Bull.*, 916, 19–20 (1942).

⁹ These PROCEEDINGS, **27**, 440–445 (1941); *Harvard Reprint* 228.

A NEW THEORY OF SECONDARY NON-DISJUNCTION IN FEMALE DROSOPHILA MELANOGASTER*

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Sturtevant and Beadle[16] have shown that female exceptions constitute some 36.6% and 45.6%, respectively, of the female classes derived from *Drosophila melanogaster* mothers heterozygous for In(1)C or In(1)dl-49 and possessing a Y-chromosome.   The female "exceptions" are so-called because they derive both their X-chromosomes from their XXY mothers, not one X-chromosome from each parent as is usually the case.   This anomalous situation is brought about by a failure of segregation, that is, by "secondary non-disjunction,"[1] of the X-chromosomes in a percentage of the oöcytic meioses, and is greatly enhanced by the presence of hetero-zygous X-chromosome inversions along with the Y-chromosome as Sturte-vant and Beadle have demonstrated.

The frequency of non-disjunction is not equal to the frequency of ex-ceptional females among the female class, however, for only half of the fertilized XX non-disjunctional eggs ordinarily emerge as adult flies, namely, the half that is fertilized by Y-bearing spermatozoa.   The other half that is fertilized by X-bearing spermatozoa gives rise to superfemales having three X-chromosomes, and under ordinary conditions superfemales

only rarely eclose from their puparia.[9, 12]   Accordingly, if p represents the *percentage* of secondary non-disjunction, or, better (XX)-(Y) segregation, and q the *frequency* of exceptional females, then

$$p = 100(2q/1 + q).$$

From this relation it follows that about 54% of all segregations in $In(1)C/+/Y$ are non-disjunctional for the X-chromosomes, and some 63% of all segregations in $In(1)dl$-$49/+/Y$ females are also of the type (XX)-(Y). The high frequencies of secondary non-disjunction in these two cases are of more than passing significance, for as Sturtevant and Beadle[16] remark, they are in sharp disagreement with predictions based upon Bridges' generally accepted and now classic hypothesis concerning the mechanism of secondary non-disjunction.
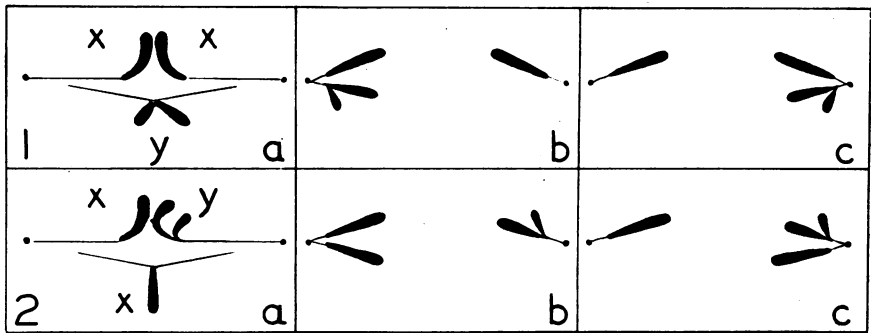

FIGURE 1

Purely diagrammatic representation of Bridges' (1916) hypothesis of secondary non-disjunction.   Line 1, the consequences of XX-conjunction, Y being excluded from association.   Line 2, the consequences of XY-conjunction, one X being excluded from association.   Equally probable anaphase patterns arising from the particular metaphase associations are represented in blocks b and c.   Secondary non-disjunction has a maximum value of 50% on this hypothesis.

Bridges' (1916)[3] hypothesis was in total accord with all of the known facts at the time at which it was proposed, and remained a satisfactory accounting of the mechanism and consequences of secondary non-disjunction until Sturtevant and Beadle's work upon heterozygous X-chromosome inversions.   Bridges assumed that in XXY females the two X-chromosomes and the Y-chromosome are in mutual competition for a pairing or conjunctive partner, and that when any two of these three homologous chromosomes succeed in pairing, the third is left unassociated. Furthermore, the univalent chromosome was supposed to distribute randomly with respect to the poles, as is known to be the case for many univalents.   The operation of the hypothesis is diagrammatically represented in figure 1.   Line 1 represents the consequences of XX-pairing,

leaving Y a univalent to assort randomly. Line 2 represents the non-disjunctive process as Bridges conceived it. One X pairs with Y, the other X being a univalent. When anaphase occurs the univalent. X goes to the Y-pole (2c) in 50% of the cases, and to the same pole of the spindle as the other X (2b) in the remaining 50% to give XX non-disjunctional nuclei. On Bridges' hypothesis, therefore, one hundred per cent pairing of Y with an X, could it be brought about, would give a maximum p of only 50%, and a maximum q of 0.33 as the frequency of exceptions among the female class of offspring. Thus the 54% non-disjunction.found in $In(1)C/+/Y$ and 63% for $In(1)dl$-$49/+/Y$ cannot be accounted for on the basis of Bridges' assumptions. As Sturtevant and Beadle[16, 17] remark, a new interpretation of secondary non-disjunction must be sought as "the general problem of the mechanism of secondary non-disjunction is unsolved." It is the purpose of this note to provide such a new interpretation of the mechanism of secondary non-disjunction, along with data on several synthetic high non-disjunctional lines upon which the new interpretation is based.

All chromosomes (including the Y) of the *Drosophila melanogaster* stocks employed in the research now reported were derived from *Canton-S*, with the exception of the inverted X-chromosomes and the possible exception of the fourth chromosomes in some strains. Special methods were adopted to guarantee that homologous large autosomes were isosequential through all the stocks employed, and this was also the case for any non-inverted X-chromosome used in the crosses. The reason for building up such special stocks, the chromosomes of which are isosequential *inter se*, lies in Sturtevant's[14] notable discovery that heterozygous inversions in the large autosomes may markedly *decrease* rates of secondary non-disjunction in X-chromosome structural heterozygotes. Experiments on comparative rates of secondary non-disjunction may be.expected to gain in sensitivity, to be more reproducible, and more revealing of unique qualities (if any) of inversions, therefore, when such potentially disturbing factors as autosomal structural (= sequential) heterozygosity are removed.[2]

The effects upon secondary non-disjunction of heterozygous combinations of $In(1)B^{M1}$, $In(1)dl$-49, and $In(1)AM$ with wild-type (+) X-sequences, and in certain combinations with one another, are given for the female classes in table 1. (Data for the male classes are in good agreement with those for the female progeny, but are not recorded here as they require corrections for exchanges within inversions, as well as for viability differences in some cases.) $In(1)B^{M1}$ is roughly ten map units long, and has its left break just to the right of B (57.0), and its right break probably to the right of .bb (66.0). $In(1)dl$-49 is perhaps thirty map units long, having its left break between rb and cv (i.e., between 7.5 and 13.7) and its right break between fw and g (between 38.3 and 44.4). $In(1)AM$ is also about thirty

TABLE 1

THE PERCENTAGES OF EXCEPTIONAL FEMALES AND OF SECONDARY NON-DISJUNCTION IN *Drosophila melanogaster* MOTHERS HETEROZYGOUS FOR THE SPECIFIED INVERSIONS AND HAVING THE Y-CHROMOSOME AND ISOSEQUENTIAL AUTOSOMES DERIVED FROM *Canton-S*. THE STANDARD OR WILD TYPE SEQUENCE FOR THE X-CHROMOSOME IS REPRESENTED BY "$+$"

CONSTITUTION OF MOTHER	TOTAL FEMALE OFFSPRING	EXCEPTIONAL FEMALES, %	(XX)-(Y) SEGREGATIONS, %
$+/+/Y$ (=control)	2121	0.9	2
$In(1)B^{M1}/+/Y$	2465	8	14
$In(1)dl-49/+/Y$	3705	54	70
$In(1)AM/+/Y$	2277	54	70
$In(1)dl-49/In(1)B^{M1}/Y$	1981	61	76
$Ins(1)dl-49, B^{M1}/+/Y$	5330	63	77
$In(1)dl-49/In(1)AM/Y$	2171	67	80

TABLE 2

THE OCCURRENCE OF EXCEPTIONAL FEMALES BY PRIMARY NON-DISJUNCTION IN *Drosophila melanogaster* MOTHERS HETEROZYGOUS FOR SPECIFIED INVERSIONS AND HAVING THE AUTOSOMES ISOSEQUENTIAL AND DERIVED FROM *Canton-S*. THE STANDARD OR WILD TYPE SEQUENCE FOR THE X-CHROMOSOME IS REPRESENTED BY "$+$." COMPARE WITH TABLE 1

CONSTITUTION OF MOTHER	TOTAL FEMALE OFFSPRING	TOTAL EXCEPTIONAL FEMALES	(XX)-(O) SEGREGATIONS, %
$+/+$ (=control)	8081	1	0.02
$In(1)B^{M1}/+$	2660	0	..
$In(1)dl-49/+$	3155	10	0.6
$In(1)AM/+$	1133	1	0.2
$In(1)dl-49/In(1)B^{M1}$	1389	4	0.6
$Ins(1)dl-49, B^{M1}/+$	5613	10	0.4
$In(1)dl-49/In(1)AM$	1159	0	..

map units in length, extending.from near *lz* (27.7) to between *B* and *Bx* (i.e., between 57.0 and 59.4).[3] It is seen that the inversion heterozygotes markedly differ in non-disjunctional rates from the control, and that secondary non-disjunction sharply rises with an increase in the structural difference existent between the two X-chromosomes. Furthermore, excepting the cases of $+/+/Y$ and $B^{M1}/+/Y$, none of the non-disjunctional rates found are interpretable upon the basis of the classical hypothesis which sets 50% as the uppermost limit of secondary non-disjunction.

Table 2 records the occurrence of primary exceptions among the offspring of females having X-chromosome constitutions identical with those of table 1, but lacking a Y-chromosome. As Sturtevant and Beadle[16] concluded from their own data, and as table 2 shows, mere heterozygosity for X-chromosome inversions has but a negligible effect upon the final outcome and regularity of X-chromosome segregation in female *Drosophila melanogaster*. Thus very high non-disjunction rates depend not only upon

the particular combination of heterozygous inversions present, but in addition *require* the presence of a *Y*-chromosome. There is no doubt that Bridges was correct in his opinion that the *Y*-chromosome somehow brings about non-disjunction by its own segregative involvements.

Now it is known that the presence of these inversions in heterozygous combinations markedly increases the proportion of non-exchange tetrads formed by the *X*-chromosomes,[10, 13, 15, 16] whether or not a *Y*-chromosome is present. Indeed, as a conservative estimate, at least 76% of the *X*-chromosome pairs in Ins(1)*dl-49*, $B^{M1}/+$, *Dp*(1; 1)*112* are non-exchange tetrads,[10] and this is also the case where the uninverted chromosome lacks a duplication. The degree to which crossing-over is eliminated may be taken as one measure of the meiotic dissimilarity of the *X*-chromosomes caused by their sequential non-homology in principally euchromatic lengths.

But as the *X*-chromosomes become sequentially dissimilar through the introduction of heterozygous combinations of inversions affecting their euchromatic lengths, so they become effectively more like *Y*-chromosomes in their pairing affinities. A moment's reflection will bring conviction that were the euchromatic lengths of two *X*-chromosomes to be made wholly dissimilar, but the so-called "inert" or chromocentral regions to remain essentially unaltered, then conjugation between the *X*-chromosomes would be predominantly a heterochromatic affair—a process occurring almost exclusively between the chromocentral regions.[4] This, however, is merely another way of stating that conjunction becomes more frequent in those regions of *X* wherein pairing with *Y* normally occurs. Hence, as is found to be the case, when a *Y*-chromosome is present non-disjunction markedly rises in *X*-chromosome inversion heterozygotes, probably because the sequential differences between the *X*-chromosomes in effect emphasize their likenesses to the *Y*-chromosome. Thus, where the *X*-chromosomes are everywhere in sequential homology, *Y* induces a rise in non-disjunction from 0.02% in +/+ to a mere 1.6% in +/+/*Y*. In other words the *X*-chromosomes share more similarities with each other than they do with the *Y*-chromosome, and *Y* becomes an effective partner to *X* in only a small proportion of the cases—perhaps less than 2% of all associations in +/+/*Y*. But where there is a great reduction in sequential homology between the two *X*-chromosomes, as in the combination In(1)*dl-49*/In(1)*AM*, non-disjunction rises from an indeterminately small fraction in *dl-49*/*AM* to about 80% when merely a *Y*-chromosome is added to the karyotype. *Y*, in other words, is a more attractive partner to either *X*-chromosome in this combination than either *X*-chromosome is to the other. The apparent paradox that each *X* in effect finds *Y* a more attractive and frequent conjunctive partner than the other *X*-chromosome, although the *X*-chromosomes remain alike in the very regions with which

they share homology with Y, is resolved by the assumption that *both arms* of the Y-chromosome share homology and may thus conjoin with an X.[5] Hence if one X pairs with one arm of Y, then the other X must conjoin with the other arm of Y if it is to have a meiotic partner at all. The mechanical aspects of this mode of bringing about an apparent non-disjunction by trivalent formation of both X-chromosomes with a Y-chromosome are diagrammed in figure 2. Since, for example, X_a pairs with arm Y_a, and X_b with Y_b, the segregation patterns are $X_a - Y_a$ and $X_b - Y_b$. But in an alternately oriented trivalent Y_a and Y_b perforce must go to one pole, since they possess the same kinetochore, and X_a and X_b undergo forced segregation to the other pole since each disjoins from Y.
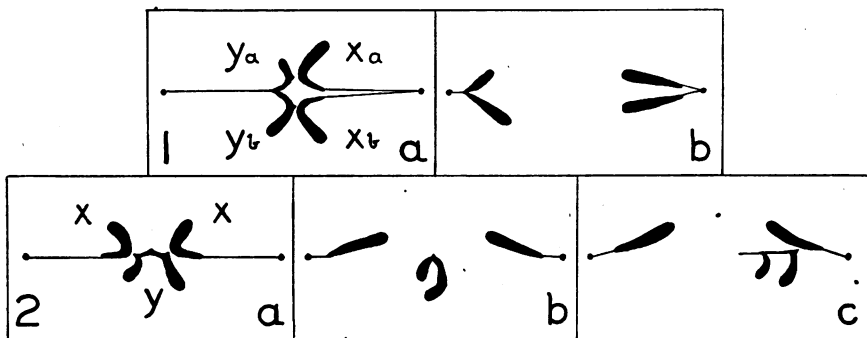

FIGURE 2

Purely diagrammatic representation of the interpretation of secondary non-disjunction proposed here. *Line 1 of figure 1* is held to represent accurately the consequences of XX-conjunction in XXY females. Line 1 of figure 2 shows an XYX trivalent and the imposed non-random segregation following alternate coörientation of the associated chromosomes. Line 2 represents a linear orientation of an XYX trivalent, and two of the possible consequences in anaphase. Such misoriented trivalents may lead to the loss of Y and other irregularities, but do not give rise to exceptional gametes. The majority of trivalents are supposed to orient alternately as in 1*a*, hence this interpretation admits a possible maximum of 100% secondary non-disjunction.

The interpretation of secondary non-disjunction that is offered here asserts, therefore, that secondary non-disjunction is not a case of non-disjunction at all, but an example of imposed non-random segregation from a sex-chromosome trivalent.[7] The mechanism proposed has a direct analogy with the segregative mechanism normally occurring for X_1, X_2 and Y in the spermatogenesis of *Drosophila miranda*.[11] It may be pointed out that XYX trivalents formed at meiosis in any XXY female of *Drosophila melanogaster* may be greater than the percentage of secondary non-disjunction owing to the probable occurrence of some linear orientations of the trivalent at first metaphase (Fig. 2, line 2).

The principal virtue of this new interpretation of secondary non-disjunction lies not alone in the fact that it wholly accounts for data not

resolvable by Bridges' hypothesis, but that it can, if false, be disproved by a direct test of its predictions. The scheme proposed predicts that (1) secondary non-disjunction must rise on the average as the euchromatic sequences of the two X-chromosomes become increasingly different; (2) the presence of two Y-chromosomes should decrease the rate of secondary non-disjunction; (3) either the long arm or short arm of Y alone could not regularly give greater than 50% secondary non-disjunction; and (4) that in those cases where secondary non-disjunction exceeds 50%, the X-chromosomes must have a partner that can pair with both X-chromosomes simultaneously (i.e., in general the partner must have two arms). And so forth.

Each of these implications has been put to test, and each has so far proved to be in accord with the experimental facts. For example, (1) tests of $+/+/Y$, $In(1)sc^7/+/Y$, $In(1)B^{M1}/+/Y$, $In(1)sc^7/In(1)B^{M1}/Y$ and $Ins(1)sc^7$, $B^{M1}/In(1)dl-49/Y$ give as secondary non-disjunction rates: 2%, 14%, 14%, 63% and 78%, respectively. This is the very order that would be predicted, and since $In(1)sc^7$ and $In(1)B^{M1}$ are of the same order of size, close similarity in their effects on raising the secondary non-disjunction rate is to be expected.

(2) On the new interpretation, if each arm of Y is equivalent to X in its pairing, and if the order of pairing is random, but pairing complete in so far as possible, then in XXY females secondary non-disjunction would be expected to approximate a p of 80%. It is interesting to note that this is the uppermost value for secondary non-disjunction so far attained in my experiments. In $XXYY$ females tetravalents may be expected if corresponding arms of Y may pair, but their characteristics of orientation is an unknown factor. However, it may be assumed that, for necessity of spatial economy on the spindle, alternate orientations (N) should exceed adjacent (M). If results for equality of these two classes of orientation be calculated, as well as for total alternate orientation, then the expectation is that secondary non-disjunction in $XXYY$ females will have a p with some value in the interval from 27 to 52%. If it be assumed that tetravalents orient wholly at random, then p should approximate 52%. Should it be the case that tetravalents never form, then p should approximate 42%. The three possibilities treated above cover the more probable conjectures about conjunction in $XXYY$ females, and the calculated values are closely similar to or include the experimental value of 36% obtained from $Ins(1)dl-49$, $B^{M1}/+/Y/Y$.[6] In the control, $Ins(1)dl-49$, $B^{M1}/+/Y$, p is 78%.

(3) An effectively one-armed Y^s-chromosome obtained through the kindness of Professor H. J. Muller gives about 24% non-disjunction in $Ins(1)dl-49$, $B^{M1}/+/Y^s$ whereas the control crosses of $Ins(1)dl-49$, $B^{M1}/+/Y$ give 77%. (The Y^s-chromosome has not yet been gotten into

a background of *Canton-S* autosomes, so this test with Y^s may have given a somewhat low value. Internal evidence supplied by the controls, which are XXY daughters of the XXY^s females, suggests, however, that the true effect of the Y^s-chromosome on secondary non-disjunction in this X-chromosome karyotype will not be found to differ appreciably from the low value so far obtained.)

(4) All Y-chromosomes in the experiments undertaken which give non-disjunctional rates over 50% (with heterozygous Ins(1)*dl-49*, B^{M1}) have two effective arms. These Y-chromosomes include Y^{cs}, Y^{sw-b}, $sc \cdot Y^L$, and others.

The interpretation here posed, therefore, is in agreement with a wide variety of new facts, as well as with those past discoveries which make up the body of available data on secondary non-disjunction in *Drosophila melanogaster* females.

Summary.—A new theory of secondary non-disjunction in female *Drosophila melanogaster* is proposed. It asserts that the process is actually a case of imposed non-random segregation from an XYX trivalent. Each arm of Y is assumed to pair with a different X-chromosome to form a trivalent which usually orients in alternate pattern at first meiotic metaphase. Thus the cytological features are envisioned as similar to the directed segregation from X_1YX_2 trivalents in the spermatogenesis of *Drosophila miranda*. Previously known facts, as well as results of genetic experiments designed to test the scheme, appear to be in full accord with this interpretation.

[1] Failure of the two X-chromosomes to segregate to opposite poles in the oögenesis of an XX-female is referred to as "*primary* non-disjunction." Inclusion of both X-chromosomes in a single polar group at meiosis of an XXY-female is referred to as "*secondary* non-disjunction." The distinction is an important one, for the mechanisms of primary and secondary non-disjunction are wholly dissimilar, the latter being dependent upon the extra Y-chromosome.

[2] Sturtevant and Beadle[16] did not deliberately clear their stocks of possible sequential disturbances of the autosomes, for no such effect of autosomal inversions upon secondary non-disjunction had been anticipated in 1936. This may well account for the significantly lower rate (63%) found by them than that (70%) reported here for secondary non-disjunction in In(1)*dl-49*/ + /*Y*. · Cf. table 1.

[3] Additional information on these inversions can be had from: Bridges, C. B., Carn. Inst. Wash. Pub. No. 552, viii + 257 pp. (1944).

[4] This conclusion perhaps deserves a general discussion of the nature of synapsis. Suffice it to say in this connection that the idea of specific forces between homologous genes is without proof, and is almost certainly not a necessary hypothesis.

[5] Proof that each arm of Y shares homology with X will be found in Neuhaus's work; v. Neuhaus, M., *Genetics*, **22**, 333–339 (1937).

[6] A noteworthy outcome of these experiments, that will be reported in detail in another paper, is that, in general, flies whose eyes are $+/+$, $+/w^a$, w^a/w^a, $+/v$, v/v, v/w^a, v/car, car/car, etc., and which possess two (or more) Y-chromosomes beyond normal for the karyotype (i.e., $XXYY♀♀$ and $XYYY♂♂$) have flecked and/or mottled eyes.

[7] Bauer suggests that the union of two Y-chromosomes to form a V made possible the general elimination of $(X_1Y_1) - (X_2Y_2)$ segregations in tetraploid earwigs. A mechanism similar to the one proposed here would result by such translocation, and give principally $(X_1X_2) - (Y_1Y_2)$ segregations at spermatogenesis.   v. Bauer, H., *Zeitschr. Naturforsch.*, 2b, 63–66 (1947).

[8] Bridges, C. B., *Genetics*, **1**, 1–52, 107–163 (1916).

[9] Bridges, C. B., *Science*, **54**, 252–254 (1921); *Am. Naturalist*, **56**, 51–63 (1922); *ibid.*, **59**, 127–137 (1925).

[10] Cooper, K. W., *Genetics*, **30**, 472–484 (1945).

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THE ORIGIN OF VOLUTIN ON THE CHROMOSOMES, ITS TRANSFER TO THE NUCLEOLUS, AND SUGGESTIONS CONCERNING THE SIGNIFICANCE OF THIS PHENOMENON*

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Volutin as a Cytological Entity.—Volutin, which is widely distributed in bacteria and fungi and is also called metachromatin, is a clearly defined cytological entity, distinguished by its ability to retain basic stains when the cells are rinsed in dilute acid.   When volutin is stained with methylene blue or toluidine blue, it retains the stain after treatment with 1 per cent sulphuric acid.   In addition to being "acid fast," volutin has the characteristic of staining red or purple with methylene blue or toluidine blue, in contrast to other basophilic components of the cell which stain blue with these dyes.   Both metaphosphates and estersulphates retain dyes after destaining and stain red or purple with toluidine blue.   Volutin is widely distributed in fungi and bacteria, while the estersulphates[1] are apparently widely distributed in higher animals.   Wiame's work[2] strongly suggests