THE ANALYSIS OF ROD DERIVATIVES OF AN UNSTABLE RING CHROMOSOME OF *DROSOPHILA MELANOGASTER*¹

CLAUDE W. HINTON²

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Received September 17, 1956

In contrast to those of corn, the ring chromosomes of *Drosophila melanogaster* are, with one exception, relatively stable. This exceptional *Drosophila* ring, called w^{vc} , is derived from a stable ring-X chromosome, and its instability is manifested through the frequent production of gynandromorphs, XO males, and dominant lethals among prospective w^{vc} /rod progeny. It has been proposed that these manifestations of w^{vc} instability are a consequence of anaphase bridge formation by the w^{vc} chromosome, whereby gynandromorphs and XO males arise from bridge loss, and dominant lethals owe their origin to bridge breakage (HINTON 1955). Since the work of MCCLINTOCK (1938) and SCHWARTZ (1953) suggests that sister-strand exchange is responsible for anaphase bridge formation by the ring chromosomes of corn, it appears that the same process might be the cause of w^{vc} instability. If this is true, the manifestations of instability should disappear upon conversion of the unstable w^{vc} ring chromosome to a rod chromosome because ring structure is a prerequisite for anaphase bridge formation as a consequence of sister-strand exchange. Studies on rod- w^{vc} chromosomes derived from unstable ring- w^{vc} chromosomes, as will be described in this paper, show that these rods are unstable. Although the manifestations of instability by w^{vc} rods are qualitatively different from those of the unstable ring- w^{vc} chromosome, they nevertheless are capable of interpretation in terms of anaphase bridge formation.

PRIMARY NONDISJUNCTION AND w^{vc} INSTABILITY

For interpretation of the results of crosses with w^{vc} rod chromosomes, one must first reconsider the relation of primary nondisjunction and instability of the w^{vc} ring chromosome. It was supposed (HINTON 1955) that patroclinous XO males produced by w^{vc} /rod females arose predominantly by w^{vc} loss during the meiotic divisions of the eggs and the early cleavage divisions of F_1 w^{vc} /rod zygotes. Primary nondisjunction was also recognized as a potential source of nullo-X eggs (and consequently XO males), but this source was assumed to be negligible since the frequency of recovered matroclinous females is very low in comparison with the patroclinous male frequency. However, if primary exceptional zygotes (w^{vc} /rod/Y) are subject to the dominant lethality experienced by their regular (w^{vc} /rod) sisters, the recovered matroclinous daughters represent only the surviving fraction of the w^{vc} /rod/Y zygotes produced. Consequently, a correction for dominant lethality applied to the observed matroclinous female frequency would provide a more reliable measure of primary nondisjunction frequency.

In each of the crosses listed in table 1 both primary exceptional females and

¹ Work performed under USAEC Contract No. W-7405-eng-26.

² Present address: Department of Zoology, University of Georgia, Athens, Georgia.

TABLE 1

The relation between w^{vc} instability and primary nondisjunction in crosses of w^{vc}/rod females. Details of calculations are found in the text

Cross	Females					Males			Corrected percentage of exceptions		Index of w^{vc} instability (per cent)
	Regular			Matroclinous		Regular		Patroclinous			
	rod	w^{vc}	Gy	w^{vc}	Gy	rod	w^{vc}	rod (XO)	Female	Male	
Ia	1534	1263	11	0	0	1036	823	39	0.00	1.85	17.7
b	2832	920	393	2	2	2190	518	376	0.15	7.91	67.5
IIa	1498	642	158	21	6	1427	115	153	1.66	5.09	57.1
b	1055	261	142	7	5	1056	51	149	1.47	6.59	75.3
IIIa	6024	1961	524	81	42	4885	265	440	2.42	4.31	67.4
b	3796	487	279	25	30	2975	44	289	3.47	4.63	87.2
IVa	8919	7469	114	50	2	6859	1947	99	0.34	0.72	16.3
b	1490	582	297	8	7	1288	71	137	0.84	5.05	60.9
Va	4100	3141	33	2	0	3428	655	17	0.03	0.25	23.4
b	1383	438	315	22	24	1079	0	117	2.96	5.14	68.3

- I. $w^{vc}, f/y w spl sn^3 \times y sc lz^a v f/Y$
 II. $w^{vc}, f/y w spl sn^3 \times dl-49, y w v f car/Y$
 III. $w^{vc}, f/dl-49, y w v f car \times y w spl sn^3/Y$
 IV. $w^{vc}, f/dl-49, y Hw m^2 g^4 \times dl-49, y w lz^a/Y$
 V. $w^{vc}, f/dl-49, y w B \times dl-49, y w lz^a/Y$

gynandromorphs could be distinguished phenotypically from their regular sisters by appropriate combinations of mutant markers. In most of the crosses, the presence of the Y chromosome in primary exceptions could be detected by its suppression of eye color variegation exhibited by w^{vc}/w females. The corrected frequency of primary exceptional females (including gynandromorphs) is calculated as equal to $(mat \text{ } \varnothing + mat \text{ } Gy) \div 2(reg \text{ } w^{vc} \text{ } \varnothing + reg \text{ } Gy) + mat \text{ } \varnothing + mat \text{ } Gy$; it is assumed that the frequency of dominant lethals is equal among w^{vc}/rod and $w^{vc}/rod/Y$ zygotes. The corrected frequency of primary exceptional males is calculated with the assumption that viability of XO males is equal to that of their regular rod/Y sibs so that the corrected frequency is equal to $pat \text{ } \sigma^7 \div 2(reg \text{ } rod \text{ } \sigma^7) + pat \text{ } \sigma^7$; regular w^{vc}/Y males cannot be considered since their viability is probably affected by Notch variegation in addition to instability of the w^{vc} chromosome. Finally, an index of w^{vc} instability provided for each cross of table 1 is calculated as $(reg \text{ } rod \text{ } \varnothing - reg \text{ } w^{vc} \text{ } \varnothing) \div reg \text{ } rod \text{ } \varnothing$. The sublines designated a and b for each of the crosses of table 1 were alike in all experimental conditions except for the w^{vc} chromosomes that were chosen from stocks having different degrees of instability. Comparisons within these sublines show that (except for Cross II) the corrected frequency of matroclinous females is higher in the subline having the higher instability index, which suggests that increased primary nondisjunction is still another manifestation of w^{vc} instability. In-

spection of the uncorrected data also provides evidence of this association between primary nondisjunction and instability.

Comparison of the corrected frequencies of primary exceptional females and males shows that for all crosses the male frequency is the higher. Since an excess of male over female exceptions is generally observed, the corrected frequency of exceptional females provides only a minimum estimate of exceptional males arising from primary nondisjunctional egg nuclei. However, different approaches are available to show that not all the exceptional males are the consequence of primary nondisjunction. Thus, on the anaphase bridge model of w^{vc} instability, bridge loss at the first cleavage mitosis produces only XO males whereas bridge loss at the second cleavage division is registered by gynandromorphs or, owing to the indeterminate distribution of early cleavage nuclei, by XO males. Another argument supporting the origin of XO males from w^{vc} loss is derived from the behavior of the w^{vc} chromosome when introduced by the male parent; in such crosses, XO males are also produced in frequencies similar to those found in progenies of w^{vc}/rod females, yet primary exceptional females (including gynandromorphs) are relatively rare in comparison. For example, the cross of $y w spl sn^3$ ♀ by $w^{vc}, f/sc^8 \cdot Y$ ♂ produced 2469 $w spl sn^3$ ♂, 743 w^{vc} ♀, 151 Gy, 179 XO ♂ (table 1 of HINTON 1955); only one of the females and two of the gynandromorphs may have carried the $sc^8 \cdot Y$ chromosome as judged by their phenotypes. From these considerations, it appears that both primary nondisjunction and w^{vc} loss are significant factors in the production of patroclinous XO males among the progeny of w^{vc}/rod females, although their relative contributions cannot be accurately evaluated.

THE CONSTRUCTION OF w^{vc} ROD CHROMOSOMES

The derivation of rods from ring- w^{vc} chromosomes is schematically outlined in figure 1. The structure of the w^{vc} ring chromosome was proposed (HINTON 1955) to be a long inversion with breakpoints between the loci of y and w and in the proximal heterochromatin. $In(1)sc^4 \cdot sc^8$ is a chromosome of inverted sequence combining the left end of $In(1)sc^4$ and the right end of $In(1)sc^8$ and lacking most of the basal heterochromatin. The B^s duplication is composed of the proximal part of the X chromosome through Bar and is derived from $T(1;4)B^s$. At intervals for more than a year, females of the constitution $w^{vc}, f/y sc^4 car m w^a sc^8/Dp B^s$ were synthesized and mass-mated to dl-49, $y w v f car/Y$ males. The progenies were scored for all females (including gynandromorphs) carrying the w^{vc} chromosome. Among 11,259 w^{vc} females, 27 were found having the Bar eye and nonforked bristle phenotype expected for the rod- w^{vc} crossover product. On testing, six of these 27 females were sterile, 16 were of other double crossover or nondisjunctional origin, and five proved to be the desired crossovers of the constitution $y sc^4 car m w^{vc} \cdot y^+ B^s/dl-49, y w v f car$. These five rod- w^{vc} chromosomes, briefly designated $w^{vc} \cdot B^s$ (where the elevated dot indicates centromere position) and distinguished from each other by the catalog numbers of the cultures in which they occurred, were established in stocks for further testing.

Before describing the behavior of the $w^{vc} \cdot B^s$ chromosomes, let us consider the origin of derivatives of these chromosomes. In $w^{vc} \cdot B^s/dl-49, y w v f car$ females, a single exchange between the B^s arm and the dl-49 chromosome will produce a com-

FIGURE 1.—The derivation of w^{vc} rod chromosomes and the generation of w^{vc} ring chromosomes from the rods. Details of the crosses involved are provided in the text. For simplicity, only two of the four chromatids of a tetrad are drawn, and the relative extents of heterochromatin (heavy line) and euchromatin (light line) are shown arbitrarily.

pound metacentric X chromosome of the constitution $y\ sc^4\ car\ m\ w^{vc}\cdot y^+\ f\ v\ w\ y$, dl-49 (abbreviated $w^{vc}\cdot dl-49$) similar to the tandem metacentric heterozygous for the dl-49 inversion studied by NOVITSKI and BRAVER (1954). Several $w^{vc}\cdot dl-49$ chromosomes were obtained from each of the five $w^{vc}\cdot B^S$ stocks. Neuroblast preparations confirmed the metacentric structure of both the $w^{vc}\cdot B^S$ and $w^{vc}\cdot dl-49$ chromosomes. It is of importance to note that both these rod- w^{vc} chromosomes retain intact the centromere and associated heterochromatic regions of the w^{vc} ring chromosome because some component of these regions appears to be responsible for w^{vc} instability (HINTON 1955).

Inspection of the pairing configurations of the w^{vc} rod chromosomes shows that a single exchange between the two arms of $w^{vc}\cdot B^S$ or $w^{vc}\cdot dl-49$ chromosomes leads to the generation of w^{vc} ring chromosomes (only half the indicated singles would generate rings since, at the four-strand stage, chromatids having a common centromere must be involved in the exchange). These generated w^{vc} rings were recovered from both the $w^{vc}\cdot B^S$ and $w^{vc}\cdot dl-49$ chromosomes from each of the five stocks. Since the original $w^{vc}/sc^4\text{--}sc^8/Dp\ B^S$ females were produced and mated in mass cultures, nothing is known about the initial instability of the individual rings that gave rise to the w^{vc}

rods; but the behavior of the generated w^{vc} rings, identical in structure to the originals, provides essential control information on this point.

THE BEHAVIOR OF w^{vc} ROD CHROMOSOMES

The results of crosses involving the $w^{vc} \cdot B^S$, $w^{vc} \cdot dl-49$ and generated w^{vc} ring chromosomes are presented in tables 2, 3 and 4. The presentation of artificial (reconstructed) tetrad analyses for these chromosomes is unwarranted since the structural and behavioral complexities involved require rather questionable assumptions and cumbersome manipulations. Instead, quantitative considerations of the results rely on comparisons of arbitrarily based frequencies of the various classes of offspring recovered.

The very rare gynandromorphs listed in table 2 from crosses of $w^{vc} \cdot B^S/dl-49$ females show that none of the five $w^{vc} \cdot B^S$ chromosomes experience somatic loss frequencies that are at all comparable to those exhibited by unstable rings. By this criterion alone, we would conclude that the $w^{vc} \cdot B^S$ chromosomes are stable. However, if the $w^{vc} \cdot B^S$ female and XO male frequencies (based on the number of $y w$ females) are examined, the five $w^{vc} \cdot B^S$ stocks can be separated into two groups. Although minor differences between stocks may be found within either group, the results from each stock have been combined to form the group total in order to facilitate comparisons. In the group consisting of stocks designated 5374, 5582, and 6850 there is a deficiency of $w^{vc} \cdot B^S$ females compared to the approximate 1:1 expectation for $y w$

TABLE 2
The behavior of $w^{vc} \cdot B^S$ chromosomes in crosses of $w^{vc} \cdot B^S/dl-49$, $y w lz^s$ females to $dl-49$, $y w v f car/Y$ males

$w^{vc} \cdot B^S$ stock number	$y w lz^s$ males	$y w$ females	Regular $w^{vc} \cdot B^S$ females	Matro- clinous $w^{vc} \cdot B^S$ females*	Gy	$y w f$ males	Generated rings			Generated compounds $w^{vc} \cdot dl-49$
							w^{vc} females	Gy	w^{vc} males	
5374	919	1222	1041	—	0	4	0	1	2	0
5582	2087	2880	2423	—	1	13	7	0	8	2
6850	1015	1364	1159	—	0	9	5	0	4	4
Total	4021	5466	4623	—	1	26	12	1	14	6
Percent		100.0	84.6	—	0.0	0.5	0.2			0.1
6848	1730	2545	1219	—	3	120	2	5	0	4
6857	1477	2095	1054	—	5	94	3	0	1	5
Total	3207	4640	2273	—	8	214	5	5	1	9
Percent		100.0	49.0	—	0.2	4.6	0.2			0.2
6850	1228	2117	1659	8	0	6	8	0	5	6
Percent		100.0	78.4	0.4	0.0	0.3	0.4			0.3
6857	838	1668	856	69	0	134	1	2	0	1
Percent		100.0	51.3	4.1	0.0	8.0	0.2			0.1

* Distinguished from regular females by the effect of the Y chromosome on eye color variegation.

and $w^{vc} \cdot B^s$ females. Part of this deficiency arises through w^{vc} ring and compound generation, but the greater part is probably attributable to hyperploidy of the B^s segment of the chromosome (which also partially accounts for the semilethality of $w^{vc} \cdot B^s$ males). In the second group, consisting of the two stocks numbered 6848 and 6857, the deficiency of $w^{vc} \cdot B^s$ females is about three times as great as in the first group, although inviability owing to hyperploidy should be the same in the two. The net difference between the $w^{vc} \cdot B^s$ female frequencies for the two groups of 35.6% is quantitatively comparable to the frequency of dominant lethals caused by the unstable ring chromosomes.

Similarly, the tenfold difference in XO male frequency between the two groups of $w^{vc} \cdot B^s$ stocks parallels that difference between stable and unstable w^{vc} ring stocks. Since neither of the $w^{vc} \cdot B^s$ stocks numbered 6848 and 6857 produce significant numbers of gynandromorphs, it is not reasonable to suppose that the XO males produced by these same stocks are the consequence of somatic loss of the $w^{vc} \cdot B^s$ chromosome. An alternative—that these XO males are chiefly of primary nondisjunctional origin—is supported by the observations on the frequency of primary exceptional daughters produced by $w^{vc} \cdot B^s$ females (matroclinous $w^{vc} \cdot B^s$ females could be accurately distinguished from their regular sisters as shown by progeny tests). Comparison of the data from stocks 6850 and 6857 (lower part of table 2) shows that the frequencies of patroclinous males and matroclinous females are positively correlated.

In the $w^{vc} \cdot dl-49$ crosses (table 3), the $sc^8 \cdot Y$ chromosome was utilized to distinguish regular (XY) males from their exceptional (XO) sibs, and the dominant B served as a convenient marker for the detection of females bearing generated ring chromosomes. Although superfemale-XO male mosaics might be expected as a consequence of $w^{vc} \cdot dl-49$ somatic loss, no mosaics of this type were positively identified, indicating that none of the $w^{vc} \cdot dl-49$ compounds experience somatic loss. However, examination of the frequencies of the various offspring classes, based on the incidence of $y w B/$

TABLE 3

The behavior of $w^{vc} \cdot dl-49$ compound chromosomes in crosses of $w^{vc} \cdot dl-49/sc^8 \cdot Y$ females to $dl-49, y w B/Y$ males

$w^{vc} \cdot B^s$ stock number	Number of compounds tested	$w B$ males	w^{vc} females	$y w B$ males	Generated rings		
					$w^{vc} B$ females	Gy	w^{vc} males
5374	3	534	437	4	50	0	29
5582	2	869	807	7	61	1	52
6850	3	618	551	7	53	0	11
Total		2021	1795	18	164	1	92
Percent		100.0	88.8	0.9	8.2		
6848	3	1045	632	31	42	16	13
6857	7	2808	1439	93	119	47	13
Total		3853	2071	124	161	63	26
Percent		100.0	53.8	3.2	5.8		

$sc^8 \cdot Y$ males, shows that the $w^{vc} \cdot dl-49$ chromosomes are also separable into two groups corresponding with the two categories of $w^{vc} \cdot B^s$ chromosomes. Comparison of the first group (derived from $w^{vc} \cdot B^s$ stocks 5374, 5582, and 6850) with the second group (derived from $w^{vc} \cdot B^s$ stocks 6848 and 6857) reveals significant differences in the recovery of females bearing $w^{vc} \cdot dl-49$ chromosomes and in the incidence of XO males. The 35% deficit of $w^{vc} \cdot dl-49$ females in the second group suggests that zygotes bearing these chromosomes are also susceptible to the dominant lethality experienced by unstable ring w^{vc} chromosomes.

The assignment of the origin of the XO males produced by the $w^{vc} \cdot dl-49/sc^8 \cdot Y$ females requires consideration of several potential sources. (1) Since no superfemale-XO male mosaics were observed, somatic loss of the $w^{vc} \cdot dl-49$ chromosome apparently cannot account for XO male production. (2) Of the interarm exchanges occurring in either the $w^{vc} \cdot B^s$ or $w^{vc} \cdot dl-49$ chromosomes, only those that involve chromatids having a common centromere will generate rings; the other half of such exchanges will cause the production of a single chromatid bridge at the second meiotic anaphase. If this bridge were lost and the functional egg nucleus fertilized by an X-bearing sperm, an XO male would be produced. However, this is not the case, as shown by the high frequency of generated rings and low frequency of XO males produced by females carrying $w^{vc} \cdot dl-49$ compounds derived from $w^{vc} \cdot B^s$ stocks 5374, 5582, and 6850. This result agrees with that of NOVITSKI and BRAVER (1954) from their study of the compound metacentric heterozygous for $dl-49$ and supports their conclusion that such bridges suffer breakage rather than loss. (3) If some mechanism other than interarm exchange (see DISCUSSION) should lead to second meiotic anaphase bridge formation by the $w^{vc} \cdot dl-49$ chromosome, it seems probable that these bridges also would be broken rather than lost. (4) Another possible source of XO males involves nondisjunction of the $w^{vc} \cdot dl-49$ and $sc^8 \cdot Y$ chromosomes to produce nullo-X-Y and $w^{vc} \cdot dl-49/sc^8 \cdot Y$ egg nuclei. Fertilization of the first type of egg nucleus by an X-bearing sperm would produce an XO male. If the latter type egg nucleus were fertilized by a Y-bearing sperm, $w^{vc} \cdot dl-49/sc^8 \cdot Y/Y$ females should be produced. From the cross of $w^{vc} \cdot dl-49/sc^8 \cdot Y$ females (derived from $w^{vc} \cdot B^s$ stock 6857) to $dl-49, y w B/Y$ males, 259 F_1 females were progeny tested and none carried the $sc^8 \cdot Y$ chromosome in addition to the unmarked Y chromosome. This result does not conclusively deny the occurrence of nondisjunction since it is possible that $w^{vc} \cdot dl-49/sc^8 \cdot Y/Y$ zygotes will not survive; on the other hand, this result is comparable to the apparently negligible nondisjunction frequency in unstable $w^{vc}/sc^8 \cdot Y$ males. (5) The most probable origin of XO males is somatic loss of generated ring chromosomes. In the group of $w^{vc} \cdot dl-49$ chromosomes characterized by a low (0.9%) XO male frequency, only one of 165 generated ring females was a gynandromorph; in the other group having an XO male frequency of 3.2%, 63 of 224 generated ring females were gynandromorphs. When generated rings undergo somatic loss to produce gynandromorphs, they may be expected to produce XO males by the same process.

From the data just considered on gynandromorph frequencies among generated ring females, it is clear that the two groups of $w^{vc} \cdot dl-49$ chromosomes are distinguishable on this basis as well as by $w^{vc} \cdot dl-49$ female recovery frequencies. This conclusion may be strengthened and extended to the $w^{vc} \cdot B^s$ chromosomes by study

TABLE 4

The behavior of generated w^{vc} ring chromosomes (from $w^{vc} \cdot B^S$ and $w^{vc} \cdot dl-49$ rods) in crosses of $w^{vc}/dl-49$, $y w lz^s$ females to $dl-49$, $y w v f car/Y$ males

$w^{vc} \cdot B^S$ stock number	Number of rings tested	w^{vc} males	$y w lz^s$ males	$y w$ females	w^{vc} females	Gy	$y w f$ males
5374	8	138	403	442	444	16	8
5582	18	640	954	1336	1174	20	10
6850	6	7	291	357	395	3	0
Total		785	1648	2135	2013	39	18
Percent				100.0	94.3	1.8	0.8
6848	11	183	628	819	572	88	36
6857	23	60	865	1624	492	250	130
Total		243	1493	2443	1064	338	166
Percent				100.0	43.5	13.8	6.8

of the generated rings in subsequent generations. Ring- w^{vc} chromosomes generated directly by the $w^{vc} \cdot B^S$ chromosomes or indirectly via the $w^{vc} \cdot dl-49$ chromosomes were collected and established in stocks for further testing; the results of these tests (table 4) are pooled according to the five original $w^{vc} \cdot B^S$ stocks. There are two distinct groups of generated rings: those derived from $w^{vc} \cdot B^S$ stocks 5374, 5582, and 6850 are nearly stable, as shown by low frequencies of gynandromorphs and XO males and by high frequencies of w^{vc} female recovery; those derived from $w^{vc} \cdot B^S$ stocks 6848 and 6857 are unstable, as shown by high gynandromorph and XO male frequencies and by low recovery of w^{vc} females. On the basis of these and other observations, the behavior of these two groups of generated rings is entirely comparable to that of the stabilized and unstable w^{vc} ring chromosomes previously described (HINTON 1955).

In summary, none of the derived rod- w^{vc} chromosomes are unstable, by the criterion of somatic elimination, since no sex mosaics are recovered and since the XO males that do appear are attributable to other sources. However, other manifestations of w^{vc} instability allow the separation of the derived w^{vc} rods into two groups. Both the $w^{vc} \cdot B^S$ and $w^{vc} \cdot dl-49$ chromosomes that generate unstable w^{vc} rings are characterized by significantly reduced recovery frequencies (dominant lethality) compared to those rod- w^{vc} chromosomes which generate stable rings. In addition, the $w^{vc} \cdot B^S$ chromosomes that generate unstable rings exhibit significantly higher primary exceptional male and female frequencies than those that generate stable rings, again paralleling the behavior of stable and unstable rings. It is concluded on the basis of the dominant lethality and primary nondisjunction manifestations of instability that rod- w^{vc} chromosomes may be either stable or unstable depending, inferentially, on whether they were derived from stable or unstable ring- w^{vc} chromosomes.

DISCUSSION

Interpretation of the experiments described in this report assumes the validity of the anaphase bridge model of w^{vc} instability. This model, as formulated to explain the instability manifestations of the ring- w^{vc} chromosomes, proposes that an ana-

phase bridge may suffer either of two fates. It may be lost; whereupon a prospective female zygote will be converted to an XO male or to a gynandromorph. Alternatively, the bridge may break, whereupon delivery of the breakage products to the daughter nuclei will usually be lethal to the zygote. These experiments also rely on the thesis that sister-strand crossing over will lead to anaphase bridge formation in a ring chromosome but will be of no consequence in a rod chromosome. If anaphase bridges formed by a ring chromosome continue to be formed when the ring is converted to a rod, then the cause of bridge formation cannot be sister-strand exchange.

For the anaphase bridge mechanism to be operative in rod- w^{ec} chromosome instability, some modification of the model is necessarily since the rod- w^{ec} chromosomes show no evidence of somatic loss; this result requires that the anaphase bridges formed by rods always break, producing dominant lethals. Application of the anaphase bridge model to rod- w^{ec} instability further requires that sister-strand exchanges be discarded as the process responsible for bridge formation. An alternative possibility is union between sister chromatids, a process comparable with that producing the reversed acrocentric compound X chromosome (SANDLER 1954); in its frequency and variability, sister-strand union in the w^{ec} chromosomes may be more appropriately compared to certain of the *Ds* events described by McCLINTOCK (1951) in corn. If sister-strand union occurs in a chromosome of ring structure, the resultant bridge would be composed of two chromatids, just as in sister-strand exchange, except that the distribution of genetic material in the bridge would be different. Whether or not bridges arising from sister-strand union are symmetrical, that is to say, composed of ties of equal strength, would depend on the position of the union. Union between sister strands of a rod chromosome would produce a bridge having centromeres connected by only a single chromatid, plus an acentric fragment. Either loss of the acentric fragment or breakage of the bridge, or a combination of both events, is presumably sufficient cause for zygotic lethality. Following NOVITSKI's analysis (1955) of meiotic anaphase bridge behavior, the difference in somatic behavior of rod- w^{ec} and ring- w^{ec} chromosomes can be visualized as follows: sister centromeres, connected by two chromatids of the same strength, may not always be able to break both chromatids, so that the entire bridge will sometimes be lost, but when these centromeres are connected by only a single chromatid breakage always occurs.

Brain smears of larval $w^{ec} \cdot B^s$ and $w^{ec} \cdot dl-49$ females were prepared to confirm the metacentric structure of these chromosomes, but in the few smears examined, no definite anaphase bridges were found. One might suppose that anaphase bridges are formed by unstable w^{ec} rods in larval brain tissue but that bridges composed of single chromatids are either so short or so weak that they fracture during early anaphase movements and so escape notice. Nevertheless, the predicted acentric fragment, which should be visible at later anaphase and telophase stages, was not seen in the few configurations suggestive of bridges or in other anaphases. This negative preliminary result is of questionable significance for even if large numbers of smears were examined, the absence of bridges in brain mitoses would not rule out their occurrence in cleavage or meiotic divisions.

The observation of bridges at the second meiotic anaphase of w^{ec} males (WELSHONS and HINTON 1955) supported the previously suggested possibility that XO males

and dominant lethals originate from meiotic as well as cleavage bridges formed by the unstable ring chromosomes. Genetic evidence in support of the meiotic origin of dominant lethals is provided by the recovery frequencies of rings generated by the $w^{sc} \cdot dl-49$ compounds (table 3). Considerations presented earlier indicated the most probable source of XO males produced by $w^{sc} \cdot dl-49$ females to be somatic loss of generated rings. Therefore, these XO males should be added to the generated ring female and gynandromorph classes to provide a measure of the total ring generation frequency, which amount to 9.1% for the stable and 9.0% for the unstable compounds. Apparently, none of the zygotes receiving newly generated rings experience dominant lethality. This result suggests that dominant lethals caused by unstable ring chromosomes are entirely of meiotic origin. If this interpretation is correct, it follows that the occurrence of an interarm exchange of the type that generates a ring precludes the subsequent occurrence of a sister-strand union necessary to produce a second anaphase bridge. Another corollary of the interpretation that dominant lethals arise solely from meiotic bridges is that somatic bridges formed by rings do not fracture. However, somatic bridge breakage is demanded by the occurrence of fragments of the w^{sc} ring in gynandromorphs (HINTON 1955). Because of the limited and apparently contradictory evidence, further discussion of this question is not justified.

It seems unlikely that anaphase bridge formation could account for the increase of primary exceptional females associated with increased instability as defined by chromosome loss and dominant lethality. According to SANDLER and BRAVER (1954), primary nondisjunction is best explained by failure of pairing in such a way that homologs proceed to the first anaphase poles at random. COOPER (1951) presented cytological evidence and NOVITSKI and BRAVER (1954) obtained genetic data supporting the view that homolog pairing is initiated by special sites located in the proximal heterochromatin of the X chromosome. It may be inferred that abnormality of this region would lead to inefficient pairing and nondisjunction. Nothing is known of the detailed heterochromatic structure of the w^{sc} chromosome; it is probable that the w^{sc} centromere is of Y chromosome origin, that at least some of the heterochromatic components are duplicated and that these are rearranged in relation to the structure of X^{c2} . Some element of this region of the w^{sc} chromosome controls its instability. This conclusion is based on the continued high somatic loss frequency of fragments derived from and comprising chiefly some of the heterochromatin of the unstable w^{sc} ring (HINTON 1955). It is suggested that the element of the w^{sc} chromosome controlling anaphase bridge formation is also responsible for meiotic pairing failure.

SUMMARY

Data have been presented that show increased primary nondisjunction to be another attribute of the unstable w^{sc} chromosome. It is suggested that primary nondisjunction is a consequence of some defect of the heterochromatic region of the w^{sc} chromosome that prevents normal homolog pairing. Previous evidence indicated that some heterochromatic element of this chromosome controls formation of anaphase bridges, which provide the basis for other manifestations of w^{sc} instability.

The primary purpose of this work, to test whether sister-strand exchange causes anaphase bridge formation by the unstable w^{sc} ring chromosome, was implemented by analysis of rod chromosomes derived from w^{sc} ring chromosomes. Although the rod- w^{sc} chromosomes do not experience somatic loss, they may be unstable by the criterion of dominant lethality. This result can be explained by assuming that sister-strand union rather than sister-strand exchange leads to anaphase bridge formation, and that a bridge formed by a ring chromosome and consisting of two chromatids is susceptible to either loss or breakage; whereas a bridge formed by a rod chromosome and composed of a single chromatid is always broken.

ACKNOWLEDGMENTS

I am indebted to DR. E. NOVITSKI, who suggested the method used to convert the ring- w^{sc} chromosomes to rods, and to DRS. D. L. LINDSLEY, W. K. BAKER, and D. SCHWARTZ, who participated in discussions of this work.

LITERATURE CITED

- COOPER, K. W., 1951 Studies on spermatogenesis in *Drosophila*. Am. Phil. Soc. Ybk., pp. 146-147.
- HINTON, C. W., 1955 The behavior of an unstable ring chromosome of *Drosophila melanogaster*. Genetics **40**: 951-961.
- McCLINTOCK, B., 1938 The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315-376.
- 1951 Chromosome organization and genic expression. Cold Spring Harbor Symposia Quant. Biol. **16**: 13-47.
- NOVITSKI, E., 1955 Genetic measures of centromere activity in *Drosophila melanogaster*. J. Cellular Comp. Physiol. **45**, Supp. 2: 151-169.
- NOVITSKI, E., and G. BRAVER, 1954 An analysis of crossing over within a heterozygous inversion in *Drosophila melanogaster*. Genetics **39**: 197-209.
- SANDLER, L., 1954 A genetic analysis of reversed acrocentric compound X chromosome in *Drosophila melanogaster*. Genetics **39**: 923-942.
- SANDLER, L., and G. BRAVER, 1954 The meiotic loss of unpaired chromosomes in *Drosophila melanogaster*. Genetics **39**: 365-377.
- SCHWARTZ, D., 1953 Evidence for sister-strand crossing over in maize. Genetics **38**: 251-260.
- WELSHONS, W. J., and C. W. HINTON, 1955 Bridges at anaphase II in ring-X males. *Drosophila* Inf. Ser. **29**: 171.