THE BEHAVIOR OF AN UNSTABLE RING CHROMOSOME OF DROSOPHILA MELANOGASTER[1, 2]

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RING-SHAPED chromosomes provide an opportune situation for the analysis of some aspects of chromosome mechanics. In particular, sister-strand crossing over eludes detection in rod chromosomes but its consequences in ring chromosomes are manifested by the formation of anaphase bridges. Thus MCCLINTOCK (1938) and SCHWARTZ (1953) have presented cytological evidence for the occurrence of exchanges between sister chromatids of ring chromosomes in mitotic and meiotic divisions in maize. On the other hand, genetic studies on the ring-X chromosomes of *Drosophila melanogaster* apparently deny the occurrence of meiotic sister-strand exchange (MORGAN 1933; NOVITSKI 1955), and although ring-X chromosomes do experience somatic loss more frequently than their rod-X homologs (MORGAN 1926; BROWN and HANNAH 1952), they are described as being stable in comparison with maize rings. This report is concerned with an atypical ring-X chromosome characterized by frequent loss and dominant lethality. Results will be presented which suggest that the instability of this chromosome is mediated through anaphase bridge formation and that this process is controlled by some heterochromatic element in the centric region of the chromosome.

ORIGIN AND STRUCTURE OF w^{vc}

The structure of the stable X^{c2} ring chromosome, as observed in the salivary glands by SCHULTZ and CATCHESIDE (1937) is consistent with the hypothesis that it arose by union of the distal end of one arm of an attached-X chromosome with the proximal region of the other to produce a minute deficiency for the tip of the X chromosome and a duplication for the proximal heterochromatin. After X-raying X^{c2} CATCHESIDE (CATCHESIDE and LEA 1945) recovered a chromosome which retained the ring structure but exhibited a variegated white Notch phenotype. Subsequent observations of the phenotype produced by this chromosome have shown that the variegation is enhanced at 18°C and suppressed by the addition of a Y chromosome, and that a spreading effect of variegation extends from the w^+ to the rst^+, spl^+, and N^+ loci. These results are typical of the V-type position effects produced by rearrangements transposing these genes from their normal position to a situation adjacent to the heterochromatin of the centromere region (LEWIS 1950). The fact that the phenotype produced by the y^+ locus shows no variegation indicates that a break occurred between the y^+ and w^+ loci (fig. 1). Cytological ex-

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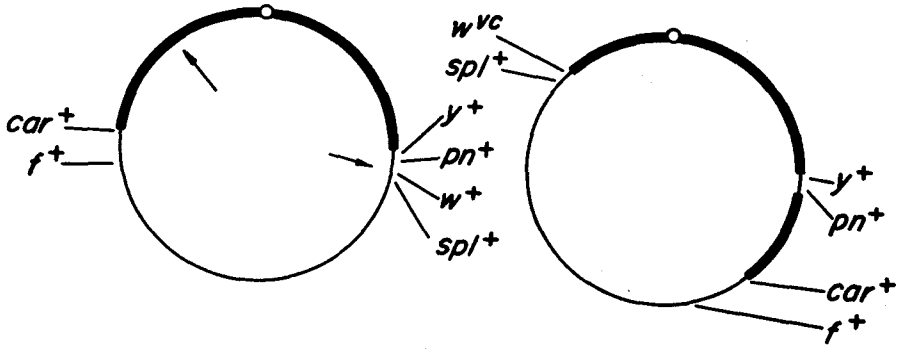

FIGURE 1.—The structure of X^{c2} and its inverted derivative, w^{vc}. The diagrams indicate euchromatin as a fine line, heterochromatin as a heavy line, the centromere as a small open circle, and the position of breaks by arrows within the X^{c2} chromosome. The position of the heterochromatic break and the relative lengths of heterochromatin and euchromatin are arbitrarily designated.

amination of this chromosome in the salivary gland nuclei reveals a long inversion configuration with the euchromatic breakpoint between the bands 3C1 and 3C5–6. Accordingly, this chromosome may be symbolized $In(1)X^{c2}$, w^{vc} and is conveniently abbreviated w^{vc}. It is not known whether the instability of w^{vc} arose as a concomitance of its inverted structure since the first record of its unstable nature appeared several years after its origin (GRIFFEN and LINDSLEY 1946).

MANIFESTATIONS OF w^{vc} INSTABILITY

In addition to the regular offspring expected from crosses involving ring chromosomes, gynandromorphs and X0 males occur very often in crosses of unstable w^{vc} (Crosses I–III, table 1). Gynandromorphs result from w^{vc} loss during the early indeterminate cleavages as shown by the extent and distribution of rod male tissue of mutant phenotype in otherwise wild type females. Gynandromorphs with noncontiguous male areas were relatively rare, and those resulting from loss of the rod-X chromosome were extremely rare.

In Cross I recognition of X0 males was facilitated by the use of the $sc^8 \cdot Y$ chromosome marked by y^+ (MULLER 1948). Regular males received this tagged Y from their father, whereas X0 males either received and subsequently lost the w^{vc} chromosome or arose from nullo-X-nullo-Y sperm. Although nullo-X-nullo-Y sperm would be produced by primary nondisjunction in the male, this process makes no significant contribution to the X0 male class since primary exceptional rod/w^{vc}/Y daughters occur only rarely, if ever, among the progeny of w^{vc} males. In Crosses II and III where the w^{vc} chromosome is introduced by the female parent, X0 males were distinguished by their patroclinous phenotype. The origin of these patroclinous males from nullo-X eggs can be considered an alternative to their origin by complete loss of the maternal w^{vc} chromosome from w^{vc}/rod zygotes. There are two mechanisms which might give rise to nullo-X eggs from w^{vc}/rod females, namely, primary nondisjunction and double first anaphase bridges resulting from 4-strand double exchange tetrads (STURTEVANT and BEADLE 1936). These processes are probably of negligible consequence for the reasons that (1) w^{vc}/*dl-49* females produce less than 1% primary

TABLE 1

The results of typical crosses with the unstable w^{vc} *chromosome (I-III) compared with the stable* w^{vc} *(IV, V) and* X^{c2} *(VI, VII) chromosomes. Calculations of ring loss and dominant lethal frequencies are explained in the text*

Cross	Female parent	Male parent	Ring male	Rod male	Rod / Rod female	Ring / Rod female	Gynandromorph	X0 male	Frequency of: Ring loss	Frequency of: Dominant lethals	Total
Ia	*y w spl* sn^3	w^{vc}, *f*/sc^8·Y	—	4808	—	2196	326	517	0.18	0.37	0.54
b			—	2469	—	743	151	179	0.13	0.57	0.70
IIa	$\frac{w^{vc}, f}{dl\text{-}49, y\ Hw\ m^2\ g^4}$	*y w spl* sn^3/Y	97	601	637	241	62	91	0.24	0.38	0.62
b			162	613	858	280	38	84	0.14	0.53	0.67
c			97	1211	1295	140	106	181	0.22	0.67	0.89
IIIa	$\frac{w^{vc}, f}{y\ w\ spl\ sn^3}$	*dl-49, y Hw* m^2 g^4/Y	365	710	1037	855	8	22	0.03	0.15	0.18
b			86	615	713	156	78	58	0.19	0.59	0.78
IV	$\frac{y\ w\ spl\ sn^3}{dl\text{-}49, y\ v\ f\ car}$	w^{vc}, *f*/sc^8·Y	—	2846	—	3022	0	1	—	—	—
V	$\frac{w^{vc}, f}{dl\text{-}49, y\ v\ f\ car}$	*dl-49, y w* lz^s/sc^8·Y	221	1446	1684	1649	0	2	0.00	0.02	0.02
VI	$\frac{y\ w\ spl\ sn^3}{dl\text{-}49, y\ v\ f\ car}$	X^{c2}, *cv v f*/sc^8·Y	—	9036	—	8441	83	72	0.02	0.05	0.07
VII	$\frac{X^{c2}, cv\ v\ f}{dl\text{-}49, y\ v\ f\ car}$	*dl-49, y w* lz^s/sc^8·Y	2703	1582	2864	2666	48	29	0.03	0.04	0.07

exceptional daughters and (2) no double crossovers are recovered from w^{vc}/*dl-49* heterozygotes. Elimination of primary nondisjunction and first anaphase bridge configurations as prime sources of nullo-w^{vc} gametes suggests that, if meiotic loss occurs, it is the result of the same process which operates during the cleavage mitoses. Therefore, regardless of their germinal or somatic origin, X0 males should be regarded with gynandromorphs as a class arising from w^{vc} loss.

One half of all gametes produced by w^{vc}/Y males should carry the w^{vc} chromosome and should be represented in the progeny of these males by the sum of females plus gynandromorphs and X0 males. However, comparison of this total with the number of regular males bearing the sc^8·Y shows a gross deficiency of the w^{vc} class instead of the 1:1 expectation (Cross I). In order to determine the source of this deficiency, counts were made of eggs from pair matings of *y v* females to w^{vc}/sc^8·Y males. The

TABLE 2

Comparison of dominant lethal frequency with the deficiency of presumptive w^{vc} *zygotes observed from pair matings of* y v *females to* $w^{vc}/sc^{8}\cdot Y$ *males. The results from each mating were obtained from five consecutive 24-hour subcultures*

Mating No.	Egg counts			Adult counts					
	No. laid	No. lethal	Dominant lethal frequency	Rod male	w^{vc} female	Gynandromorph	X0 male	w^{vc} Zygote deficiency	w^{vc} Lethal frequency
1	262	79	0.30	129	15	5	12	97	0.75
2	243	68	0.28	113	11	7	10	85	0.75
3	252	67	0.27	129	29	11	4	85	0.66
4	273	71	0.26	139	14	5	13	107	0.77
5	259	58	0.22	124	24	8	16	76	0.61
6	261	58	0.22	125	46	11	14	54	0.43
7	228	44	0.19	108	41	8	14	45	0.42
8	182	20	0.11	82	40	2	16	24	0.29
9	172	15	0.09	80	52	5	10	13	0.16
10	258	22	0.09	113	70	8	15	20	0.18
11	238	17	0.07	112	73	8	14	17	0.15
12	219	14	0.06	105	69	10	10	16	0.15
13	94	1	0.01	46	44	0	0	2	0.04

number of eggs which failed to hatch agreed closely with the w^{vc} adult deficiency measured in the same cross (table 2). The results of this experiment show that the w^{cv} deficiency is primarily attributable to zygotic or embryonic lethality rather than gametic or postembryonic loss. When the female parent contributes the w^{vc} chromosome it is also clear that the number of presumptive w^{vc}/rod zygotes recovered as adults is strikingly reduced from the expected number based on rod/rod females. These observations lead to the conclusion that instability of the w^{vc} chromosome is characterized not only by its loss but by dominant lethality as well.

An estimate of the frequency of zygotes affected by w^{vc} instability can be made on the assumption that w^{vc} gametes and gametes bearing the w^{vc} homolog (either rod-X or Y) are produced in a 1:1 ratio. Thus from the cross of rod/rod females by w^{vc} males, the rod male class provides an estimate of the number of w^{vc} gametes produced; consequently, (Gy + X0 ♂) ÷ rod ♂ represents the proportion of w^{vc}/rod zygotes in which loss occurs, and (rod ♂ − w^{vc} ♀ − Gy − X0 ♂) ÷ rod ♂ gives the dominant lethal frequency among w^{vc}/rod zygotes. In the reciprocal cross of w^{vc}/rod females by rod males, the accuracy of these estimates is probably improved by substituting the rod/rod female class for the rod male class as the basis of expectation. No other attempt to correct for differential viability not associated with w^{vc} instability has been made, and in general the effect of errors from this source is not sufficient to alter conclusions based on these estimates. It can be seen on inspection of the estimates of w^{vc} loss and lethality listed for Crosses I–III (table 1) that there is considerable variability associated with both attributes of w^{vc} instability, even in the same cross performed at different times (cf. Cross Ia with Ib, etc.). Although Crosses I and II are not strictly reciprocal, there appears to be no essential difference in the pattern of w^{vc} behavior dependent upon maternal or paternal origin.

In controlled experiments with sib w^{vc}/rod females in comparison tests, it was demonstrated that the total instability is not affected by autosomal or sex-linked modifiers or by addition of a Y chromosome. However, w^{vc} instability is significantly decreased at 18°C as compared with 26°C developmental temperature, and the F_1 w^{vc} instability increases with increasing age of w^{vc} mothers. These results supported the conclusion that the variability is an inherent property of the w^{vc} chromosome which is subject to environmental or physiological modification (HINTON 1954).

Crosses IV and V (table 1) illustrate the behavior of a w^{vc} chromosome which became stable in stock culture; this chromosome is more stable than the standard stable X^{c2} chromosome (Crosses VI and VII) by the criteria of loss and lethality. Three other cases of w^{vc} stabilization have been observed under controlled conditions. Stabilization resembles a mutational event in the w^{vc} chromosome since only one individual of a generation exhibits the change from high instability to near or complete stability and no segregating modifiers are demonstrable. In all cases, the original w^{vc} phenotype and ring structure are maintained. No instances of the reverse change from the completely stable to the unstable condition have been recorded, although it has been possible to select highly unstable w^{vc} chromosomes from lines characterized by low instability.

THE OCCURRENCE AND BEHAVIOR OF w^{vc} DUPLICATIONS

From crosses of various yellow, attached-X females to unstable w^{vc} males, a few yellow mosaic daughters were obtained from which five different free duplications derived from the w^{vc} chromosome were isolated. Examination of larval brain smear metaphases showed that four of these duplications are small ring chromosomes (one duplication was lost before cytological examination could be made). The euchromatic composition of each of the duplications, determined in tests of their ability to cover various mutant loci, is limited to the loci immediately adjacent to the heterochromatin on one or both sides of the centromere (Part A of table 3 and fig. 1). The frequency of yellow mosaics produced from the cross of *Dp/y w f*/Y females by *dl-49, y w v f car*/Y males varies with the particular duplication and ranges from 100% down to 5.4% (table 4). This lower frequency is comparable to that given by $Dp(1;f)X^{c2}$ which is a small ring-shaped duplication derived from X^{c2}. The size of mosaic areas also varies widely and is clearly correlated with the mosaic frequency; the modal size of yellow spots ranges from a few hairs for $Dp(w^{vc})6094a$ and $Dp(1;f)X^{c2}$ to over half the hypodermis for $Dp(w^{vc})6101$. If the duplication is one carrying the w^{vc} locus, mosaicism for eye pigmentation is registered in accordance with the yellow mosaicism of the hypodermis surrounding the eye.

SCHULTZ reported (BRIDGES and BREHME 1944) that the frequency of yellow mosaicism produced by $Dp(1;f)X^{c2}$ could be partially suppressed by the addition of a Y chromosome, suggesting a position effect component of the mosaicism. This effect has been tested with $Dp(w^{vc})6094b$ which, when present in eye tissue, permits detection of the Y chromosome through the latter's effect on the position effect variegation of the w^{vc} locus of the duplication. Among *64* $Dp(w^{vc})6094b/dl\text{-}49$, *y w v f car*/0 males, 27 were mosaic, while 102 of 198 $Dp(w^{vc})6094b/dl\text{-}49$, *y w v f car*/Y males were mosaic. Since these frequencies are not significantly different, position

TABLE 3

The euchromatic constitution of duplications derived from the unstable w^{vc} *chromosome. The duplications listed under Part A were recovered from attached-X females; those listed under Part B were observed in sterile gynandromorphs or X0 males*

Presumptive zygotic genotype	Identification or number of duplications	Fraction of tested duplications covering mutant loci										
		y	*w*	*spl*	sn^3	lz^s	*v*	m^2	g^4	*f*	*car*	*bb*
Part A												
y f:=/w^{vc}, *B*	*Dp*(w^{vc})*4097*	1/1	1/1*	1/1*						0/1		
y w/w^{vc}, *f*	*Dp*(w^{vc})*5279*	1/1	1/1	0/1	0/1		0/1				0/1	
y w f/w^{vc}, *f*	*Dp*(w^{vc})*6094a*	1/1	0/1	0/1	0/1							
	Dp(w^{vc})*6094b*	1/1	1/1	0/1	0/1		0/1				0/1	0/1
	Dp(w^{vc})*6101*	1/1	0/1									
Part B												
y w spl sn^3 *(bb)*/w^{vc}, *f*	9	9/9	5/7	0/7	0/9							0/2
dl-49, y Hw m^2 g^4/w^{vc}, *f*	7	7/7						0/4	0/3			
dl-49, y w lz^s/w^{vc}, *f*	6	6/6	4/4			0/4						
dl-49, y w v f car/w^{vc}, (*f*)	6	6/6	2/3				0/1			0/2	0/1	
dl-49, y v f car/w^{vc}, *f*	2	2/2					0/1				0/1	
w^{m4}, *y w sn m*/w^{vc}, *f*	1	1/1			0/1							
Total	36	36/36	14/19	1/11	0/13	0/4	0/4	0/4	0/3	0/3	0/4	0/3

* Constitution inferred from Confluens phenotype of *y f*:= females.

TABLE 4

The frequency of yellow mosaics produced from the cross of Dp/y w f/Y *females by* dl-49, y w v f car/Y males

Duplication	*Dp/y w v f car* ♂♂			*Dp/y w f* ♀♀		
	Nonmosaic	Mosaic	Percent mosaic	Nonmosaic	Mosaic	Percent mosaic
Dp(1;f)X^{c2}	18	1	5.3	213	21	9.0
Dp(w^{vc})*4097*		Lethal		97*	97*	50.0
Dp(w^{vc})*5279*		Lethal		0	57	100.0
Dp(w^{vc})*6094a*	403	53	11.6	565	32	5.4
Dp(w^{vc})*6094b*	133	129	49.2	270	192	41.6
Dp(w^{vc})*6101*	0	35	100.0	0	85	100.0

* These females carried the *y f*:= double-X chromosome of MULLER.

effect variegation probably does not contribute to the mosaicism displayed by the w^{vc} duplications.

Among the progeny of crosses involving unstable w^{vc} and various *y* rod chromosomes, very rare gynandromorphs and X0 males have been observed in which part or all of the male tissue was phenotypically wild type rather than yellow as expected; in those instances where the rod also carried the *w* allele, a variegated male eye phenotype was sometimes found in conjunction with the surrounding wild type male hypodermis. Despite their occurrence in sterile individuals, sufficient information is available to interpret these phenotypes on the basis of small w^{vc} duplications comparable to those described in the preceding paragraphs. The data itemized

below are pertinent to the origin, frequency, and constitution of these duplications. (1) Of 31 cases, 13 occurred among 13,929 gynandromorphs and 18 were found among 12,360 X0 males. The duplications which occurred in gynandromorphs must have been of somatic origin since the zygote received a whole w^{vc} chromosome; although this interpretation may also be applicable to those duplications observed in X0 males, it is possible that they were of gametic origin. (2) The frequency of w^{vc} duplication occurrence was higher when the w^{vc} chromosome was introduced by the male parent (13 cases out of 3,772 individuals) than when it was of maternal origin (18 cases out of 22,527) individuals. The reason for this difference is not apparent. (3) In 19 of the 31 cases, the duplication was present in only part of the X0 male tissue, the remainder being of the mutant phenotype expected from the particular rod used. Mosaicism of these duplications may be either a consequence of their formation or indicative of loss after formation (see discussion). (4) The genetic constitution of the duplications was determined with reference to the mutant alleles carried by the rod chromosome, provided that the duplication was present in the tissue affected by the particular mutant. Examination of table 3, which summarizes the constitution of these 31 duplications (Part B) and the 5 cases described earlier (Part A), shows that all 36 duplications included the y^+ locus, that 14 included and 5 excluded the w^{vc} locus, that only 1 of 11 probably carried the spl^+ locus, and that no other mutant locus over the entire euchromatic length of the X chromosome was covered by any of the duplications. These data, considered in reference to the structure of the w^{vc} chromosome, suggest that these 36 duplications comprise in common the w^{vc} centromere and part of the adjacent heterochromatin bounded on either side by the w^{vc} and y^+ markers; in addition, some fraction of the heterochromatic segment marked by y^+ and car^+ may be included in the duplications.

This analysis of w^{vc} duplications leads to two conclusions regarding w^{vc} behavior. First, the production of the duplications must be considered a fourth manifestation of w^{vc} instability. Second, the maintenance of instability by some of the recovered duplications shows that control of the instability mechanism must reside in that part of the w^{vc} chromosome present in the duplications.

DISCUSSION AND INCIDENTAL RESULTS

Two distinct mechanisms have been previously proposed to account for ring chromosome loss during the cleavage divisions of *Drosophila melanogaster*. The first of these, advanced by MORGAN (1926), is exclusion of the ring chromosome from telophase nuclei as a consequence of lagging on the anaphase spindle. The second means by which the ring chromosome might be lost from a cleavage nucleus is through the formation of an anaphase bridge. Thus GRIFFEN and LINDSLEY (1946) suggested that the w^{vc} chromosome might form anaphase configurations composed of interlocked single rings as the consequence of a 360° shift in the plane of chromosome reduplication. This result is similar to that produced by two progressive exchanges between sister chromatids of a ring chromosome at the time of its reduplication as envisaged by MCCLINTOCK (1938) in her cytogenetic study of somatic ring chromosomes in maize. A dicentric double ring anaphase bridge would result from a single exchange between sister chromatids.

Evidence in favor of the anaphase bridge hypothesis of ring chromosome loss has

been presented by BRAVER and BLOUNT (1949) who found bridge configurations in 12 and 22% of the larval brain anaphases of $X^{rc^2}/dl\text{-}49$ and $w^{rc}/dl\text{-}49$ females, respectively. In conjunction with the present study, aceto-orcein smear preparations were made of the brains from unstable w^{rc} and stabilized w^{rc} male larvae. Bridges were observed in 10.1% (N = 179) of the late anaphases and early telophases of the unstable w^{rc} material whereas the stabilized w^{rc} produced 1.6% (N = 310) bridges. Although many rather diagrammatic examples of dicentric double ring bridges were observed in these preparations and in w^{rc} female brain smears, no unequivocal figures of interlocked single ring bridges were recorded. The disagreement between this observation and the report of BRAVER and BLOUNT that most of the bridges in their preparations were composed of interlocked single rings may not be surprising in view of the cytological difficulty of the material. Another interesting cytological feature is that X0 cells are only very rarely found in larval brains of unstable w^{rc}/rod females, which indicates that the result of anaphase bridge formation at this stage of development is not ring chromosome loss. This difference in the fate of anaphase bridges might be correlated with the syncytial structure of the early embryo as compared with the cellular structure of the larval brain. It is evident that the relation of anaphase bridge configurations in larval brain mitoses to those hypothesized to occur during the cleavage divisions remains to be clarified.

The analysis of w^{rc} behavior requires consideration of an hypothesis to account for the production of gynandromorphs, X0 males, dominant lethals and unstable duplications derived from the w^{rc} chromosome. In addition, such an hypothesis should account for the changes in the degree of w^{rc} instability and thus provide a clue to the difference in behavior of unstable w^{rc} and the typical stable ring-X chromosomes. Although gynandromorphs and X0 males might arise from anaphase lagging, this behavior is not sufficient to produce dominant lethals or w^{rc} duplications which appear to require breakage of the chromosome. On the other hand, anaphase bridge formation furnishes an adequate model of the w^{rc} instability mechanism.

The first possibility to be discussed is that the initial bridge configuration is a dicentric double ring of the type produced by a single exchange between sister chromatids. Such a bridge would appear to present ample opportunity for loss since at least two breaks are required to deliver any part of the bridge to a daughter nucleus. Given the required breaks in each member of the bridge, most of the products would be lethal because of genetic unbalance arising either at this time or following a bridge breakage fusion cycle. The only products which could be expected to survive would be those reconstituting the original euchromatic composition, those of the original composition plus or minus a small euchromatic segment, and small free duplications. Although changes in the heterochromatic constitution of the chromosome would probably not cause serious viability defects, these events would not ordinarily be recognized.

Alternatively, the primary anaphase bridge might be composed of interlocking single ring chromosomes. It seems unlikely that dominant lethals or w^{rc} duplications could arise directly from interlocked rings since a single break would suffice to resolve the interlock. If however, the break is followed by torsional restitution or sister chromatid union initiating a bridge breakage fusion cycle, the consequences of inter-

locked ring bridges become indistinguishable from those of a primary bridge formed by a dicentric double ring. Although it is generally assumed that chromatid type bridge breakage fusion cycles contribute to the production of radiation induced dominant lethals in Drosophila, there is no direct evidence on this point; in Habrobracon, however, WHITING (1945) has observed bridge configurations in cleavage mitoses following irradiation of oocytes. In any event, the anaphase bridge model of w^{rc} instability may not require the operation of bridge breakage fusion cycles unless the primary bridge is of the interlocked ring type.

No special crosses have been designed to detect w^{rc} chromosomes having hyperploid euchromatic segments, but deficient w^{rc} chromosomes would have been detectable in routine crosses involving rods bearing mutant markers. Possible examples of deficient w^{rc} chromosomes may be represented by nine $w^{rc}/y\ w\ldots$ females which displayed completely white rather than the expected white-variegated eyes; in eight of the nine cases the w^{rc} chromosome was introduced by the male parent. Since one of the exceptional females had a small sector of variegated tissue in one eye, and since four of the nine produced only typical variegated w^{rc} offspring, it is suggested that their phenotypes were the results of early somatic events. One of the "white" w^{rc} females was sterile. The remaining four transmitted w^{rc} chromosomes which continued to give a white eye phenotype in subsequent generations and which presented the normal ring configuration in larval brain metaphases. The deficiency hypothesis for the origin of these altered w^{rc} chromosomes is supported by the fact that two of them are male lethal. It appears unlikely that the two cases which are male viable might also represent deletions of the w^{rc} locus, and thus mutation of w^{rc} to w remains an alternative interpretation of these cases. No examples of w^{rc} chromosomes apparently deficient for other loci have been recorded.

The anaphase bridge hypothesis of w^{rc} instability predicts the formation of small free w^{rc} duplications as bridge breakage products. Some of or all those duplications observed in X0 males may have been derived from bridges formed at the second meiotic division in either sex. The interpretation of the recovery of those duplications which were observed in gynandromorphs is less direct. Since the reciprocal product of the cleavage bridge, if included in the sister telophase nucleus, would be lethal by all expectations, it becomes necessary to assume that either the reciprocal product was lost at the next mitosis, or that it behaved as a nuclear rather than zygotic lethal. A fourth possibility, suggested by the recovery of both *Dp*(w^{rc})*6094a* and *Dp*(w^{rc})*6094b* from a single attached-X female, is that two small duplications are produced simultaneously, one at each end of the bridge.

The simultaneous incorporation of both markers bounding the w^{rc} centromere region rules out the origin of w^{rc} duplications from double ring dicentrics formed by centromere misdivision since this event would place both y^+ loci at one pole of the bridge and both w^{rc} loci at the opposite end. The euchromatic constitution of the w^{rc} duplications may also have some bearing on the distribution of break points in double ring dicentrics. Clearly, the amount of euchromatin recoverable in a duplication is limited by viability considerations. However, this should not influence the observation that 14 of 19 tested duplications included the w^{rc} locus whereas only 1 of 11 apparently included the spl^+ allele separated from w^{rc} by only 4 or 5 salivary chro-

mosome bands. Secondly, although duplications carrying the w^{vc} locus but lacking the y^+ locus could have been detected, all 36 duplications included y^+. It may be worth repeating in this context that only w^{vc} chromosomes apparently deficient for the w^{vc} locus were observed although others could have been recognized. These results suggest the possibility that double ring dicentrics tend to break at localized positions.

Anaphase bridge formation also provides an adequate basis for understanding the instability exhibited by some of the duplications. Since there has been no suggestion of lethality caused by unstable w^{vc} duplications beyond that expected from duplicated euchromatin *per se*, it is possible that bridges formed by small rings are more susceptible to loss than to breakage as McCLINTOCK (1938) found to be the case with the small rings in maize. One of the strongest bases for McCLINTOCK's suggestion that sister-strand exchange is the primary cause of bridge formation by maize rings was her observation that bridge frequency is dependent on ring size. Such a direct comparison has not been possible between w^{vc} and its small ring counterparts.

On the basis of the instability and genetic constitution of the w^{vc} duplications, it was concluded that control of w^{vc} instability must reside in that part of the chromosome which is chiefly heterochromatic. Inclusion of the euchromatin marked by the y^+ allele may be a necessary, but not sufficient, factor in controlling w^{vc} instability; inclusion of the w^{vc} locus is clearly not necessary. If it is considered that instability of the w^{vc} chromosome is dependent on the dosage of some heterochromatic element of the w^{vc} centromere region, then the changes in degree of w^{vc} instability may be explained in terms of anaphase bridge formation. Either increases or decreases in the dosage, or complete loss, of this element might be represented in single rings derived from double ring dicentrics, and these changes would be reflected by altered instability of the recovered rings. It is neither required nor excluded that the events leading to the formation of anaphase bridges be restricted to that segment of the w^{vc} chromosome which controls its instability.

Although the results of this analysis are satisfied by the hypothesis of anaphase bridge formation, they provide little information relevant to the time, frequency, or cause of this event. Only gynandromorphs and certain w^{vc} duplications demand their origin from cleavage events; X0 males, dominant lethals and other w^{vc} duplications might be the consequence of dicentric double ring formation at anaphase II of meiosis as well as during the subsequent cleavage mitoses. Bridge formation at different times in embryonic development is clearly illustrated by the wide range of mosaic spot sizes produced by loss of w^{vc} duplications. Since anaphase bridges might be formed with different probabilities in any of several successive mitoses, and because of potential variables determining whether a bridge will break or be lost, it seems doubtful that the observed w^{vc} loss and lethal frequencies can be quantitatively related to frequencies of cytological events. In general, w^{vc} behavior appears to parallel that of maize rings; however, the evidence presented here does not demonstrate the occurrence of sister-strand exchange in the unstable w^{vc} chromosome.

SUMMARY

The structure of $In(1)X^{c2}$, w^{vc}, an unstable ring-X derived from the stable X^{c2} chromosome of *Drosophila melanogaster*, is described. Instability of the w^{vc} chro-

mosome is manifested by the frequent production of gynandromorphs, X0 males, and dominant lethals among w^{vc}/rod zygotes. Observations on unstable small ring duplications derived from the w^{vc} chromosome suggest that some heterochromatic element of the w^{vc} centromere region is responsible for w^{vc} instability.

The consequences of w^{vc} instability can be interpreted on the hypothesis that the unstable ring chromosome forms dicentric double ring configurations at anaphase of the cleavage mitoses and perhaps of the preceding meiotic division as well. Gynandromorphs and X0 males register loss of the dicentric, whereas dominant lethals arise by inclusion of bridge breakage products in the cleavage nuclei. The origin and continued instability of w^{vc} duplications are also explicable in terms of anaphase bridge formation, and it is postulated that changes in the degree of w^{vc} instability may be related to heterochromatic duplication or deficiency products of bridge breakage.

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