A CYTOLOGICAL STUDY OF w^{vc} CHROMOSOME INSTABILITY IN CLEAVAGE MITOSES OF DROSOPHILA MELANOGASTER[1]

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INSTABILITY of the w^{vc} ring-X chromosome of *Drosophila melanogaster* is attributable to anaphase bridge formation during the cleavage mitoses where loss of the bridge from $w^{vc}/+$ embryos results in gynandromorphs or X0 males and breakage of the bridge results in lethality (HINTON 1955). Rod-w^{vc} chromosomes derived from unstable w^{vc} ring chromosomes also cause lethality, but are not lost during cleavage. If lethality is due to anaphase bridge breakage in both the unstable ring and rod chromosomes, the bridges must arise from sister strand fusion rather than sister strand crossing over. Structural differences between bridges formed by sister strand fusion may account for the difference with respect to gynandromorph production by the two kinds of chromosomes (Figure 1); the ring-w^{vc} double chromatid bridge may be subject to either breakage or loss while the rod-w^{vc} single chromatid bridge may always break (HINTON 1957).

This model of w^{vc} instability is supported by the differences in bridge frequencies for stable and unstable rings observed in larval brain cells (BRAVER and BLOUNT 1949; HINTON 1955) and in secondary spermatocytes (WELSHONS and HINTON 1955). Because of their limited nature and the questionable relevance of observations on these tissues, the results of a cytological study of ring and rod-w^{vc} embryos of the third through eighth cleavages are reported in this paper. These results provide partial confirmation of the anaphase bridge model in that the frequencies of bridges and other mitotic abnormalities are associated with the degree of instability manifested by the ring and rod chromosomes.

METHODS

For convenience of readers not immediately familiar with Drosophila genetics symbolism, it may be appropriate to describe briefly the chromosomes utilized in this study, as follows: two standard rod-X chromosomes, one of normal sequence bearing the mutant yellow (y) and one of the inverted sequence (dl-49) bearing the mutants yellow, white (w) and lozenge (lz^s); two ring-X chromosomes, one of normal sequence (X^{c2}) bearing the mutants crossveinless (cv), vermilion (v) and forked (f) and one of inverted sequence (w^{vc}) having a variegated position effect at the white locus; a rod-w^{vc} chromosome (w^{vc}-B^s) derived from the w^{vc} ring and bearing the proximal segment of the Bar of Stone translocation as a hyperploid second arm; finally, two Y chromosomes, one standard, the other

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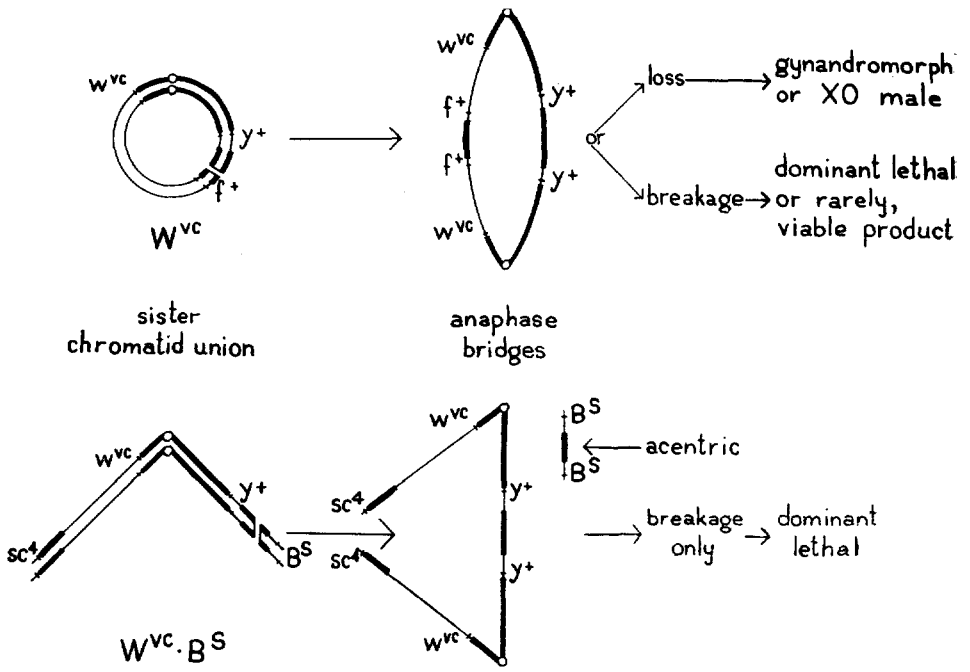

FIGURE 1.—The anaphase bridge model of w^{vc} chromosome instability comparing the consequences of bridge formation in ring and rod chromosomes. The relative proportions of euchromatin (light line) and heterochromatin (heavy line) are shown arbitrarily.

(sc^8·Y) bearing the tip of the X chromosome and including the wild type allele of yellow.

Genetic and cytological observations were made on two sets of crosses. In one set y/y females were crossed to either unstable w^{vc}/sc^8·Y or stable X^{c2}, *cv v f*/sc^8·Y males. For the second set w^{vc}·B^S/dl-49, *y w lz*^{s} females were crossed to y/Y males, where the w^{vc}·B^S chromosome (Figure 1) of the female was either stable or unstable. Genetic data were collected from these crosses in the usual manner, and eggs deposited by the females on heavily yeasted food chips were collected and prepared by a Feulgen whole mount technique (VON BORSTEL and LINDSLEY 1959).

During preliminary examination, technically good eggs in the third through eighth cleavages were selected for further study; subsequent observations were made from slides coded with respect to stability or instability but not with respect to ring or rod. The cleavage nuclei were classified according to mitotic stage and recorded under either of two groups, prophase-metaphase or anaphase-telophase. The number of prophase-metaphase figures observed is based upon counts (which agreed closely with the geometric increase expected from synchronous mitoses) for the third through sixth cleavage embryos and upon the expected numbers of 64 and 128 for the seventh and eighth cleavage embryos where accurate counts could not be made readily. The anaphase-telophase data include only

those figures classifiable with respect to presence or absence of anaphase bridges, defined as extensive chromatin strands connected with or isolated between the two polar groups of chromosomes of late anaphase or telophase stages. It is possible that use of this definition resulted in the omission of some broken bridges, which may have had the same appearance as chromosome lags often encountered in early anaphase. Because of the compact arrangement of the chromosomes in mitotic figures, the chromosomes involved in bridges could not be identified; for the same reason, it was not possible to distinguish that half of the embryos expected to carry the chromosome under investigation. This latter defect precludes treatment of the data by ordinary statistical tests.

RESULTS AND DISCUSSION

Genetic results: The differences between the stable and unstable ring crosses and between stable and unstable w^{vc}·B^{s} crosses seen in Table 1 are comparable with those previously reported (HINTON 1955, 1957). The unstable w^{vc} ring cross produced a high frequency of gynandromorphs, X0 males and dominant lethals (= rod ♂ − w^{vc} ♀ − Gy − X0 ♂/rod ♂), while these manifestations of instability were negligible in the stable X^{c2} cross. There were no gynandromorphs and relatively few X0 males produced by either of the two w^{vc}·B^{s} crosses illustrating the lack of somatic loss of this chromosome. Dominant lethality appeared in both the w^{vc}·B^{s} crosses; that of the stable cross is primarily attributable to hyperploidy of the B^{s} arm of the chromosome, and an equal amount of lethality from this source probably exists also in the unstable w^{vc}·B^{s} cross. The remaining dominant lethality (0.569 − 0.103 = 0.466) is a measure of the total instability of the unstable w^{vc}·B^{s} chromosome. The total instability of the unstable w^{vc}·B^{s} chromosome was lower than that of the unstable w^{vc} chromosome. However, this difference is not significant in that the w^{vc}·B^{s} chromosome was not derived directly from the unstable w^{vc} stock used in this study.

Cytological results: The cytological observations are presented according to cleavage stage in Table 2 for each of the four chromosomes investigated. The

TABLE 1

Genetic results from crosses of y/y *females to ring/*sc^{s}·*Y males and of* w^{vc}·B^{s}*/dl-49*, y w lz^{s} *females to* y/*Y males*

Chromosome	Normal rod males	Normal rod females	w^{vc} females	Gy	XO males	Dominant lethals
Unstable w^{vc}	4519	...	1268	279	455	
Percent	100.0		28.1	16.2		55.7
Stable X^{c2}	2544	...	2533	3	0	
Percent	100.0		99.6	0.1		0.3
Unstable w^{vc}·B^{s}	260	292	121	0	5	
Percent		100.0	41.4	1.7		56.9
Stable w^{vc}·B^{s}	945	1341	1195	0	8	
Percent		100.0	89.1	0.6		10.3

TABLE 2

Cytological results from crosses of y/y *females to ring/*sc^{s}*-Y males and of* w^{vc}*-*B^{s}*/dl-49,* y w lz^{s} *females to* y/*Y males*

Chromosome	Cleavage	Number of embryos Observed	Number of embryos Abnormal	Prophase-metaphase Number of figures Observed	Prophase-metaphase Number of figures Abnormal	Anaphase-telophase Number of figures Classifiable	Anaphase-telophase Number of figures With Bridges
Unstable	3	9	6	36	15	0	..
w^{vc}	4	12	4	96	25	0	..
	5	8	6	96	27	19	2
	6	22	10	512	29	100	2
	7	29	13	1088	58	454	25
	8	12	2	768	0	579	4
	Total	92	41	2596	154	1152	33
	Percent	..	44.6	..	5.9	..	2.9
Stable	3	4	1	8	1	6	0
X^{c2}	4	8	1	32	0	27	1
	5	5	0	32	0	23	0
	6	8	1	96	0	99	1
	7	8	5	192	0	213	5
	8	6	0	392	0	177	0
	Total	39	8	752	1	545	7
	Percent	..	20.5	..	0.1	..	1.3
Unstable	3	11	5	40	13	2	0
w^{vc}*-*B^{s}	4	19	3	128	12	24	0
	5	16	1	240	16	16	0
	6	12	4	288	1	30	3
	7	21	4	1024	18	84	3
	8	31	1	3456	0	181	6
	Total	110	18	5176	60	337	12
	Percent	..	16.4	..	1.2	..	3.6
Stable	3	11	1	40	0	4	1
w^{vc}*-*B^{s}	4	14	0	96	0	16	0
	5	18	0	240	0	25	0
	6	14	2	320	3	50	1
	7	14	0	896	0	0	..
	8	16	2	1408	0	175	2
	Total	87	5	3000	3	270	4
	Percent	..	5.7	..	0.1	..	1.5

frequency of embryos having one or more nuclear abnormalities was higher in the unstable than in the stable crosses for both the ring and rod chromosomes; this gross consideration does not take into account the fact that there were many more embryos with more than one nuclear abnormality in the unstable than in the stable crosses. It may be noted that the highest frequency of abnormal embryos was less than 50 percent as expected if all the nuclear abnormalities are reflections of instability of the w^{vc} chromosome which should be present in only half the embryos. One might expect the incidence of embryos with nuclear abnormalities to increase exponentially with cleavage stage if the probability of nuclear

abnormality remains constant with cleavage stage; the data, however, suggest no such increase in incidence of abnormal embryos.

The nuclear abnormalities included not only anaphase bridges as previously defined, but a second more frequent class characterized by pycnosis, chromosome fragmentation, increased amounts of chromatin, and tripolar spindles (Figure 2). Nuclei possessing these qualitatively and quantitatively variable characteristics were found adjacent to normal mitotic figures (for a description of the normal mitotic cycle in cleavage nuclei, see SONNENBLICK 1950). The presence of spindles and the oriented arrangement of chromatin in some abnormal figures suggested abortive divisions, which may account for the increased amounts of chromatin. Mitotic derangements similar to those seen in this material have been described previously in Drosophila embryos having excessive polyspermy (HUETTNER 1927), produced by species hybrid females (KAUFMANN 1940), arising from X-rayed gametes (SONNENBLICK 1940), having no X chromosome (POULSON 1940) and treated with ether (CORNMAN 1944). The frequencies of abnormalities for prophase-metaphase mitoses in the stable ring and rod crosses were hardly higher than one might expect in any "normal" material, whereas embryos from both unstable crosses possessed a high abnormality frequency which declined with cleavage stage. No abnormalities were recorded among eighth cleavage prophase-metaphase figures; this fact may be due to including in the data only those nuclei that had completed peripheral migration while those

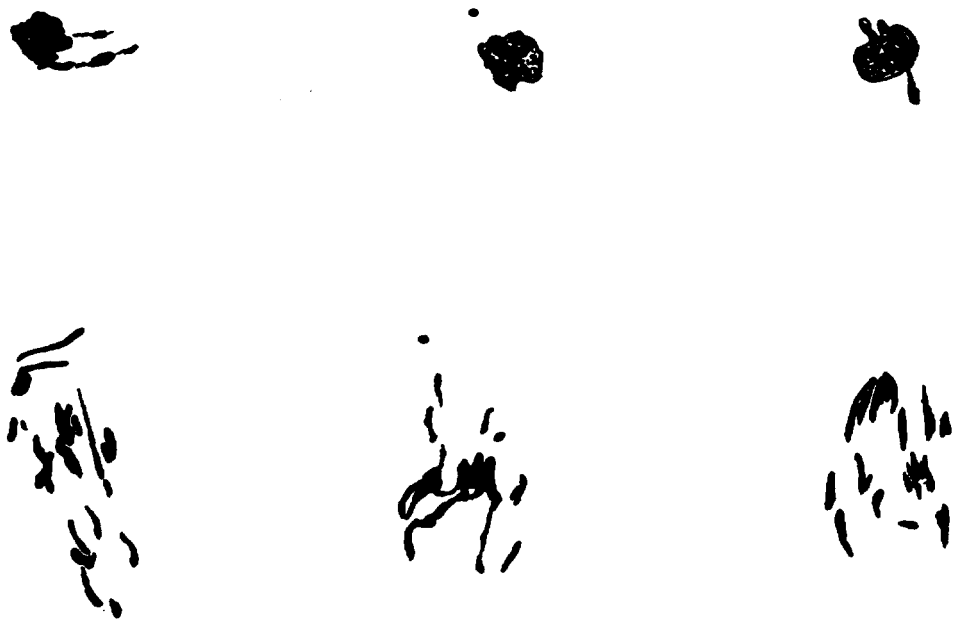

FIGURE 2.—Camera lucida drawings (ca. 2730 ×) of cleavage nuclei showing pycnosis, chromosome fragmentation, and increased amounts of chromatin.

nuclei having defects remained unrecorded in the yolk mass. Omission of the yolk nuclei was deliberate in that frequent mitotic disturbances have been reported to occur regularly among them (RABINOWITZ 1941).

The data on anaphase bridges suggest no consistent frequency variation with cleavage stage. The bridge frequencies were higher for the unstable than for the stable ring and rod crosses, although the statistical significance of these differences is difficult to assess. The twofold difference in bridge frequency between the X^{c2} and w^{vc} material parallels that found by BRAVER and BLOUNT (1949) in brain smears of X^{c2}/dl-49 and w^{vc}/dl-49 larvae where the bridge frequencies were 12 and 22 percent, respectively. Summing the data from all four crosses, there were 56 bridges observed among 81 embryos having 2306 classifiable late anaphase or telophase figures. If it is assumed that only half of these figures contained a ring or w^{vc}·B^{s} chromosome and that bridges were limited to this half of the figures, the over-all bridge frequency could be calculated as 56/1153 or 4.8 percent. However, 29 of the bridges were seen in just four embryos having a total of 206 figures (the individual embryo counts were 5/50, 8/24, 10/58 and 6/74 bridges per figures, where the first three cases were seventh cleavage w^{vc} embryos and the fourth was an eighth cleavage w^{vc}·B^{s} embryo). The remaining 27 bridges were distributed one or two per embryo. This apparent clustering of bridges in particular embryos suggests the occurrence of a bridge breakage fusion cycle of the chromatid type (MCCLINTOCK 1941). In the four embryos cited above, the distribution of figures with and without bridges appeared to be not in sectors as one might expect if the several bridges were related but rather at random. This might be explained in either of three ways: there may be intermingling of the products of normal and bridge anaphases during nuclear proliferation, or some of the breaks may heal rather than fusing, or as SCHWARTZ and MURRAY (1957) have suggested, the fusion forming secondary bridges may be incomplete and weak so that breakage occurs so early in anaphase as to escape cytological detection.

The anaphase bridge model makes two predictions which are supported by the observations. First, bridges formed by ring chromosomes should be double, while bridges formed by rod chromosomes should be single. Of the 16 bridges found in embryos from the w^{vc}·B^{s} cross, none were visibly double, while 15 of the 40 bridges recorded from the ring crosses were distinctly double; all 15 double bridges were from the unstable w^{vc} cross. As suggested by the drawings of typical bridges (Figure 3), it was frequently difficult to distinguish single and double bridges because of the proximity of the two strands of double bridges. The second prediction supported by the observations is elimination of bridges formed by rings but not of those formed by rods; five of the 40 bridges from the ring crosses were situated between the polar groups of chromosomes so as to suggest their being lost, but none of the 16 w^{vc}·B^{s} bridges were of this configuration. Other than the differences noted here, there were no recurrent qualitative differences in bridges or other mitotic abnormalities for the different crosses.

The anaphase bridge model also predicts that the bridges produced by sister chromatid union in the w^{vc}·B^{s} chromosome should be accompanied by an acentric

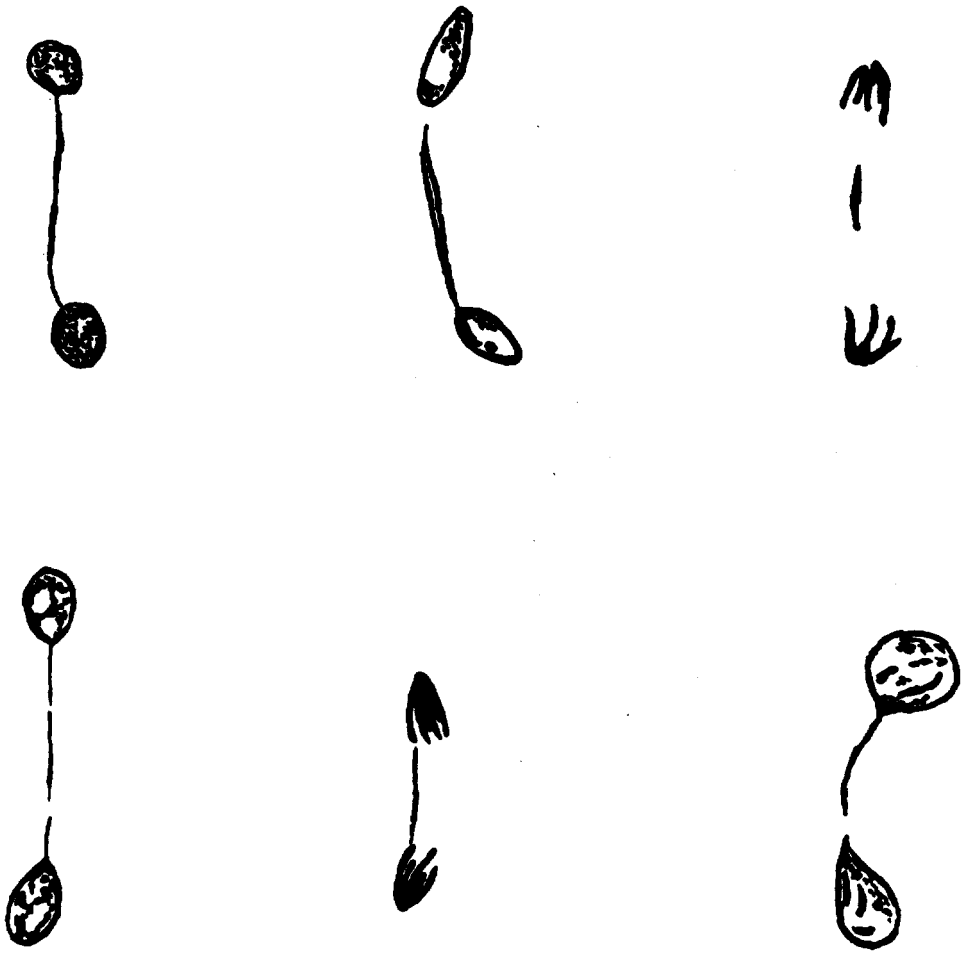

FIGURE 3.—Camera lucida drawings (ca. 2730 ×) of representative bridge configurations found in cleavage mitoses of embryos from the unstable w^{vc} (top three) and w^{vc}·B^{S} (lower three) crosses.

fragment, but such fragments could be detected in none of the w^{vc}·B^{S} bridge figures seen in this study. This result was preindicated by a similar failure to find acentrics (or bridges) in a preliminary study of w^{vc}·B^{S} larval brain smears (HINTON 1957). The possibility that the acentrics are too small to be seen was tested by examination of anaphase figures in unstable w^{vc}·dl-49 larval brains where the acentric should include at least the entire euchromatic portion of the dl-49 arm of the chromosome. Of 129 anaphase figures observed, four exhibited what may be interpreted either as broken bridges or lagging chromosomes, and five others showed two rod-shaped elements lying isolated between the polar groups of chromosomes (Figure 4). It seems probable that these elements were the pre-

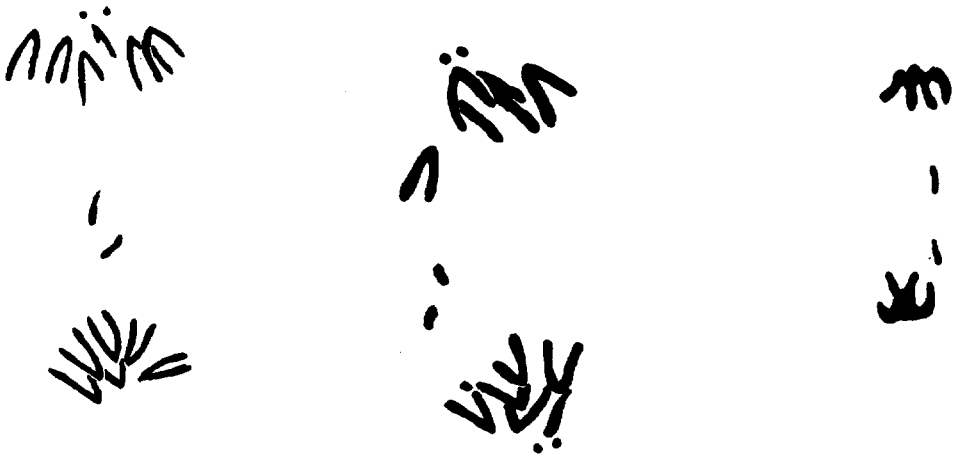

FIGURE 4.—Camera lucida drawings (ca. 2730 ×) of w^{vc}·dl-49 larval brain smear anaphases showing two elements isolated between the polar groups of chromosomes.

dicted acentrics. That there were two such elements per anaphase suggests that the distal chromatid union illustrated in Figure 1 either does not occur or that it persists only briefly, but apparently neither of these alternatives is compatible with production of double dicentrics by unstable ring chromosomes. In this case, one might suppose that the absence of the centromere either prevents the distal sister union or alters its consequences.

An obvious interpretation of these cytological observations in terms of the anaphase bridge model of instability is that bridge breakage results in aberration of the mitotic mechanism which at some later time causes death of the embryo. Because of the statistical inadequacy of the data, their use in attempting to support or deny a causal relation between bridges and other mitotic abnormalities would appear overzealous, especially in view of the lack of information on events occurring between cytologically visible bridges and other mitotic abnormalities. Similarly, the observations offer no direct means of relating the mitotic abnormalities to dominant lethality. Although genetic results (HINTON 1955) have shown that death occurs before larval emergence, none of the embryos, including many in developmental stages later than the eighth cleavage, prepared in this study were obviously necrotic.

SUMMARY

A cytological study was made of Feulgen whole mount preparations of third through eighth cleavage embryos of *Drosophila melanogaster*. These embryos were derived from four crosses involving stable X^{c2}, unstable w^{vc}, and stable and unstable w^{vc}·B^{s} chromosomes. Mitotic abnormalities were scored and included pycnosis, chromosome fragmentation, increased amounts of chromatin and anaphase bridges; the abnormalities were more frequent among embryos from the unstable than from the stable ring and rod chromosome crosses. These results and

other observations were interpreted in terms of the anaphase bridge model of w^{vc} chromosome instability. Evidence was found which suggests the occurrence of a chromatid type breakage fusion bridge cycle in this material.

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